THE AUTONOMIC NERVOUS SYSTEM

This document arises from a WHO meeting held in Bethesda, MD, USA, on 10-12 December 1998. It considers current research on the autonomic nervous system, directions for future investigation, and implications for clinical medicine and public health.

Edited by C. L. Bolis and J. Licinio

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LIST OF CONTRIBUTORS

**J. Antunes Rodrigues**, A.L.V. Favaretto and S.M. McCann
(1) Department of Physiology, School of Medicine of Ribeirao Preto, Sao Paolo, Brazil
(2) Pennington Biomedical Research Center, Louisiana, USA

**C. Liana Bolis** and M. Piccolella
(1) Laboratory of Comparative Biology, University of Milan, Italy
(2) Consultant, Office of Research Policy and Cooperation, World Health Organization, Geneva, Switzerland

**Stefan R. Bornstein and Antje Böttner**
National Institute of Child Health and Human Development, National Institute of Health, Bethesda, Maryland, USA, and Department of Internal medicine III, University of Leipzig, Germany

**Ary L. Goldberger**
Cardiovascular Division, Department of Medicine, Beth Israel Deaconess Medical Center; Harvard Medical School, Boston, Massachusetts, USA.

**David S. Goldstein**
Clinical Neuroscience Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

**Julio Licinio**
Unit of Clinical Research, Clinical Neuroendocrinology Branch, National Institute of Mental Health, Bethesda, Maryland, USA

**Frans H. Messerli and Ehud Grossman**
Department of Internal Medicine, Section on Hypertensive Diseases, Ochsner Clinic and Alton Ochsner Medical Foundation, New Orleans, Louisiana, USA
Markus Meyer, Thomas Flüge and Wolf-Georg Forssmann
Niedersächsisches Institut für Peptid-Forschung, Hannover, Germany

Massimo Pagani and Daniela Lucini
Dipartimento di Scienze Precliniche LITA di Vialba, Università di Milano,
Ospedale L.Sacco, Milan, Italy

Quentin J. Pittman
Neuroscience Research Group, Department of Physiology and Biophysics,
Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

Francesco E. Pontieri and Cesare Fieschi
Dipartimento di Scienze Neurologiche, Università degli Studi di Roma
“La Sapienza”, Rome, Italy

Virend K. Somers
Department of Internal Medicine, University of Iowa, USA

Yvette Taché
UCLA Department of Medicine, CURE, Digestive Diseases Research
Centre, Los Angeles, California, USA
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The autonomic nervous system (ANS), with its two main branches, the sympathetic and parasympathetic, consists of an integrated neuronal network that reaches all organs and tissues, and is essential for the maintenance of homeostasis. The ANS ensures rapid adjustment of biological functions that are crucial for survival, adaptation and evolution. It integrates afferent information that originates from both the environment and the internal milieu and, through its efferents, regulates the functions of various organs and systems according to the needs of the organism as a whole. Because of its role in the regulation of biological functions that are essential to life, the ANS was the subject of some of the earliest research work in neurology and neuroscience. The earliest recorded reference to the visceral nervous system was made by Galen in the second century. The considerable amount of neuroanatomical and neurophysiological work that was done over the last 18 centuries has helped elucidate the structure and key functions of the ANS. However, much remains to be done.

Recent progress in molecular medicine and imaging has led to new approaches in neuroscience research, and nervous system function is now viewed in a more mechanistic way. Elegant descriptions of the ANS and how it functions are giving way to increasingly sophisticated approaches that use the tools of contemporary biology to dissect the mechanisms through which the ANS ensures rapid and effective integration between cortical and visceral inputs, thereby establishing and maintaining homeostasis.

Dysregulation of such a key system is associated with disease. ANS dysfunction can be of two types. Primary ANS dysfunction causes disease by a direct dysfunction of this crucial regulation system. These disorders have been classified as (a) generalized ANS disorders such as Parkinson's disease, Guillain-Barré syndrome and familial dysautonomia; (b) segmental ANS disorders such as the peripheral neuropathy that is associated with amyloidosis, porphyria and alcoholism; and (c) focal ANS disorders such as Horner's syndrome. Additionally, ANS dysfunction can
be an element in the pathophysiology of complex disorders such as cardiovascular diseases, asthma, and depression.

The role of the ANS in disease goes beyond primary and secondary dysfunction. Pharmacological approaches to important diseases are based on drugs that are agonists and antagonists of various components of ANS function. The treatment of conditions that are highly prevalent and represent public health problems worldwide involves the use of drugs that modulate ANS function. Those diseases include various types of cardiovascular disorders, such as heart failure, arrhythmias, ischaemia and myocardial infarction, as well as diseases such as asthma, migraine, urinary retention and shock. Drugs that regulate the ANS are essential for surgical anaesthesia. Thus a better understanding of ANS biology and further work in this field will lead to improved knowledge of biology, pathophysiology, and pathogenesis in general. It will also lead to more efficacious treatment for human diseases of major public health significance.

On 10-12 December 1998 a World Health Organization meeting took place in Bethesda, MD, USA to consider the status of current research on the autonomic nervous system, directions for future investigation, and implications for clinical medicine and public health. As an outcome of that meeting, this book provides a progress report on ANS biology research and its implications for health.

C. Liana Bolis and Julio Licinio
THE AUTONOMIC NERVOUS SYSTEM IN HUMANS AND ANIMALS: COMPARATIVE ASPECTS

C.L. Bolis and M. Piccoliella

When comparative studies were first made on mammals and other animals, the researchers paid attention principally to differences in anatomy. It was only later, when technology improved, that they started to study comparative physiology and biochemistry.

The earliest recorded reference to the visceral nervous system was made by Galen in the second century. He gave the first account of the paravertebral sympathetic chains, but he made the mistake of describing the sympathetic and vagal trunks as one structure originating within the cranium. This gave rise to an error which persisted for fifteen hundred years. Galen was the first to note that the denervated heart maintained its beat. Following these observations of Galen, little progress was made for fourteen centuries until the time of Vesalius (1543), who depicted a combined vagosympathetic trunk arising from the brain stem. Stephanos (1545) and later Eustachius (1563) were the first to distinguish the two separate nerves.

In the seventeenth century Willis (1664) published a remarkably clear account of the ganglionated chains and their connections with the intercostal nerves. He described the cardiac branches and stated that the great mesenteric plexus sent its nerve fibres like rays in all directions; hence, it came to be called the solar plexus. He considered that its function was to place the heart and viscera in connection with the brain so that they would act in harmony. The modern nomenclature of the cranial nerves originated with Willis. In addition, he gave an accurate description of the vagus or “wandering nerve” with a true understanding of its apparent union with the cervical sympathetic in some of the lower mammals and its separate course in man. He even noted the branch given off to the aortic arch “so it may react to changes in the pulse”.
According to Sheehan (1936), who described the concept of the autonomic nervous system at the end of the seventeenth century, the “intercostal” (sympathetic) and “wandering” (vagus) nerves were clearly separated anatomically. Considering that this concept was based almost entirely on anatomical observations, it constituted a remarkable hypothesis.

In 1732 the Danish anatomist Winslow gave the name “sympathetic” to nerves which he demonstrated by dissection to lie outside the main cerebrospinal pathways. Neubauer (1772) published an illustration of the vagus and sympathetic nerves in the neck and thorax which ranks as one of the best anatomical plates ever produced.

As in other fields of medical science, anatomical knowledge developed far ahead of physiological experiment. In 1669 Lower published the earliest observations on stimulation of the vagus. Further experiments by Ens (1745) and by the Webers (1846) a century later finally established the role of the vagus in inhibition of the heart. The discovery that the sympathetic trunks originate below the cranium and not from the brain stem, as described by Galen and all subsequent anatomists, was made by Petit in 1727. He was likewise the first to observe the pupillary paralysis which follows cervical sympathectomy, thus antedating Bernard and Horner by over a hundred years.

The first appreciation of involuntary movements and visceral sensation developed out of the experiments of Whytt (1751). He was the first to gain an insight into such fundamental concepts as the tone of skeletal muscle, the reflex responses of the pupils to light, and the fact that “the distension of hollow muscles has a remarkable influence towards exciting them into action”. The ultimate expression of this theory had been reached in the writing of Willis, that “sympathy” was due to communications of the nerve tubes, which issued from the cerebellum and the brain stem, more especially those belonging to the “eighth pair” and the “intercostal nerve”. Whytt (1765) revised this traditional view by stating that “sympathy” presupposed feeling and must therefore be dependent on nerves.

Although earlier workers had recognized that the viscera were not under the voluntary control of the nervous system, they had not observed the structural differences between skeletal muscle and the muscular coats of the hollow viscera. This discovery was made by Muller (1840). Even he did not recognize that arteries possess a true muscular coat. The histological description of the muscular layer in the media was given by von Kolliker (1849) and its innervation by means of a periarterial sympathetic plexus was described by Henle (1868).
Langley's final establishment of the two great divisions of the involuntary nerves depended on Hirschmann's discovery in 1863 that moderate doses of nicotine prevent pupillary dilatation when the cervical sympathetic trunk is stimulated. In fact, the term “autonomic nervous system” was proposed by Langley in 1898 to describe “...the sympathetic system and the allied nervous system of the cranial and sacral nerves, and for the local nervous system of the gut” (Langley, 1898). Early work on the functional anatomy of the autonomic nervous system included studies on tetrapods other than mammals, for instance amphibians and birds. Young studied both teleost and elasmobranch fish, describing the morphology of the autonomic innervation of the iris (Young, 1931a; 1933a,b) and viscera (Young, 1931b; 1933c; 1936).

The comparative approach makes it possible to understand how animals can adjust various physiological parameters in response to changing environmental or internal cues. Evolutionary changes governed by natural selection have occurred in vertebrates over 400 million years. For example, all vertebrates have an alimentary canal of some sort, but the control systems are not identical in all the animal groups. Comparative studies have been very important in furthering understanding of different pathways that are fundamental in human beings.

The mammalian autonomic nervous system has been divided into a sympathetic, a parasympathetic and an enteric portion. In the original definitions, the sympathetic pathways run from thoracic and lumbar segments of the spinal cord, while the parasympathetic pathways run in the cranial nerves and also from the sacral segments. This anatomical division was supported by the perceived functional differences, and the terms sympathetic and adrenergic have become almost synonymous in the literature dealing with mammals. The discovery of a vast number of non-adrenergic, non-cholinergic neurotransmitters, notably peptides, clearly challenges the idea that “sympathetic = adrenergic”.

Similarly, a functional subdivision based on transmitter content of the neurons can be made for some types of nerve.

**FISHES**

In fishes, the cranial autonomic pathways are probably restricted to the oculomotor and vagus nerves, although autonomic fibres in the facial and glossopharyngeal nerves in elasmobranchs and teleosts have been postulated by some workers (Nicol 1952). There are no lacrimal or salivary glands in fish, and the main targets for the cranial autonomic pathways within the head are the eye and the gills.
The autonomic nervous system

The paired sympathetic chains are very well developed in teleosts. In contrast to the situation in other vertebrates, these chains continue into the head, bearing ganglia in contact with the cranial nerves. In the elasmobranchs, the paravertebral ganglia are not always longitudinally connected to form proper sympathetic chains of the teleost or tetrapod type. The autonomic nervous system of cyclostomes is fragmentary.

In the African and South American lungfish, cranial autonomic pathways are restricted to the vagus, but in the Australian lungfish there may be additional pathways to the eyes running in the oculomotor nerve (Nicol, 1952).

The transmitter substances found in the fish groups are similar to those found in the tetrapods, with the notable exception of the neuropeptides, where the amino acid sequences differ (Holmgren and Jensen, 1994). Thus, both acetylcholine and the catecholamines (adrenaline and noradrenaline) occur in autonomic nerves of fishes.

Adrenaline is the dominant catecholamine in adrenergically innervated organs of teleosts and amphibians, while noradrenaline dominates in the other groups. Contrary to popular belief, noradrenaline is not the sole catecholamine in mammalian adrenergic neurons (von Euler, 1946). In fact, adrenaline release from mammalian adrenergic nerves can be quite substantial, and this is also true for birds. Serotonin is now recognized as an important transmitter substance in the autonomic nervous system of both mammals and non-mammalian vertebrates.

There are clear differences in the level of organization of the ganglia associated with the spinal autonomic outflow in the different classes of fish.

Cyclostomes

The spinal autonomic pathway in cyclostomes is sparse and confusing. This must be due to the ill-defined nature of tissues that could be considered to be components of these pathways (Nicol, 1952). Cyclostomes do not possess sympathetic ganglia like those seen in mammals, and it is most probable that they do not have autonomic ganglion cells closely associated with the roots of their spinal nerves.

Although there is no obvious widespread system of spinal autonomic neurons, there are defined aggregations of chromaffin cells throughout the body of the lamprey (Gaskell, 1912). The largest collection of chromaffin cells is located in the walls of the anterior part of the posterior cardinal veins. Some of the chromaffin cells are multipolar and are innervated by fibres running from the dorsal rather than
the ventral roots. Although these fibres have been considered to be autonomic, there is no functional evidence that this is really the case.

**Elasmobranchs**

Sympathetic ganglia and their corresponding spinal pathway clearly are present in elasmobranchs. They still possess some of the characteristics of the cyclostome spinal autonomic system, the most notable being more or less segmentally arranged aggregations of chromaffin tissue. Sympathetic ganglia are absent from the cranial and caudal regions (Shore, 1889). Adjacent ganglia are usually interconnected by a loose network of fine nerve trunks, but there is not a well-demarcated sympathetic chain as seen in mammals, and some ganglia may lack direct connections with their neighbours. On the other hand, there may be connections between ganglia on each side of the animal. Although there are some ganglion cells scattered around the urogenital tract, there are no well-defined pelvic ganglia. Postganglionic fibres are thought not to travel with the spinal nerves. Rather, they follow segmental blood vessels directly in the splanchnic nerves to the gastrointestinal tract and perhaps other viscera (Nilsson and Holmgren, 1988).

**Teleosts**

The sympathetic ganglia of teleost fish are generally arranged in well-organized paravertebral sympathetic chains, adjacent ganglia being connected by a well-defined sympathetic trunk.

In most teleost species, there are usually five pairs of cranial sympathetic ganglia associated with the cranial nerves (Chevrel, 1889). The first two ganglia are associated with the trigeminal-facial sensory ganglion complex.

The great majority of the neurons in the cranial sympathetic ganglia synthesize catecholamines (Nilsson, 1983). Postganglionic sympathetic fibres run out with their corresponding cranial nerves to supply blood vessels, pigment cells, and perhaps glands in the cranial and branchial region (Chevrel, 1889). Like the cranial sympathetic ganglia, the postganglionic neurons in the spinal sympathetic ganglia directly enter their corresponding spinal nerve via communicating rami to innervate blood vessels, pigment cells and perhaps cutaneous glands.

Many teleost species possess some form of coeliac ganglion that is absent from the primitive chondrostean fish, as is the case in elasmobranchs (Chevrel, 1894). Most of the postganglionic neurones in the sympathetic chains, coeliac ganglion, or scattered along the splanchnic nerves contain catecholamines (Nilsson, 1983). However,
there is pharmacological evidence that the pigment cells in the skin of
the catfish are innervated by cholinergic sympathetic neurons, pres-
sumably arising from the chain ganglia.

**Dipnoans**
As in teleosts, the sympathetic ganglia of lungfish are arranged in
well-defined paravertebral sympathetic chains. However, like
tetrapods, they have no cranial sympathetic ganglia.

**AMPHIBIANS**
There are marked differences in the organization of the spinal au-
tonomic pathways in the three major groups of extant amphibians, rang-
ing from the primitive urodeles through the familiar anurans to the
highly specialized legless amphibians.

Urodeles are the amphibian group with the most primitive ar-
rangements of the sympathetic ganglia. In some species the more cra-
nial sympathetic trunk on each side is doubled, with a component
lying either side of each lateral aorta. The largest sympathetic ganglia
occur in the region of the branchial plexus, with a relatively large
ganglion lying cranial and caudal to the origin of the subclavian ar-
tery. These ganglia are interconnected with each other and other smaller
ganglia to form a subclavian plexus.

In contrast with the urodeles, the sympathetic chains of anuran
amphibians are well organized, with prominent segmentally arranged
ganglia. Anurans have a greatly reduced number of vertebrae and
spinal segments, and there are usually only ten pairs of sympathetic
ganglia, corresponding to the number of spinal nerves (Pick, 1970).
There are no cranial sympathetic ganglia, although the sympathetic
trunk extends into the head as far as the ciliary nerves (Morris, 1975).
The two most cranial pairs of ganglia tend to be larger than the rest
(Huber, 1900).

The sympathetic chain in caecilians is much reduced compared
with other amphibians and with any other tetrapods. This reduction
of the sympathetic system may be a consequence of their limbless
condition with a concomitant reduction in the necessity for specific
regional control of the circulation, for example.

**REPTILES**
In general, the spinal sympathetic pathways of reptiles and birds are
well organized, with clearly defined paravertebral chains. Some re-
tiles and birds share features not seen in other vertebrates.
The paravertebral sympathetic chains of the lizard may contain from 15 to 25 pairs of ganglia, each one corresponding to a spinal nerve. The most cranial ganglion is at the level of the branchial plexus and is considerably larger than the rest (Hirt, 1921; Adams, 1942). Virtually all of the postganglionic neurones in the sympathetic chain of lizards synthesize catecholamines. The great majority of them are likely to innervate the cardiovascular system, although most of the viscera do have a sympathetic innervation (McLean and Burnstock, 1967a,b). Furthermore, in chameleons there is a well-documented sympathetic innervation of the cutaneous chromatophores (Sand, 1935).

The arrangement of the sympathetic ganglia in chelonia seems to be similar to that in lizards (Hirt, 1934). There is a particularly large ganglion at the level of the branchial plexus, which gives rise to cardiac and pulmonary nerves. The cervical sympathetic trunk may bear two to three small ganglia; these ganglia also may contribute to the cardiac nerves, as well as to the innervation of cranial structures (Gaskell and Gadow, 1884; Terni, 1931).

The spinal sympathetic ganglia of crocodilians have a number of peculiarities in their arrangement (Hirt, 1934). The most cranial of the thoracic ganglia lies at the level of the branchial plexus, and as in the case of the lizard, it is much larger than the other paravertebral ganglia. In addition to feeding into the branchial plexus, the ganglion gives rise to a prominent cardiac nerve. Consequently, it has been named the "cardiac ganglion" (Gaskell and Gadow, 1884), although it clearly corresponds to the stellate ganglion of mammals.

**BIRDS**

The sympathetic chains of birds extend from upper cervical to sacral levels. The ganglia are well formed and are segmentals arranged with short communicating rami to their corresponding spinal nerves. As in crocodiles, ganglia are present in the cervical sympathetic trunk, which runs through the transverse foramina of the cervical vertebrae. There is convincing functional and histological evidence that preganglionic outflow to the sympathetic chains has a much more restricted distribution, extending only from the last cervical segment to the fourth lumbar segment (Langley, 1904; Terni, 1931). The major targets of these neurons are blood vessels and the penna-motor muscles, which may be innervated by two functionally distinct classes of neurons: one raising the feathers and one lowering them (Langley, 1904). In contrast with reptiles, there are well-developed prevertebral ganglia in birds (Pick, 1970; Bennett, 1974).
It seems to be clear that the increase in organizational complexity of the peripheral autonomic nervous system across vertebrate phylogeny is parallel to that seen in the central nervous system. Presumably, it is related most closely to the requirements for increasingly efficient control of the cardiovascular system, gut and other internal organs in response to the high energy demands imposed by lifestyles progressively more independent of environmental constraints (Bray, 1985; Carroll, 1988; Little, 1990).

**NEUROTRANSMITTERS: COMPARATIVE ASPECTS**

**Cholinergic neurotransmitter**
Acetylcholine has been considered the transmitter of old preganglionic neurons, and of most postganglionic, parasympathetic neurons of the autonomic nervous system in old vertebrate groups (Nilsson, 1983). The earliest experiments leading to the biochemical identification of acetylcholine were made by Loewi (1921) on the amphibian heart.

**Adrenergic neurotransmitter**
Adrenergic neurons use the catecholamines noradrenaline and adrenaline as transmitters. Catecholamines are synthesized in adrenergic nerves as in an adrenal medulla. Noradrenaline is formed from tyrosine and adrenaline is formed from noradrenaline. Adrenaline is synthesized intraneuronally and can act as a transmitter in some species. Assays have shown that adrenaline predominates over noradrenaline in many organs of amphibians, holosteans and teleosts (Holtzbaumer and Sharman, 1972; Abrahamson and Nilsson, 1976). Noradrenaline is the predominant catecholamine in mammalian adrenergic nerves. But analyses of perfusates from the mammalian intestine after stimulation of mesenteric nerves showed that 5-25 % of the catecholamines were adrenaline (Mann and West, 1951).

**Neuropeptides**
Peptides were recognized as neurotransmitters of the autonomic and sensory nervous systems at a relatively late stage. Several of the humoral substances released from endocrine cells were peptides. The demonstration of the substance P-like peptide in the intestine of both an elasmobranch, the spiny dog fish, *Squalus acanthias*, and a teleost, the cod, *Gadus morhua*, was the first finding of a regulatory peptide in a non-mammalian vertebrate (von Euler and Ostlund, 1956). Like its mammalian counterpart the non-mammalian peptide occurred in
both brain and gut. Several properties of the fish polypeptide were similar to those of the mammalian peptide substance P. It was established at an early stage that brain-gut peptides existed in non-mammalian species as well as in mammals. Indeed neuropeptides occur in the nervous system of all animal groups possessing a nervous system, from the most simple of coelenterates (Thorndyke and Goldsworthy, 1988; Nilsson and Holmgren, 1989). Compared to other known types of transmitters there is an enormous potential diversity for the individual in using peptides as neuronal messengers.

**Bombesin-like peptides**

Although first demonstrated in the amphibian skin, bombesin or related peptides have subsequently being demonstrated in autonomic and central neurons as well as endocrine cells of the gut and lung in number of non-mammalian species, both vertebrates and invertebrates. It has been agreed that the generic family name “bombesin-like peptides” should be used for all peptides with a C-terminal aminoacid sequence similar to bombesin (Erspaner and Go, 1988).

During the last two decades, the characterization of many neuropeptides and their receptors has given new impetus to exploring how the central nervous system (CNS) regulates visceras function through autonomic nervous system pathways.

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10 The autonomic nervous system


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12 The autonomic nervous system
STRUCTURAL AND CHEMICAL ORGANIZATION OF THE AUTONOMIC NEUROEFFEKTOR SYSTEM

David S. Goldstein

OVERVIEW OF PARASYMPATHETIC AND SYMPATHETIC NEUROTRANSMISSION

The autonomic nervous system contains two components—the parasympathetic and sympathetic nervous systems (Figure 1). The parasympathetic system consists of two sets of nerves, one derived from the brainstem, constituting the cranial parasympathetic outflow, and the other derived from the intermediolateral columns of the sacral spinal cord, constituting the sacral parasympathetic outflow. In contrast, sympathetic nerves emanate from the thoracic and lumbar spinal cord.

Whereas increased sympathetic nervous preganglionic outflows expend metabolic energy, such as via epinephrine-induced glycogenolysis, increased parasympathetic outflows tend to replenish energy stores during periods of quiescence. Thus, parasympathetic stimulation promotes digestion, by releasing gastrin and insulin and increasing gut motility, and decreases the force and rate of cardiac contraction. Conversely, parasympathetic activity decreases during many but by no means all situations involving emotional distress. In general, when sympathoneural activity increases, parasympathetic activity decreases.

The main parasympathetic nerve, the vagus nerve, emanates from the medulla of the brainstem as the tenth cranial nerve. The vagus innervates the heart, bronchioles, skin, and mesenteric organs. In contrast with sympathetic ganglia, strung in chains alongside the spinal column, parasympathetic ganglia lie close to or inside the innervated organs. The post-ganglionic nerves therefore are short or non-existent, and the vagus consists of pre-ganglionic fibres. Stimulation of the right vagus nerve slows the discharge rate of the sinus node, while stimulation of the left induces less slowing of the sinus rate but more interference with atrioventricular conduction.
All parasympathetic nerves release acetylcholine as the neurotransmitter. Acetylcholine is synthesized from the choline acetyltransferase-catalysed transfer of acetyl-coenzyme A to choline and is inactivated by acetylcholinesterase almost immediately after release of the transmitter from nerve endings. Other cholinergic nerves participate in neuromuscular transmission, adrenomedullary and sweat gland secretion, and, in some species, sympathetic vasodilation.

Specific receptors mediate the effects of acetylcholine. Classically they have been divided into nicotinic and muscarinic (Dale, 1914). Atropine blocks muscarinic receptors selectively and hexamethonium and related drugs nicotinic receptors selectively. Nicotinic receptors also mediate cholinergic transmission from pre- to post-ganglionic sympathetic neurons in the sympathetic ganglia and cholinergic stimulation of adrenomedullary secretion. Muscarinic agonists stimulate gut smooth muscle contraction and glandular secretion and inhibit norepinephrine release from sympathetic nerve terminals. At skeletal neuromuscular junctions, acetylcholine binds to nicotinic receptors distinct from those in the autonomic nervous system.

Acetylcholine usually produces vasodilation. The vasodilator effect of acetylcholine depends at least partly on intact endothelium (Furchgott, 1983). Acetylcholine increases local generation of the endothelium-derived relaxing factor, nitric oxide. N-methylarginine, by blocking nitric oxide production, therefore prevents acetylcholine-induced vasodilation (Figure 2), and denudation of endothelium not only unmasks a vasoconstrictor effect of acetylcholine but also potentiates responses to norepinephrine (Urabe et al., 1991).

The sympathetic nervous system, the neuronal component of what Walter B. Cannon termed the sympathico-adrenal system (Cannon, 1929), consists of nerve networks. Sympathetic nerves derive from cells in ganglia rather than from cells in the spinal cord or brainstem and therefore consist mainly of post-ganglionic neurons. Peripheral nerves contain sympathetic fibres that supply the vasculature of the skeletal muscle and skin, innervate sweat glands, and carry afferent traffic from nociceptors. The fibers enmesh the adventitial and adventitial-medial layer of arteries and form lattice-like networks in the myocardium and in glands (Figure 3).

Sympathetic nerve stimulation constricts arterioles almost instantaneously, increasing regional resistance to blood flow and diverting blood flow to other regions with less resistance. Diffuse sympathetic stimulation increases total arteriolar resistance to blood flow, and since sympathetic activation in the heart increases the force and rate of
cardiac contraction, blood pressure increases from both increased peripheral resistance and increased cardiac output. Stimulation of sympathetic nerves to the salivary glands increases salivation, to the eye dilates the pupil, to sweat glands increases sweating, to hair follicles evokes piloerection, to the kidneys inhibits sodium excretion, and to skeletal muscle causes trembling.

The key chemical transmitter at sympathetic nerve endings is norepinephrine. Sympathetic stimulation releases norepinephrine, and binding of norepinephrine to adrenoceptors on cardiovascular smooth muscle cells causes the cells to contract.

In contrast with sympathetic nerves, adrenomedullary cells secrete catecholamines – in humans mainly epinephrine – directly into the bloodstream. The adrenomedullary system therefore is hormonal. Epinephrine affects the function of most body organs (Figure 3). Exogenously administered epinephrine rapidly increases the rate and force of cardiac contraction; increases myocardial cell automaticity; dilates bronchioles and increases the rate of breathing; redistributes blood volume toward the heart, brain, and skeletal muscle and away from the skin, kidneys, and gut; enhances the aggregability of platelets; relaxes smooth muscle of the uterus and gut; increases blood glucose by a variety of means including glycogenolysis and antagonizing insulin; dilates pupils; increases activity of the renin-angiotensin-aldosterone system; decreases serum potassium concentrations; increases the metabolic rate; and produces psychological effects such as increased alertness, decreased fatigue, and intensification of emotions (Schachter and Singer, 1962).

These effects would be expected usually to enhance survival in emergencies such as traumatic haemorrhage, hypoglycaemia, asphyxiation, cardiac collapse, or emotional distress, when the individual senses an overall threat to well-being or survival.

The spinal cord is the most distal site of the central nervous system that generates patterns of sympathetic activity. The final common pathway for sympathetic outflow is the preganglionic neuron. The sympathetic preganglionic neurons discharge spontaneously at only a slow rate. Their tonic activity depends instead mainly on input from chemoreceptor, somatic, and visceral afferent nerve traffic to the spinal cord, but more importantly on descending input from supraspinal structures.

Whereas feedback from the periphery contributes relatively little to direct regulation of sympathetic preganglionic neuron activity, feedback becomes a prominent feature at the level of the medulla,
where visceral afferent fibres synapse. Medullary cardiovascular centres therefore subserve simple homoeostatic reflexes.

After exiting the spinal cord, axons from sympathetic preganglionic neurons travel via white rami to the preaortic and paravertebral chains of sympathetic ganglia. In contrast, fibres innervating the adrenal medulla typically pass through the splanchnic ganglia without synapsing. Adrenal nerve activity therefore at least partly reflects preganglionic outflow, whereas activity in, for instance, the renal nerve reflects postganglionic outflow. Adrenomedullary chromaffin cells resemble ganglionic sympathetic neurons in that the adrenomedullary nerves release acetylcholine as the neurotransmitter.

Whereas mechanisms of release of acetylcholine at skeletal neuromotor junctions have received extensive research attention, mechanisms of acetylcholine release at parasympathetic junctions – especially the roles of cholinergic receptors on vagal terminals in modulating acetylcholine release – remain obscure.

**MECHANISMS OF NOREPINEPHRINE SYNTHESIS, RELEASE, UPTAKE AND METABOLISM**

Norepinephrine biosynthesis begins with uptake of the amino acid tyrosine (Figures 4 and 5). Tyrosine hydroxylase catalyses the conversion of tyrosine to dihydroxyphenylalanine (L-DOPA). This is the enzymatic rate-limiting step in catecholamine synthesis. The enzyme is almost saturated under normal conditions and is stereospecific. Concentrations of tetrahydrobiopterin, Fe++, and molecular oxygen regulate tyrosine hydroxylase activity. Dihydropteridine reductase catalyses the reduction of dihydropterin, produced during the hydroxylation of tyrosine (Figure 5). Since the reduced pteridine, tetrahydrobiopterin, is a key co-factor for tyrosine hydroxylase, dihydropteridine reductase deficiency decreases the amount of tyrosine hydroxylation for a given amount of tyrosine hydroxylase enzyme. Phenylalanine hydroxylase, tryptophan hydroxylase, tyrosine hydroxylase, and nitric oxide synthase all require tetrahydrobiopterin as a co-factor. Sympathetic activation augments the synthesis and tissue concentrations of tyrosine hydroxylase, helping to maintain tissue norepinephrine stores. Multiple and complex mechanisms contribute to this activation. Short-term mechanisms include feedback inhibition and phosphorylation of the enzyme, the latter depending on membrane depolarization, contractile elements, and receptors. Long-term mechanisms include changes in tyrosine hydroxylase synthesis, probably determined by trans-synaptic induction and retrograde transport. Even with diminished norepinephrine stores after
prolonged sympathoneural activation, increased nerve traffic can maintain extracellular fluid levels of the transmitter.

L- aromatic-amino-acid decarboxylase (also called DOPA decarboxylase) catalyses the rapid conversion of DOPA to dopamine. Many tissues contain this enzyme – especially the kidneys, gut, liver, and brain. Activity of the enzyme depends on pyridoxal phosphate. Although L- aromatic-amino-acid decarboxylase metabolizes most of the DOPA formed in catecholamine-synthesizing tissues, some of the DOPA enters the circulation unchanged, providing the basis for using plasma DOPA levels to examine catecholamine synthesis (Goldstein et al., 1987).

Dopamine-β-hydroxylase catalyses the conversion of dopamine to norepinephrine. Dopamine-β-hydroxylase is confined to monoamine-storing vesicles. Dopamine-β-hydroxylase contains, and its activity depends on, copper. Because of this dependence, children with Menke’s disease, a rare, X-linked recessive inherited disorder of copper metabolism, have neurochemical evidence for decreased dopamine-β-hydroxylase and compensatorily increased tyrosine hydroxylase (Kaler et al., 1993). Patients with absent dopamine-β-hydroxylase have virtually undetectable levels of both norepinephrine and its neuronal metabolite dihydroxyphenylglycol (Goldstein et al., 1989). Dopamine-β-hydroxylase activity also requires ascorbic acid, which provides electrons for the hydroxylation.

Phenylethanolamine-N-methyltransferase catalyses the conversion of norepinephrine to epinephrine. S-adenosyl methionine constitutes the methyl donor for this reaction.

Vesicles in sympathetic nerves actively remove and trap axoplasmic amines, via a vesicular monoamine transporter protein. Vesicular uptake favors L- over D-norepinephrine, Mg²⁺ and ATP accelerate the uptake, and reserpine effectively and irreversibly blocks it. The vesicular uptake carrier protein resembles the neuronal uptake carriers.

Adrenomedullary chromaffin cells, much easier to study than sympathetic nerves, have provided the most commonly used model for studying mechanisms of catecholamine release. Agonist occupation of nicotinic acetylcholine receptors releases catecholamines from the cells.

According to the exocytotic theory of norepinephrine release, acetylcholine depolarizes the terminal membranes by increasing membrane permeability to Na⁺ (Figure 6). The increased intracellular Na⁺ levels directly or indirectly enhance transmembrane influx of Ca²⁺, via voltage-gated Ca²⁺ channels. The increased cytoplasmic Ca²⁺ concentration evokes a cascade of as yet incompletely defined
biomechanical events resulting in fusion of the vesicular and axoplasmic membranes. The interior of the vesicle exchanges briefly with the extracellular compartment, and the soluble contents of the vesicles diffuse into the extracellular space.

As predicted from this model, manipulations besides application of acetylcholine that depolarize the cell, such as electrical stimulation or increased K+ concentrations in the extracellular fluid, also activate voltage-gated Ca++ channels and trigger exocytosis. During cellular activation, simultaneous, stoichiometric release of soluble vesicular contents — ATP, enkephalins, chromogranins, and dopamine-ß-hydroxylase — without similar release of cytoplasmic macromolecules, provides biochemical support for the exocytosis theory. Consistent with transient exposure of the interior of the vesicle to the extracellular fluid, after intravenous injection of fluorescein-labelled antibodies to dopamine-ß-hydroxylase, labelled antibodies accumulate in the terminals (Jacobowitz et al., 1975). Electron micrographs occasionally show an “omega sign,” with an apparent gap in the cell membrane at the site of fusion of vesicle with the axoplasmic membrane (Thureson-Klein et al., 1979).

Sympathetic nerve endings have the capacity also to release norepinephrine by Ca++-independent, non-exocytotic mechanisms. The hydrophilic nature of catecholamines and their ionization at physiological pH probably prevent norepinephrine efflux by simple diffusion.

Compounds besides norepinephrine released by sympathetic stimulation may function as neurotransmitters. ATP, adenosine, neuropeptide Y, acetylcholine, and epinephrine have received the most attention.

Unlike acetylcholine, which undergoes inactivation mainly by enzymatic degradation extracellularly, norepinephrine undergoes inactivation mainly by cellular uptake, with subsequent intracellular metabolism or storage. Reuptake into nerve terminals — Uptake-1 — is the predominant means of terminating the actions of released norepinephrine. Uptake-1 is energy-requiring, carrier-mediated, and can transport catecholamines against large concentration gradients. The only common structural feature of all known substrates for Uptake-1 is an aromatic amine, with the ionizable nitrogen moiety not incorporated in the aromatic system. Uptake-1 does not even require a catechol nucleus. Alkylation of the primary amino group decreases the effectiveness of the transport, explaining why sympathetic nerves take up norepinephrine more efficiently than they do epinephrine (Eisenhofer et al., 1990) and why they do not take up isoproterenol,
an extensively alkylated synthetic catecholamine, at all. O-Methylation of the phenolic hydroxyl groups also markedly decreases susceptibility to Uptake-1, and so sympathetic nerves do not take up O-methylated metabolites such as normetanephrine.

Neuronal uptake absolutely requires intracellular K⁺ and extracellular Na⁺ and functions most efficiently when Cl⁻ accompanies Na⁺. Transport does not require ATP directly; however, maintaining the trans-membrane ionic gradients depends on ATP, and the carrier uses the energy expended in maintaining the transmembrane Na⁺ gradient to co-transport amines with Na⁺.

Norepinephrine removed from the extracellular fluid by Uptake-1 is subject to two fates – translocation into storage vesicles and deamination by monoamine oxidase-A (MAO-A). The combination of enzymatic breakdown and vesicular uptake constitutes an intraneuronal “sink”, keeping cytoplasmic concentrations of norepinephrine very low.

MAO catalyzes oxidative deamination of dopamine to form dihydroxyphenylacetic acid and norepinephrine to form dihydroxyphenylglycol. Because of efficient uptake and reuptake of catecholamines into the axoplasm of catecholaminergic neurons, and because of the rapid exchange of amines between the vesicles and axoplasm, the neuronal pool of MAO, located in the outer mitochondrial membrane, figures prominently in the overall functioning of catecholaminergic systems.

Pharmacological characteristics and genetic cloning have revealed two isozymes of MAO, MAO-A and MAO-B. Clorgyline blocks MAO-A, and deprenyl and pargyline block MAO-B. MAO-A predominates in neural tissue, whereas both subtypes exist in non-neuronal tissue. Thus, inhibitors of MAO-A potentiate the pressor effects of tyramine, whereas inhibitors of MAO-B do not. Norepinephrine and epinephrine are substrates for MAO-A, and dopamine is a substrate for both MAO-A and MAO-B. The deaminated products are short-lived aldehydes. For dopamine, the aldehyde intermediate is converted rapidly to dihydroxyphenylacetic acid by aldehyde dehydrogenase; for norepinephrine, the aldehyde intermediate is converted mainly to dihydroxyphenylglycol by an aldehyde reductase. The formation of the aldehydes reduces a flavine component of the enzyme. The reduced enzyme reacts with molecular oxygen, regenerating the enzyme but also producing hydrogen peroxide, which may be toxic to cells, because the peroxidation releases free radicals. MAO-B inhibitors appear to delay neurological degeneration in patients with Parkinson’s disease, possibly by limiting oxidative injury.
Non-neuronal cells remove norepinephrine actively by a process
called Uptake-2, characterized by the ability to transport isoproter-
enol, susceptibility to blockade by metanephrines, corticosteroids, and
β-haloalkylamines, and an absence of susceptibility to blockade by
the Uptake-1 blockers cocaine and desipramine. In contrast with
Uptake-1, Uptake-2 functions independently of extracellular Na⁺. The
Uptake-2 carrier has little if any stereoselectivity and has low affinity
and specificity for catecholamines. For instance, extraneuronal cells
remove imidazolines such as clonidine by Uptake-2. The affinity of
Uptake-2 for dobutamine averages about 100 times that of DA.
Whereas reverse transport via the Uptake-1 carrier requires special
experimental conditions, one can readily demonstrate reverse trans-
port via the Uptake-2 carrier. Thus, during infusion of a catecholamine
at a high rate, the catecholamine can accumulate in extraneuronal
cells, with re-entry of the catecholamine into the extracellular fluid
via the Uptake-2 carrier after the infusion ends.

Catechol-O-methyltransferase (COMT) catalyzes the conversion
of norepinephrine to normetanephrine (NMN) and epinephrine to
metanephrine. Uptake-2 and COMT probably act in series to remove
and degrade circulating catecholamines. The methyl group donor for
the reaction is S-adenosyl methionine. Immunohistochemical studies
have indicated mainly extraneuronal localization of COMT, which ex-
ists at high concentrations in the liver and kidney. In these organs,
COMT catalyses O-methylation of dihydroxyphenylglycol (DHPG)
to form methoxyhydrox-phenylglycol (MHPG) and of dihydroxy-
phenylacetic acid (DOPAC) to form homovanillic acid (HVA). In
contrast, since COMT exists at surprisingly high concentrations in
adrenomedullary chromaffin cells, plasma levels of free (unconjugated)
metanephrines in humans derive mainly from the adrenal medulla,
and elevated plasma metanephrine levels provide a sensitive means
to detect phaeochromocytomas, tumors that synthesize catechol-
amines (Eisenhofer et al., 1998).

PHARMACOLOGICAL MODULATION OF SYMPATHETIC
NEUROEFFECTOR FUNCTION
Several classical neuropharmacological agents modulate release of
norepinephrine from sympathetic terminals. Drugs that block gan-
glionic neurotransmission decrease the rate of bursts of post-gangli-
onic sympathetic post-ganglionic nerve traffic to virtually zero
(Grossman et al., 1991). Loss of sympathetic vasoconstrictor tone
can explain decreased total peripheral and forearm vascular resis-
tance, dilation of conjunctival blood vessels, and nasal congestion dur-
ing ganglion blockade. Drugs that block ganglionic neurotransmission (e.g. trimethaphan, hexamethonium, pentolinium) also produce symptoms and signs of parasympathetic inhibition, including dry mouth and decreased gastrointestinal and urinary bladder motility.

The effects of acute administration of nicotine, which augments ganglionic neurotransmission, result mainly from increased adrenergic-medullary secretion of epinephrine. Concurrent parasympathetic stimulation can increase salivation, secretion of insulin and gastrin, and gastrointestinal motility.

Catecholamines and DOPA feedback inhibit tyrosine hydroxylase, and α-methyl-para-tyrosine inhibits the enzyme competitively.

α-MethylDOPA, an effective drug in the treatment of high blood pressure, inhibits L-aromatic-amino-acid decarboxylase and therefore norepinephrine synthesis. α-Methylnorepinephrine, formed from α-methylDOPA in catecholamine-synthesizing tissues, stimulates α₂-adrenoceptors in the brain and thereby inhibits sympathetic outflows, explaining the anti-hypertensive efficacy of α-MethylDOPA. Other inhibitors of L-aromatic-amino-acid decarboxylase include carbipoda and benzerazide. These catechols do not readily penetrate the blood-brain barrier, and by inhibiting conversion of DOPA to dopamine in the periphery, they enhance the efficacy of L-DOPA treatment of Parkinson’s disease.

Reserpine blocks the vesicular monoamine transporter, and ongoing leakage of norepinephrine from vesicles into the cytoplasm leads rapidly to depletion of norepinephrine stores. Reserpine also increases activity of tyrosine hydroxylase and thereby catecholamine synthesis. After reserpine injection, plasma dihydroxyphenylglycol levels increase rapidly, reflecting marked net leakage of norepinephrine from vesicular stores, and then decline to very low levels, reflecting the abolition of vesicular uptake and β-hydroxylation of dopamine (Eisenhofer et al., 1988).

Each molecule of norepinephrine synthesized from dopamine by the actions of dopamine-β-hydroxylase consumes a molecule of intragranular ascorbic acid; loss of granular ascorbic acid stops norepinephrine synthesis.

Sympathomimetic amines such as amphetamines and tyramine displace norepinephrine from vesicular stores and appear to release norepinephrine mainly non-exocytotically, since tyramine releases norepinephrine independently of Ca²⁺ and does not release dopamine-β-hydroxylase. As predicted from the ionic requirements for neuronal reuptake, increases in intracellular Na⁺ concentrations, such as produced by ouabain, enhance carrier-mediated efflux of norepinephrine.
Phrine. Myocardial ischaemic hypoxia also evokes Ca\textsuperscript{++}-independent release of norepinephrine (Schomig et al., 1987).

Bretylium, called for in current algorithms in advanced cardiac life support for treating ventricular fibrillation, inhibits exocytosis, by poorly understood mechanisms. Hypotensive effects therefore can limit the use of bretylium as an anti-arrhythmic drug.

Many drugs or in vitro conditions inhibit Uptake-1, including cocaine, tricyclic antidepressants, low extracellular Na\textsuperscript{+} concentrations, and Li\textsuperscript{+}. Cocaine increases norepinephrine concentrations in extracellular fluid and therefore at post-synaptic adrenoceptors; tricyclic antidepressants do not, because their administration produces marked concurrent decreases in rates of post-ganglionic sympathetic nerve traffic (Eisenhofer et al., 1991). Desipramine-induced blockade of neuronal uptake of norepinephrine augments pressor responses during sympathetic stimulation or administration of catecholamines.

Desipramine and other tricyclic antidepressants block uptake by noradrenergic neurons more effectively than they block uptake by dopaminergic neurons, suggesting different membrane transporters for norepinephrine and dopamine. Recent molecular genetic studies have confirmed this distinction. The human norepinephrine transporter protein includes 12-13 hydrophobic and therefore probably membrane-spanning domains. This structure differs substantially from that of adrenoceptors and other receptors coupled with G-proteins but is very similar to that of the dopamine, \gamma-aminobutyric acid, serotonin, and vesicular transporters, suggesting a family of neurotransmitter transporter proteins.

Drugs that inhibit MAO-A decrease oxidative metabolism of norepinephrine and dopamine in sympathetic terminals. MAO inhibitors are effective antidepressants. A phenomenon known as the "cheese effect" limits their clinical use. In patients taking MAO inhibitors, administration of sympathomimetic amines such as in many non-prescription decongestants, or ingestion of foods such as aged cheese, wine, or meat, which contain tyramine, can produce paroxysmal hypertension. Since tyramine and other sympathomimetic amines displace norepinephrine from sympathetic vesicles into the axoplasm, blockade of MAO in this setting causes axoplasmic norepinephrine to accumulate, and outward transport of the norepinephrine stimulates cardiovascular smooth muscle cells, producing intense vasoconstriction and hypertension. When given alone, MAO inhibitors usually decrease blood pressure and produce orthostatic hypotension, by unknown mechanisms.
Pharmacological stimulation of a large variety of receptors on noradrenergic terminals affects the amount of norepinephrine released during cellular activation. Compounds inhibiting norepinephrine release include acetylcholine (at muscarinic receptors) dopamine, gamma-aminobutyric acid (at \( \gamma \)-aminobutyric acid \(_1\) receptors), prostaglandins of the E series, opioids, adenosine, nitric oxide, and norepinephrine itself. Compounds enhancing norepinephrine release include angiotensin II, acetylcholine (at nicotinic receptors), corticotropin, \( \gamma \)-aminobutyric acid (at \( \gamma \)-aminobutyric acid \(_2\) receptors), and epinephrine (via stimulation of pre-synaptic \( \beta_2 \)-adrenoceptors).

In general, whether at physiological concentrations these compounds exert modulatory effects on release of endogenous norepinephrine remains unproved, especially in humans. Substantial evidence, however, has established that endogenous norepinephrine can regulate its own release, by stimulating inhibitory \( \alpha_2 \)-adrenoceptors on sympathetic nerves. Conversely, systemic administration of the \( \alpha_2 \)-adrenoceptor blocker, yohimbine, produces much larger proportionate increases in forearm norepinephrine spillover than in directly recorded skeletal muscle sympathoneural activity, providing strong evidence that in human limbs, \( \alpha_2 \)-adrenoceptors on sympathetic nerve endings exert tonic inhibitory modulation of norepinephrine release (Grossman et al., 1991).

In addition to local feedback control of norepinephrine release, reflexive "long-distance" feedback pathways, via high- and low-pressure baroreceptors, elicit reflexive changes in sympathoneural impulse activity. Alterations in receptor numbers or of intracellular biomechanical events after receptor activation also affect responses to agonists. These factors may regulate norepinephrine release by trans-synaptic local and reflexive long-distance mechanisms.

**ADRENOCEPTORS AND SYMPATHETIC NEUROEFFECCTOR FUNCTION**

Effects of endogenous transmitters depend importantly on the numbers and types of receptors for those transmitters in different organs and tissues. Adrenoceptors in the brain and periphery mediate the physiological effects of catecholamines. The myriad different effects exerted by only three endogenous catecholamines – norepinephrine, epinephrine, and dopamine – in different organs depend on the numerous types and subtypes of adrenoceptors and intracellular mechanisms. Distinctions based on genetic sequences for identifying types and sub-types of adrenoceptors have outpaced
those based on pharmacological techniques such as ligand binding. As a result, the functional importance of many of these distinctions remains unclear.

All adrenoceptors identified so far share several structural characteristics – an amino-terminal, glycosylated polypeptide chain from the cell membrane extending into the extracellular fluid; 7 polypeptide membrane-spanning domains, each domain consisting of about 20-28 hydrophobic amino acids in an α-helical arrangement, with highly conserved sequences; and a long carboxyl-terminal polypeptide chain extending from the internal surface of the cell membrane into the cytoplasm. The membrane-spanning domains determine the ligand binding characteristics of the receptor. The cytoplasmic domains, comprising three loops and the tail ending in the carboxy terminus, regulate the specific coupling with G proteins and phosphorylating enzymes in the cascade of intracellular events leading to cellular activation or inhibition. The carboxy terminal tail, which contains a serine- and threonine-rich domain, is a site of phosphorylation by protein kinases such as protein kinase C, βARK, and cyclic AMP-dependent protein kinase.

Adrenoceptors also share the same process for transducing signals to alter cellular functions – via G-proteins (guanine-nucleotide regulatory proteins), located near the receptor on the inner portion of the cell membrane (Figure 7). G-protein complexes consist of an α subunit, responsible for the specificity of the G-protein, and β and γ subunits. The heterotrimeric G-proteins constitute a “superfamily”, with multiple subunits.

Differences in the effects of norepinephrine and epinephrine result from different actions at two types of receptor – α and β. In general, β-adrenoceptors mediate the positive inotropic and chronotropic effects of catecholamines in the heart; stimulation of vascular α-adrenoceptors produces vasoconstriction; and stimulation of vascular β-adrenoceptors – especially in skeletal muscle – produces vasodilation. Non-specific α-blockers include phenoxybenzamine and phentolamine, non-specific β-blockers include propranolol and timolol, non-specific α-agonists include norepinephrine, and non-specific β-agonists include isoproterenol. As noted above, epinephrine stimulates both α- and β-adrenoceptors. The antihypertensive drug, labetalol, blocks both α- and β-adrenoceptors.

G is the G-protein responsible for cellular activation upon occupation of β-adrenoceptors. The α subunit of the β-adrenoceptor is designated α. Adjacent to the G-protein complex is adenyl cyclase, which spans the cell membrane. Under resting conditions,
guanosine diphosphate (GDP) binds to the G-protein. Binding of the agonist to the receptor results in a receptor-ligand complex. In the presence of guanosine triphosphate (GTP), receptor occupation results in substitution of GTP for GDP at the binding site of the G-protein. This activates the G-protein. The activated α subunit separates from the β and γ subunits and stimulates adenyl cyclase, which catalyses the synthesis of cyclic AMP from ATP. As long as GTP is bound to the α subunit, cyclic AMP can be generated. The process ceases when the GTP bound to the α subunit is converted to GDP, inactivating both the G-protein and the cyclase. Cyclic adenosine monophosphate, or cyclic AMP, an intracellular “second messenger” (the first messenger being the hormone binding to the receptor), stimulates cyclic AMP-dependent protein kinase (protein kinase A), a tetramer including two regulatory and two catalytic subunits. Binding of cyclic AMP to the regulatory subunits of protein kinase A releases them, leading to phosphorylation of many proteins, evoking changes in cellular activity such as contraction or secretion. Protein kinase A also catalyses phosphorylation of the receptor, desensitizing β-adrenoceptor-mediated processes. The phosphorylation appears to change the conformation of the α subunit, interfering with the function of the G-protein. Other mediators of desensitization include β-adrenergic receptor kinase (βARK), β-arrestin – an intracellular protein – and protein kinase C.

Agonist occupation of α1-adrenoceptors leads to a different cascade of intracellular events from that consequent to agonist occupation of β-adrenoceptors (Figure 7). The α1-adrenoceptor is linked to a different G-protein, Gj. Occupation of the receptor by the agonist leads to activation of the G-protein by GTP hydrolysis. This activates phospholipase C, which catalyses the hydrolysis of phosphatidylinositol 4,5-diphosphate to form two active subunits, inositol triphosphate and diacylglycerol. Diacylglycerol activates protein kinase C, leading to cellular activation by as yet unknown mechanisms. Inositol triphosphate binds to another receptor on the endoplasmic reticulum, releasing Ca++ from the stores into the cytoplasm, also activating the cell.

Agonist occupation of α2-adrenoceptors inhibits adenyl cyclase by interaction with an inhibitory G-protein, G. This decreases intracellular formation of cyclic AMP and therefore decreases activity of protein kinase A. The mechanism for vasoconstriction induced by stimulation of α2-adrenoceptors has not been established.
The following table summarizes classification schemes for human adrenoceptor subtypes, lists the associated G-proteins and intracellular messengers that couple adrenoceptor occupation with cellular functional changes, and provides examples some specific agonists and antagonists.

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<td>Phenylephrine</td>
<td>Prazosin</td>
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<td>( \alpha )Methyl-</td>
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<tr>
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<td>( G_o )</td>
<td>Inh. adenylyl cyclase</td>
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<tr>
<td>( \alpha_{1},^{(*)} )</td>
<td>( G_i, G_o )</td>
<td>Inh. adenylyl cyclase</td>
<td>Prazosin*</td>
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\( ^{(*)} \) Prazosin also blocks \( \alpha_1 \)-adrenoceptors non-selectively.

\( \beta_1 \) | \( G_i \) | Adenylyl cyclase | Isoproterenol | Propranolol |
| \( \beta_2 \) | \( G_i \) | Adenylyl cyclase | Dobutamine    | Metoprolol  |
| \( \beta_3 \) | \( G_i \) | Adenylyl cyclase | Terbutaline   |              |
| \( \beta_3 \) | \( G_i \) | Adenylyl cyclase | BRL 37344    |              |

\( \text{D1-like} \) | Adenylyl cyclase | Fenoldopam | SCH 33390 |
\( \text{D1} \) (D1A) | Adenylyl cyclase |            |           |
\( \text{D5} \) (D1B) | Adenylyl cyclase |            |           |

\( \text{D2-like} \) | Inh. adenylyl cyclase | Bromocriptine | Raclopride   |
\( \text{D2} \) | Inh. adenylyl cyclase |            | Haloperidol |
\( \text{D3} \) | Inh. adenylyl cyclase |            | Aj 76      |
\( \text{D4} \) | Inh. adenylyl cyclase |            | Clozapine |

\( ^{(*)} \) Not found in mammals, including humans.

Stimulation of either \( \alpha_1 \)-receptors or \( \alpha_2 \)-receptors on vascular smooth muscle cells elicits vasoconstriction. In humans, \( \alpha_1 \)-adrenoceptors play relatively minor roles in mediating catecholamine effects in the heart and kidneys but play an important role in the regulation of vascular tone. The \( \alpha_1 \)-adrenoceptor sub-type or sub-types responsible for vasoconstriction remain unidentified. There is little convincing evidence for pre-synaptic \( \alpha_1 \)-adrenoceptors.
In the periphery, α₂-adrenoceptors are located mainly pre- and extra-synaptically and can exert either stimulatory or inhibitory effects, depending on the cell type on which they are located. Thus, occupation of α₂-adrenoceptors on vascular smooth muscle cells elicits muscular contraction, whereas occupation of α₁-adrenoceptors on sympathetic nerve terminals inhibits exocytotic release of norepinephrine. The three subtypes of α₂-adrenoceptor all couple via pertussis toxin-sensitive Gᵢ and Gₒ proteins to inhibit adenyl cyclase, activate receptor-operated membrane K⁺ channels, and inhibit voltage-sensitive Ca²⁺ channels. α₂A-Adrenoceptors have low affinity for prazosin and high affinity for oxymetazoline and yohimbine, whereas α₂B-adrenoceptors have high affinity for prazosin and low affinity for oxymetazoline and yohimbine. The α₂A-adrenoceptor appears responsible for centrally-mediated hypotensive responses to α₂-adrenoceptor agonists (in contrast with suggestions that these agonists work by way of interactions with imidazoline receptors) and for sedative, anaesthetic-sparing, and analgesic effects of α₂-adrenoceptor agonists. Whether the presynaptic α₂-adrenoceptors are structurally unique is unknown, since as of the time of writing, the receptor has not been cloned.

As indicated in the table, agonist occupation of all β-adrenoceptor subtypes stimulates adenyl cyclase. Whereas activation of β₁-adrenoceptors on myocardial smooth muscle cells stimulates cellular contraction, activation of β₁-adrenoceptors on vascular smooth muscle cells causes vascular relaxation. Fat cells such as those in brown adipose tissue have high concentrations of β₁-adrenoceptors (Emorine et al., 1989). A single residue substitution (Trp to Arg) at position 64 has been reported as associated with a tendency to obesity in humans (Goldstein, 1998; Strosberg, 1997); however, this association has been questioned recently (Buettner et al., 1998), and whether this substitution affects caloric balance and lipolysis remains unknown.

Blockade of ganglionic neurotransmission augments pressor responses to norepinephrine. Although these findings are consistent with α₁-adrenoceptor up-regulation after blockade of exocytotic norepinephrine release, interference with baroreflex buffering of blood pressure can also explain them.

The terms “up-regulation” and “down-regulation” have been used to describe changes in both the numbers of membrane-bound receptors, as quantified from ligand-binding studies, and changes in total numbers of receptors in the cells. Adrenoceptor desensitization reflects the effects of several processes, including phosphorylation of
the receptor, sequestration (agonist-induced dissociation of the receptor from the cell membrane), inactivation of intracellular messengers, and decreased synthesis of receptor protein. One can readily demonstrate desensitization of β-adrenoceptor-mediated responses (e.g., cyclic AMP generation in response to application of a β-adrenoceptor agonist) in *in vitro* preparations, and a large body of research has concentrated on the mechanisms of this phenomenon. In contrast, relatively few studies have concentrated on desensitization of responses mediated by α-adrenoceptors, and the available literature about α-adrenoceptor desensitization has been inconsistent. Desensitization of α,-adrenoceptor-mediated responses is thought to be analogous to desensitization of β-adrenoceptor-mediated responses, with the receptor uncoupled from the G-protein due to phosphorylation of the receptor by PKA or PKC. Whether α,-adrenoceptor numbers or affinities are subject to up- and down-regulation has been unclear.

Homologous desensitization refers to a situation where production of an intracellular second messenger, such as cyclic AMP, decreases in response to stimulation of specific receptors but not in response to stimulation of other receptors using the same second messenger. Homologous desensitization therefore is agonist-specific. Heterologous desensitization entails attenuated responses to all agonists using the same second messenger. Heterologous desensitization is therefore agonist-nonspecific. Several mechanisms of homologous desensitization have been proposed, and whereas evidence for each has been obtained from *in vitro* systems, the roles of these mechanisms *in vivo* is incompletely understood. One mechanism is internalization, where the number of receptor binding sites in the cell membrane decreases and the number in the cytosol increases. A second form of homologous desensitization is by uncoupling, where the receptor dissociates from its G-protein. Uncoupling is an integral part of the normal cascade of events after occupation of receptors that use G-proteins, because after the protein binds GTP, not only is the formation of second messenger enhanced, but also the G-protein-GTP complex decreases the affinity of the receptor for the agonist. Phosphorylation of the receptor can produce this form of desensitization by uncoupling. βARK phosphorylates the agonist-occupied form of the β-receptor, decreasing the affinity of the receptor for the agonist. Homologous desensitization by βARK is thought to occur only when the receptor is occupied, and the phosphorylation may depend on another endogenous compound, β-arrestin.
Clinically relevant research about sensitization and desensitization has so far been limited mainly to adaptive responses to exogenously administered pharmacological agents such as pressor amines, antidepressants, and β-adrenoceptor blockers. Mechanisms of desensitization resulting from chronically repeated episodes of sympathetically-mediated norepinephrine release, and mechanisms of denervation supersensitivity, originally described by Bernard and Cannon (Cannon, 1939), remain obscure.

Resensitization occurs rapidly upon removal of the agonist, by the actions of phosphatases that catalyse dephosphorylation of the receptor (Lefkowitz et al., 1998). The phosphatases are latent, membrane-associated members of the PP-2A family. One, designated the G protein-coupled receptor phosphatase (GRP), has the ability to dephosphorylate not only the βARK-phosphorylated β-adrenoceptor but also the βARK-phosphorylated α̂_r̂-adrenoceptor. Resensitization by dephosphorylation appears to require sequestration of the receptor from the membrane, into a cytoplasmic population of vesicles.

Acetylcholine released from parasympathetic terminals exerts several effects in different organs. In the heart, cholinergic stimulation decreases the cardiac rate, inhibits atrioventricular conduction, and decreases the inotropic state (in the atria more than the ventricles). All these effects depend to some extent on the concurrent sympathetic tone.

Molecular cloning studies have identified five subtypes of muscarinic receptors, encoded by five separate genes and designated \( M_1 \)-\( M_5 \). Identification of subtype-specific drugs has lagged behind. According to current thinking, occupation of \( M_1 \) receptors excites ganglionic neurons and stimulates norepinephrine release from sympathetic nerves; \( M_2 \) receptors mediate vagal bradycardia and decreased cardiac inotropy; and stimulation of \( M_3 \) receptors contracts smooth muscle and increases glandular secretion.

Cloning experiments have also revealed multiple subtypes of nicotinic receptors. The receptors are acetylcholine-gated cation channels, consisting of multiple subunits, designated α, β, γ, (in adult mammals ε replaces γ), and δ, in pentamers containing 2 α subunits. In the mammalian nervous system, eight α and four β subunit genes have been cloned. The α subunits contain the acetylcholine binding site and so determine the ligand binding characteristics of the receptor. Binding of acetylcholine to the α subunit produces conformational changes in the receptor, increasing the membrane conductance for \( Na^+ \) and depolarizing the cell. Pharmacological tools have allowed partial identification of subunits in native nicotinic receptors. Alpha-
bungarotoxin blocks $\alpha_7$, $\alpha_9$, or $\alpha_9$ subunits; cytisine activates $\alpha$, or $\beta_4$
subunits; and neuronal bungarotoxin blocks a $\beta_2$ subunit. In auto-
nomic ganglia, the nicotinic receptors consist of $\alpha_3$, $\alpha_4$, $\alpha_7$, $\beta_2$, and $\beta_4$
subunits (Pacak et al., 1998).

Acetylcholine acts as a vasodilator, probably at least partly via oc-
cupation of $M_3$ receptors and local generation of nitric oxide. In the
gastrointestinal tract, acetylcholine stimulates secretory activity and peri-
stalsis, the enhanced motility producing symptoms such as nausea,
cramps, and an urge to defecate. Cholinergic agonists act by a variety
of mechanisms to stimulate bladder contraction and urination. Acetyl-
choline also stimulates secretory activity by most glands receiving
parasympathetic innervation, including the salivary glands, lacrimal
glands, and sweat glands. In the eye, acetylcholine induces miosis.

Acetylcholine itself is not used as a drug in clinical medicine, be-
cause of the diverse toxic effects and susceptibility to rapid degrada-
tion. Exogenously administered cholinergic agonists (e.g. bethanechol)
are used to treat non-obstructive urinary retention and gastric atony.
These drugs stimulate digestive processes and intestinal motility, in-
crease salivation, and increase sweating, with relatively little change
in heart rate. Drugs that inhibit acetylcholinesterase (e.g. neostig-
mime, physostigmine, edrophonium) reverse central nervous depres-
sion due to overdose of anticholinergics. Edrophonium rapidly
stimulates cardiac cholinergic receptors and is used to treat paroxys-
mal supraventricular tachycardia. Anticholinergics are used clinically
in several conditions, including asthma, insomnia, peptic ulcer dis-
 ease, symptomatic bradycardia or heart block, diarrhoea, hyperhid-
rosis, Parkinson's disease, and mushroom poisoning.

Agonist occupation of nicotinic receptors increases entry of ion-
ized calcium, whereas agonist occupation of muscarinic receptors leads
to complex effects mediated by G-proteins. Stimulation of $M_3$ or $M_4$
receptors produces hydrolysis of phosphoinositides and increased in-
tracellular mobilization of ionized calcium, via $G_\alpha$. Stimulation of $M_3$
or $M_4$ receptors inhibits adenyl cyclase, via pertussis toxin-sensitive G-
proteins ($G_\alpha$, $G_\beta$). The negative inotropic effect of occupation of mus-
carinic $M_2$ receptors appears to depend on generation of nitric oxide
and cyclic GMP with consequent activation of potassium channels.

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FIGURE 1
Schematic diagram illustrating principles of autonomic neurotransmission. Open circles indicate cell bodies of preganglionic sympathetic and parasympathetic neurons in the intermediolateral columns of the spinal cord. Parasympathetic nerves emanate from the brainstem and sacral cord, whereas sympathetic nerves emanate from the thoracolumbar cord. Closed circles indicate post-ganglionic cell bodies. Sympathetic post-ganglionic cells lie in the chain of para-spinal ganglia, whereas parasympathetic post-ganglionic cells lie near or in the innervated organs. Sympathetic innervation of the adrenal gland includes preganglionic nerves. In several organs, effects of activation of sympathetic nerves antagonize effects of activation of parasympathetic nerves.
FIGURE 2
Endothelium-dependent vasodilation produced by acetylcholine. According to this model, occupation of acetylcholine receptors leads to cyclic GMP-mediated activation of nitric oxide synthase (NOS), generating nitric oxide by catalysing conversion of arginine to citrulline.
FIGURE 3 (See next page)
Overview of effects of activation of the sympathetic nervous system, where norepinephrine acts as the main neurotransmitter, and of activation of the adrenomedullary hormonal system, where epinephrine acts as the main hormone. Because the sympathetic nervous system is a neural network, sympathetic nerves to different regions can be activated differentially during stress, producing redistribution of blood flow to body organs. Norepinephrine released from sympathetic nerves generally contracts smooth muscle cells, eliciting glandular secretion, vasoconstriction, and myocardial contraction. Renal vasoconstriction produces an anti-natriuretic effect. Cutaneous vasoconstriction produces pallor. The net effect of systemic vasoconstriction is to increase the total peripheral resistance to blood flow in the body. This, combined with myocardial stimulation, increases blood pressure. In humans, the main hormone released by the adrenal medulla is epinephrine (adrenaline). Epinephrine affects the function of most organs and produces generalized effects such as increased metabolic rate, increased blood glucose levels, hypokalaemia, increased lactate production, increased alertness, increased platelet aggregability, and intensification of emotions. Abbreviations: Inc. = increase; Dec. = decrease; Aggreg. = aggregation; RAS = renin-angiotensin-aldosterone system; Cut. = cutaneous; BP = blood pressure.
Sympathetic Nervous System (SNS)

Norepinephrine

Glands

Myocardium

Vascular Smooth Muscle

Salivation

Inc. Heart Rate
Inc. Automaticity
Inc. Contractility

Vasoconstriction

Pallor

Sodium Retention

Inc. BP

Adrenomedullary Hormonal System

Epinephrine

Inc. Glucose
Glycogenolysis

Dec. Gut Motility
Uterine Relaxation

Skeletal Vasodilation
Cut. Vasoconstriction

Inc. Metabolic Rate
Inc. Lactate Production
Hypokalemia

Inc. RAS Activity

Inc. Platelet Aggreg.
Alertness

Brochodilation
Hyperventilation

Emotional Intensification
Pupillary Dilation

Inc. Heart Rate
Inc. Automaticity
Inc. Contractility
FIGURE 4
Biosynthetic and metabolic cascades for endogenous monoamines. Abbreviations: THF = tetrahydrofolate; MTHFR = 5,10-methylenetetrahydrofolate reductase; Phe = phenylalanine; PAH = phenylalanine hydroxylase; Tyr = tyrosine; TYH = tyrosine hydroxylase; Trp = tryptophan; TRH = tryptophan hydroxylase; 5-HTP = 5-hydroxytryptophan; LAAAD = L-aromatic-amino-acid decarboxylase; MAO = monoamine oxidase; COMT = catechol-O-methyltransferase; 3-MT = 3-methoxytyrosine (3-O-methylDOPA); PNMT = phenylethanolamine-N-methyltransferase; DA = dopamine; 5-HT = serotonin; 5-HIAA = 5-hydroxyindoleacetic acid; NA = norepinephrine; DHPG = dihydroxyphenylglycol; DOPAC = dihydroxyphenylacetic acid; EPI = epinephrine.
FIGURE 5
Overviews of (left) steps in norepinephrine synthesis, release, reuptake, and metabolism; and of (right) drugs acting selectively at these steps. Hydroxylation of the amino acid, tyrosine (TYR), is the enzymatic rate-limiting step in catecholamine biosynthesis. Tyrosine hydroxylase (TH) catalyses the conversion of tyrosine to DOPA. L-aromatic-amino acid decarboxylase (LAADC) catalyses the conversion of DOPA to dopamine. Dopamine-β-Hydroxylase is localized to large dense-core vesicles in noradrenergic cells. After uptake of axoplasmic dopamine into the vesicle, DBH catalyses the conversion of dopamine to norepinephrine. According to the exocytosis theory, increased transmembrane entry of ionized calcium fosters fusion of the vesicles with the axonal membrane. Endogenously released norepinephrine is taken back up into sympathetic nerve terminals by Uptake-1 and taken up into non-neuronal cells by Uptake-2. Norepinephrine in the axoplasm can be translocated back into the vesicles or converted to dihydroxyphenylglycol (DHPG) by monoamine oxidase (MAO) in the outer
mitochondrial membrane. Norepinephrine in non-neuronal cells is converted to normetanephrine (NMN) by catechol-O-methyltransferase (COMT). DHPG can diffuse into the bloodstream or can be taken up by non-neuronal cells and converted to methoxyhydroxyphenylglycol (MHPG). MHPG is a major end-product of norepinephrine metabolism. Dopamine in the axoplasm can be translocated into vesicles and converted to norepinephrine or can be converted to dihydroxyphenylacetic acid (DOPAC) by MAO. DOPAC can diffuse into the bloodstream or can be converted extraneurally to homovanillic acid (HVA), the main end-product of dopamine metabolism. The processes of catecholamine uptake and metabolism are generally independent of actions by adrenoceptors on the nerve terminals or non-neuronal cells. Drug abbreviations: TRI = trimethaphan; Nic. = nicotine; TYR = tyramine; RES = reserpine; AMPT = 4-methyl-para-tyrosine; NMN = normetanephrine; COMTI = inhibitor of COMT; MAOI = inhibitor of MAO.
FIGURE 6
A model of exocytosis from sympathetic nerve terminals. Depolarization of sympathetic nerve terminals leads to trans-membrane entry of ionized calcium. The ionized calcium fosters migration, docking, fusion, and portation of the vesicle at the axonal membrane, via conformational changes in complexes of vesicle-associated, membrane-associated, and cytoplasmic proteins. A break in the membrane at the site of fusion allows discharge of the soluble vesicle contents (norepinephrine, ATP, DBH, enkephalins, and chromogranin A) into the extracellular fluid. The vesicles reconstitute and may recycle by endocytosis. Abbreviations: DBH = dopamine-β-hydroxylase; Enk = enkephalin; NE = norepinephrine; NPY = neuropeptide Y; Chr A = chromogranin A.
FIGURE 7
Intracellular mechanisms after binding of catecholamines to adrenoceptors. (Left) Intracellular mechanisms of β-adrenoceptor-mediated smooth muscle cell activation. Occupation of the receptor by an agonist leads to G-protein-coupled activation of adenylyl cyclase, increasing generation of cyclic AMP (cAMP) and activating protein kinase A (PKA). PKA phosphorylates calcium channels, and the increased intracellular ionized calcium concentration leads to cellular activation. (Right) Intracellular mechanisms of α₁-adrenoceptor-mediated smooth muscle cell activation. Occupation of the receptor by an agonist leads to G-protein-coupled activation of phospholipase C, which cleaves phosphatidylinositol 4,5-diphosphate (PIP₂) to yield two active subunits, inositol triphosphate (IP₃) and diacylglycerol (DG). DG activates protein kinase C (PKC), and IP₃ binds to a receptor on the endoplasmic reticulum to increase release of ionized calcium into the cytoplasm, leading to cellular activation.
The autonomic nervous system
PEPTIDES IN THE AUTONOMIC NERVOUS SYSTEM

Quentin J. Pittman

INTRODUCTION

Studies on the autonomic nervous system in the early decades of the twentieth century provided much important experimental evidence of the transmitter roles of norepinephrine, epinephrine and acetylcholine. The accessibility of the peripheral autonomic nervous system to pharmacological study facilitated studies to an extent which would not have been possible in the much more complex central nervous system. Similarly, while recent techniques have allowed us to investigate the roles of peptides throughout widespread areas of the neural axis, the accessible peripheral nervous system has again provided a substrate for much work contributing to our understanding of peptide actions.

Well over 100 peptides (short chain amino acids) have now been identified, and more are continuing to be identified. Peptides appear to be phylogenetically old; putative peptide transmitters have been discovered in the hydra, for example (Haynes, 1980), and the recent cloning of the genome of C. elegans has revealed the existence of numerous peptides including at least 56 FMRF-amide-like peptides and more than 58 other neuropeptides (Bargmann, 1998). It was not until the middle to late 1970s that peptides began to attract serious interest among neuropharmacologists as transmitter candidates, and even then they were considered as add-ons to the basic work-horse transmitters which were the amines and amino acids. One might ask why it was only in the last two decades that peptides began to take their place as important players in normal communication. The reasons for this are several. First of all, peptides appear to be present in much smaller quantities than the amines and amino acids. Biochemical techniques did not mature sufficiently until the late 1970s to permit precise identification of the composition of peptides and it was only with
the advent of solid-state peptide synthesis on a commercial scale that sufficient quantities of peptides were available for pharmacological study. Previously, pharmacological studies depended upon the use of purified extracts of uncertain composition, some of which varied from laboratory to laboratory or even from preparation to preparation within a laboratory. Similarly, until sufficient quantities of peptides were available to permit immunization of animals and the elaboration of specific antibodies, it was difficult to measure peptide levels and again, less precise (although sometimes amazingly sensitive) bioassays were the best available tool. Peptides have also proven challenging to work with as regards developing specific antagonists; peptidergic antagonists often have poor bioavailability and the presence of multiple receptor subtypes has made it difficult to develop antagonists specifically targeting one receptor subtype. Finally, with respect to the autonomic nervous system in particular, redundancy of many pathways has made it difficult to specifically block a pathway even for which there is good evidence for a peptide transmitter. Recent studies using knockout animal models have been of limited utility because of this problem.

Despite these problems a number of features of peptide transmitters have been identified; many of these are similar to what is also known for other classes of transmitters, whereas some features are unique. A major characteristic of the peptides is their synthesis in ribosomes as translated products of messenger RNA. Because of ultimate splicing of mRNA, a variety of related messages may be available for any particular peptide or peptide receptor. Thus, a single gene product may lead to a diversity of peptide products (Mains and Eipper, 1981a). All peptides identified to date also appear to be synthesized as an initial pro or pre-pro peptide which is then processed by peptidase action into final products. Thus, an initial precursor can lead to many different peptide products in a cell (Mains and Eipper, 1981b). Furthermore, because different cells may contain different processing enzymes, the products that appear in a cell may vary even given an identical precursor (Sánchez et al., 1997). The best example of this is the proopiomelanocortin system which can lead to beta-endorphin, alpha-MSH or ACTH in varying quantities in different cell populations (Eipper and Mains, 1978).

In addition to this diversity of transmitter candidates, there is also evidence that the spectrum of transmitters synthesized and secreted may vary over the lifetime of the neuron. For example, under the influence of various hormones certain neurons in the hypothalamus may phenotypically be corticotrophin-releasing hormone positive or
CRH and arginine vasopressin (AVP) positive (Levin and Sawchenko, 1993). There is also evidence that when cells are injured they may alter their biosynthetic programmes and preferentially express peptide trophic factors rather than transmitters (Pichl et al., 1998).

On a second-to-second or minute-to-minute basis, there is also evidence that different levels of activity may preferentially release different transmitters. Many neurons contain not only peptides but also a variety of so-called classic transmitters (amines, amino acids, etc.). With low levels of activity one transmitter may be preferentially released, and this is often the classical transmitter. Only under conditions of high activity are the peptide transmitters released. This appears to be particularly evident in the sympathetic innervation of blood vessels, where low levels of nerve activity produce contractions which can be attributed entirely to catecholamines, whereas more intense stimuli applied to the innervating nerve result in the additional release of the peptide neuropeptide Y (NPY) (Lundberg et al., 1989).

CENTRAL CONTROL OF THE AUTONOMIC NERVOUS SYSTEM

While the motor axons of the parasympathetic and sympathetic nerves which course through the periphery have long been recognized as effector components of the autonomic nervous system, the control of activity in these fibres rests in the central nervous system. Because of its pre-eminent position as a nucleus influencing the autonomic nervous system, the hypothalamus has often been termed the “head ganglion of the autonomic nervous system” (Pittman, 1991). In addition to this area of the brain, a number of other important brain stem nuclei and limbic system nuclei play important roles in regulating autonomic output. In all such areas, peptides have been shown to constitute an important transmitter population and fibres immunoreactive for peptides and receptors are found throughout all of these areas. While the cellular actions of peptides are still relatively unknown in many areas of the brain, a wealth of physiological and pharmacological studies indicate the important physiological roles of peptides in controlling autonomic behaviours. The following brief outline deals with a peptide that we have investigated extensively within the brain. Arginine vasopressin (AVP) was the first peptide to be structurally characterized and synthesized and it has been long known as a peptide released from the pituitary to act on the periphery to control blood pressure and water balance (among other roles). In addition, the paraventricular nucleus of the hypothalamus and a number of other limbic and brain stem nuclei contain AVP-synthesizing neurons which project throughout much
of the brain and spinal cord. A variety of roles have been ascribed to AVP within the brain; we will discuss some of our studies concerning its role in fever and antipyresis and in central control of blood pressure. A number of recent reviews cover this area in more detail (Pittman et al., 1993; 1998)

**AVP AND FEVER**

Fever is a regulated rise in body temperature which can be induced by a variety of immune stimuli. There is now good evidence that in addition to a regulated rise in temperature, the defervescence which ensues after a fever may also be a regulated event with the peptide AVP acting within the brain to reduce fever. Brain mapping studies indicate that the ventral septal area (VSA) and the amygdala are two important loci where exogenously introduced AVP can reduce fever (Naylor et al., 1988; Federico et al., 1992). Pharmacological studies indicate that these actions of AVP occur through a receptor-mediated process, activated by the V1-type receptor. If the release of AVP into the brain is impaired via depletion of AVP or by destruction of cell bodies, high fever ensues. Such effects can be brought about by long-term castration (Pittman et al., 1988), as synthesis of AVP in nuclei involved in the reduction of fever is entirely dependent upon circulating sex steroids. AVP antagonists are also available, and their introduction into appropriate areas of the brain also results in high fever (Chen et al., 1997).

Conversely, activation of endogenous AVP release can reduce fever. This can be effected either by electrically stimulating appropriate nuclei (Naylor et al., 1988) or by other physiological and pharmacological stimuli which can activate central AVP release. For example, animals which are hypertensive (e.g. one kidney one clip Goldblatt hypertensive rats) also show reduced fevers due to an overly active brain AVP system (Fyda et al., 1992). Acute reduction of blood pressure also sends afferent signals to the brain, causing the brain to secrete AVP not only into the circulation to raise blood pressure but also into the brain, where it acts to suppress fever (Pittman and Wilkinson, 1992).

There appear to be states in which fever is normally suppressed. An example is in the neonatal animal, which appears not to develop a fever due to an action of AVP (Kasting and Wilkinson, 1987). There is also some evidence that there is a suppression of fever at the time of parturition which can be correlated with an enhanced AVP system in the brain (Eliason and Fewell, 1998; Zeisberger et al., 1981).

Fever is now thought to be an important component of the host defence response to disease. Readily available antipyretic drugs such
as aspirin are very effective in suppressing fever; thus it is unlikely that there is significant potential for an AVP-like molecule to suppress fever. However, in states where fever is suppressed, it may prove possible at some time in the future to develop a molecule to block AVP receptors in the brain and return the febrile response to aid in fighting disease.

**CENTRAL CONTROL OF BLOOD PRESSURE**

AVP-immunoreactive fibres innervate many autonomic nuclei important in blood pressure control. Injection of AVP into such nuclei causes increases in blood pressure and heart rate which are neurally mediated (Pittman and Bagdan, 1992). When AVP is collected by microdialysis or push-pull perfusion from such nuclei, release is elevated in response to hypotensive stimuli, suggesting that endogenous AVP is released to attempt to return blood pressure to normal. These studies on cardiovascular actions of AVP bring out an important aspect of autonomic control shared by many peptides. That is, peptides may have similar roles in the periphery and in the brain to maintain homeostasis and control autonomic function. AVP is a peptide which, given centrally, raises blood pressure. Given peripherally, or following its release from the pituitary, the peptide acts on receptors on the arterial smooth muscle to raise blood pressure and to cause water reuptake from the kidney to increase body water volume and aid in restoration of blood pressure. Thus, such a peptide has a “holistic” effect on blood pressure.

There have been many studies attempting to find a role for brain AVP in hypertension. Although some studies have been promising, evidence obtained both from our laboratories and others does not make a strong argument for overactivity of this system in blood pressure control (Szczepanska-Sadowska et al., 1993; Earle and Pittman, 1995). Possibly this is due to the fact that many types of hypertension have a multifaceted etiology. Nonetheless, the idea is attractive, partly because we have evidence from a number of systems that the brain AVP receptor system can show exaggerated responses following intermittent exposure to the peptide.

**SENSITIZATION**

Where as exposure to a drug or agonist is usually associated with downregulation of a receptor, exposure to AVP results in a peculiar phenomenon known as sensitization. That is, if one is measuring an antipyretic effect, a cardiovascular effect or indeed any action of AVP tested to date, a second exposure to the peptide, given centrally, is
associated with an amplified response (Poulin et al., 1994; Willcox et al., 1992; Poulin and Pittman, 1993a). While the mechanism(s) responsible for this sensitization have not yet been clarified, there is evidence that it is associated with an increase in intracellular inositol phosphate hydrolysis without changes in receptor number or affinity (Poulin and Pittman, 1993a). The significance of the sensitization phenomenon is not yet well understood, but it is apparent that stimuli that cause release of AVP into the brain can result in long-lasting alteration in other responses which involve AVP receptors (Burnard et al., 1983; Poulin and Pittman, 1993b).

There is also the possibility that sensitized AVP receptors may have pathological consequences. For example, a convulsive-like disorder which can be seen after repetitive AVP injections has been suggested to resemble febrile convulsions (Kasting et al., 1980). Indeed, AVP release during fever is adequate to sensitize the brain to a subsequent AVP injection such that the animal responds to exogenously injected AVP as if it were a second exposure (Poulin and Pittman, 1993b).

**PEPTIDES AS TARGETS FOR THERAPEUTIC INTERVENTION**

Because of the multitude of peptide transmitters in both the central and peripheral components of the autonomic nervous system, they provide tempting targets for pharmacological intervention (Grundemar and Hakansson, 1994; Greene et al., 1996). As indicated earlier, the design of peptide antagonists has been challenging due to the complexity of the molecules and their limited bioavailability. Nonetheless, relatively specific antagonists of some peptides are available. Perhaps the best known is the opioid antagonist naloxone, which has been used both experimentally and clinically to treat drug overdoses. It is interesting that naloxone, while acting as a peptide antagonist, is not itself a peptide. In recognition of this, combinatorial chemistry approaches to the design of peptide mimetics have recently gained popularity (Moore et al., 1995).

The fact that peptides are synthesized in the ribosomes after translation of mRNA has prompted experiments utilizing antisense oligonucleotides as agents to downregulate synthesis of either peptide ligands or their receptors. The theory is that a short-chain oligonucleotide complementary to the mRNA for a peptide or protein will bind to the mRNA and prevent peptide synthesis either by physically blocking the amino acid incorporation or by activating enzymes that destroy the mRNA. This field of study is extremely controversial (Stein and Cohen, 1988; Stein and Cheng, 1993). There are ques-
tions about specificity of the effect and uncertainty about the mechanisms by which the antisense molecules exert their effects (Neumann, 1997; Phillips and Gyrurko, 1997). With increasing understanding of how to control viral vectors to carry such antisense molecules into neurons, one can anticipate more rapid progress in the use of these molecules as therapeutic agents.

An additional possible means to intervene in the action of peptides is through interference with their degradation (Waters and Davis, 1997). Unlike many other transmitters such as the monoamines, which are inactivated largely by reuptake, peptides are degraded enzymatically by a number of classes of peptidases. While such peptidases are not specific for individual peptides, they have specificity at certain peptide structures and components (and thus are characterized as aminopeptidases, carboxypeptidases, endopeptidases, etc.). Peptidase activity appears to be very high in the nervous system and inactivation of specific peptidases can markedly enhance peptide action (Saleh et al., 1996; Kramer et al., 1991). There is some evidence that chronic degenerative diseases may be associated with alterations in peptidase activities (Waters and Davis, 1997), but the contribution of this factor to the pathology of the diseases is not yet apparent.

CONCLUSIONS

Peptide transmitters outnumber all other transmitters identified to date and the diversity of peptide numbers, receptors and actions is overwhelming. With the availability of molecular biological approaches it is now possible to identify peptide candidates and receptors faster than physiologists, pharmacologists and anatomists can properly characterize them. Intensive effort is demanded in this area in order to reveal their roles not only in normal physiological functions but also in pathology. While this review has briefly touched upon some of the studies that have been carried out in the author's laboratory, it must be emphasized that these represent but a very small window on the world of peptides in the autonomic nervous system. In almost every homeostatic function, peptides are the major players and it can be anticipated that abnormalities in the control of any physiological process are likely to involve peptides; most likely, pharmacological interventions will also have to focus on these molecules.

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THE AUTONOMIC NERVOUS SYSTEM AND ITS IMPACT ON NEUROENDOCRINE REGULATION

Julio Licinio

INTRODUCTION
The term “stress” was borrowed from physics and introduced into medicine by Selye, who proposed that a variety of nocuous agents could elicit a general adaptive response (Selye, 1936). Selye’s concepts were a progression of a line of thought originating in antiquity that postulated that the organism existed in a healthy and stable state that would be disrupted in response to threats from the environment or to perturbations in the internal milieu, a term introduced by Claude Bernard in the 19th century. These would lead to an organized state of reactivity, the flight or fight response. Current work has questioned Walter Cannon’s concept of “homoeostasis,” which was coined to describe a stable state of equilibrium or balance between opposing forces in the body and mind with respect to various functions and to the chemical compositions of body fluids and tissues (Licinio, 1998a). The work of several groups has shown that the baseline status of health is not static. On the contrary, Goldberger and colleagues have documented that a high level of complexity exists in healthy, normal cardiac function, and that the onset of stability in heart rate variability is a predictor of sudden death (Ho et al., 1997; Peng et al., 1995).

When an organism is exposed to a threat to its basal state of health a variety of adaptive neurobiological and neuroendocrine responses take place. The specificity of those responses depends on the type of stressor and the duration of exposure. In addition to rapid activation of the sympathetic nervous system, there is rapid activation of the hypothalamic-pituitary-adrenal (HPA) axis. In conjunction with the HPA axis the sympathetic system provides a rapid and integrative response to stress. This chapter will focus on the role of HPA regulation in the neurobiology and neuroendocrinology of the response to stress and its interactions with the autonomic nervous system.
Activation of the HPA axis originates in the central nervous system (CNS). The subgenual prefrontal cortex and the hippocampus provide negative feedback to the paraventricular nucleus of the hypothalamus (PVN). PVN neurons synthesize corticotrophin-releasing hormone (CRH) (Vale et al., 1981). The synthesis of CRF by PVN neurons is a prime example of neuroendocrine transduction, a key event in neuroendocrinology that consists of the transformation of electric impulses into peptides that are secreted and can act at distant sites, promoting the integration of neuroendocrine responses (Licinio and Gold, 1997). CRH is synthesized in the PVN, transported to the median eminence and secreted into the hypophyseal-portal circulation. CRH reaches the anterior pituitary gland, where it promotes proopiomelanocortin (POMC) gene transcription, translation, and the secretion of the POMC fragment ACTH (adrenocorticotropic hormone). ACTH binds to receptors in the adrenal gland, eliciting the production of the steroid hormone cortisol or corticosterone. Cortisol circulates and reaches both the pituitary and the brain, providing an important negative feedback signal that contributes to cessation of HPA activation (Licinio and Gold, 1997). Disorders in which there is persistent HPA hyperactivity, such as major depression, are conceptualized as conditions in which persistent negative feedback provided by glucocorticoids fails to normalize the excessive activity of the HPA axis (Gold et al., 1988).

THE CRH NETWORK
CRH receptors bind not only CRH but also a new ligand, urocortin (Vaughan et al., 1995). Discovered by Vale’s group in 1995, urocortin is a new peptide that is related to urotensin (63% sequence identity) and CRH (45% sequence identity). Synthetic urocortin evokes secretion of adrenocorticotropic hormone (ACTH) both in vitro and in vivo and binds and activates transfected type-1 CRF receptors, the subtype expressed by pituitary corticotropes. Vale and his group have proposed that the coincidence of urotensin-like immunoreactivity with type-2 CRF receptors in brain, and the observation that urocortin is more potent than CRF at binding and activating type-2 CRF receptors, as well as at inducing c-Fos (an index of cellular activation) in regions enriched in type-2 CRF receptors, indicate that this new peptide could be an endogenous ligand for type-2 CRF receptors (Vaughan et al., 1995). Spina et al. have shown that urocortin is more potent than CRF in suppressing appetite, but is less potent than CRF in producing anxiety-like effects and activation. Those authors have suggested
that uroctin may be an endogenous CRH-like factor in the brain responsible for the effects of stress on appetite (Spina et al., 1996).

Uroctin is expressed in high levels in the nucleus of Edinger-Westphal. We have also shown uroctin gene expression in the hippocampus and in the pituitary gland (Wong et al., 1996). Based on those data we hypothesized that uroctin synthesized by the pituitary may modulate pituitary function, and that adrenocorticotropic hormone (ACTH) secretion is dependent on input not only from the hypothalamus as previously described, but it may also be regulated by uroctin synthesized locally. The finding of uroctin gene expression in the pituitary may help explain why proopiomelanocortin (POMC) mRNA levels are not decreased during hypothalamo-pituitary disconnection, and also describes a new level of complexity in the regulation of hypothalamo-pituitary function.

Thus, the CRH network is currently conceptualized as a complex system of two main ligands, CRH and uroctin, and several binding sites that include not only CRH receptors type 1α, 1β, and 2α, 2β, and 2γ, but also to the CRH-binding protein (CRH-BP). CRH-BP exists not only in the circulation, but also in the CNS, bound to neuronal cell membranes, and with a widespread but discrete pattern of distribution. CRH-BP in the plasma and cerebrospinal fluid (CSF) serves as a decoy for bioactive CRH. A novel therapeutic strategy has been proposed that consists of occupying the CRH-BP with specific ligand so as to enhance the bioavailability of endogenous CRH and/or uroctin. De Souza and his group have shown that ligands that dissociate CRH from CRH-BP increase brain levels of ‘free CRH’ in Alzheimer’s disease to control levels and show cognition-enhancing properties in models of learning and memory in animals without the characteristic stress effects of CRH receptor agonists (Behan et al., 1995). That group has also shown that the same strategy significantly blunted exaggerated weight gain in Zucker obese subjects and in animals withdrawn from chronic nicotine. In contrast to the effects of a CRH-receptor agonist, the CRH-BP ligand inhibitor did not stimulate adrenocorticotropic hormone secretion or elevate heart rate and blood pressure. Those data provide support for the hypothesis that the CRF-BP may function within the brain to limit selected actions of CRH and/or uroctin (Heinrichs et al., 1996).

AUTONOMIC NERVOUS SYSTEM - NEUROENDOCRINE INTERACTIONS
Stress activates both neuroendocrine responses and sympathetic activity. Soon after CRH was discovered by Vale’s group (Vale et al.,
1981), that group conducted further studies showing that CRH injected into the brains of rats produces hyperglycaemia and an increase in plasma concentrations of glucagon, epinephrine, and norepinephrine. Those results demonstrated that CRH acts within the brain to stimulate sympathetic outflow, resulting in the development of hyperglycaemia. In contrast to other peptides that act within the central nervous system, e.g. bombesin, thyrotropin-releasing hormone (TRH), and beta-endorphin, whose hyperglycaemic actions depend exclusively on adrenal epinephrine secretion, CRH-induced hyperglycemia is secondary to the enhanced secretion of both epinephrine and norepinephrine (Brown et al., 1982). Thus, soon after its discovery, it became clear that CRH could activate the sympathetic nervous system. The next question in this field was: Could noradrenergic function in turn stimulate CRH secretion by the hypothalamus?

It is not feasible to ascertain in vivo the relative contributions of the autonomic nervous system to activation of the paraventricular nucleus of the hypothalamus (PVN), resulting in CRH secretion. To accomplish this, Calogero et al. (1988) used an in vitro rat hypothalamic organ culture system in which CRH secretion from single explants was evaluated by a specific RIA (IR-rCRH). Norepinephrine (NE) stimulated IR-rCRH secretion dose dependently, with peak effects in the nanomolar range. The effect of NE was antagonized by the mixed alpha antagonist phentolamine, the alpha 1 antagonist prazosin, and the alpha 2 antagonist yohimbine, but not by the beta blocker L-propanolol. Comparable with these data were the findings that the alpha 1 agonist phenylephrine and the alpha 2 agonist clonidine both stimulated IR-rCRH secretion in a dose-dependent fashion. In their studies epinephrine (E) stimulated IR-rCRH secretion; this occurred only at higher concentrations, and was antagonized by phentolamine, but not by L-propanolol. The authors concluded that NE and E stimulate hypothalamic IR-rCRH secretion via alpha 1 and alpha 2 receptors. The effect of NE upon IR-rCRH secretion is not apparently mediated by serotonergic or cholinergic interneurons, but is modulated by the inhibitory neurotransmitter GABA. These data support the idea that the central catecholaminergic systems are excitatory rather than inhibitory upon CRH secretion when acting directly at the hypothalamic level. Using a different technique, namely an in vitro rat mediobasal hypothalamus perfusion system, Orliaguet et al. found that norepinephrine stimulated CRH secretion, with peak effects at 10^{-8} M concentration, whereas epinephrine had no effect on CRH secretion. The effect of
norepinephrine was antagonized by the mixed alpha antagonist phentolamine and by the mixed beta antagonist propranolol. They concluded that norepinephrine, but not epinephrine, stimulated hypothalamic CRH secretion via alpha and beta receptors. Thus, both studies confirm the hypothesis that the central noradrenergic systems are excitatory upon hypothalamic CRH secretion when acting directly at the hypothalamic level. However, the role of epinephrine on CRH secretion is still controversial.

Studying conscious, freely moving male Sprague-Dawley rats, Bagdy et al. (1989) showed that sympathoadrenomedullary and hypothalamo-pituitary-adrenocortical axis functions are markedly stimulated by three different serotonin agonists with different structures and 5-HT receptor binding profiles [the 5-HT1A agonist, 8-hydroxy-2-(di-n-propylamino)tetrinal (8-OH-DPAT), the 5-HT1C agonist, m-chlorophenylpiperazine (m-CPP), and the 5-HT2/5-HTIC agonist, 1-(2,5-dimethoxy-4-iodophenyl)2-amino-propane (DOI)]. Those three compounds produced substantial dose-dependent increases in plasma epinephrine and ACTH concentrations. That interesting work suggested that both the sympathoadrenomedullary system and the hypothalamo-pituitary-adrenocortical axis can be activated via 5-HT1 and 5-HT2 receptors and that these two systems may have common or similar regulatory mechanisms triggered by these stimuli. Thus, serotonergic systems could be a link between activation of both noradrenergic and neuroendocrine responses. Noradrenergic activation would in turn further contribute to activate the HPA axis, thereby preparing the organism to deal with acute stressors of various types.

Pacak et al. (1995) have described the use of in vivo microdialysis in rat brain regions such as the paraventricular nucleus (PVN) of the hypothalamus, the central nucleus of the amygdala (ACE), the bed nucleus of the stria terminalis (BNST), and the postero-lateral hypothalamus in order to examine aspects of catecholaminergic function and relationships between altered catecholaminergic function and the HPA axis and sympathoadrenal system activation in stress. Exposure of animals to immobilization (IMMO) markedly and rapidly increases rates of synthesis, release, and metabolism of norepinephrine (NE) in all the brain areas mentioned above and supports previous suggestions that in the PVN NE stimulates release of corticotropin-releasing hormone (CRH). Moreover, data obtained from adrenalectomized rats, with or without glucocorticoid replacement, and from hypercortisolemic rats suggest that glucocorticoids feedback to inhibit CRH release in the PVN, via attenuation of noradrenergic acti-
vation. Results from rats exposed to different stressors have indicated substantial differences among stressors in eliciting PVN noradrenergic responses as well as in responses of the HPA, sympathoneural, and adrenomedullary systems.

An important peripheral site for CRH-sympathetic interactions is the adrenal medulla. Immunoreactive and bioactive corticotropin-releasing factor has been identified in the adrenal gland of dogs, rats and humans. Radioimmunoassay and immunohistochemical experiments have clearly demonstrated that localization of the peptide is confined to the adrenal medulla (Bruhn et al. 1987). Bruhn et al. have reported that electron microscopic studies suggest that CRH is secreted at blood vessels within the adrenal medullary vasculature. CRH has also been identified in phaeochromocytomas. The amount of the peptide made by such tumours is highly variable as the CRF content of phaeochromocytomas may be 20 - 100 times higher or lower than that of normal adrenal tissue. Additional studies on chronically cannulated, awake dogs have shown that CRH is secreted into adrenal venous blood. A gradient exists between adrenal venous and peripheral arterial blood, as CRH is undetectable peripherally under resting conditions. Haemorrhage, a haemodynamic stimulus known to activate a sympathetic adrenal response, increases the CRF secretory rate. The time course of CRF secretion in response to this stimulus parallels that of epinephrine secretion. Bruhn et al. (Bruhn et al. 1987) have proposed that CRF may affect local blood flow within the adrenal medulla and may modify catecholamine secretory rates via this mechanism. The localization of CRF cells in close apposition to blood vessels appears to support this hypothesis.

**CRH-SYMPATHETIC-IMMUNE INTERACTIONS**

Immunity is a key biological function that requires modulation during the acute stress response. Corticotropin-releasing hormone can modulate immunity through activation of the HPA axis and cortisol secretion; cortisol is a potent endogenous immunosuppressive agent. However, several authors have shown that CRH can directly affect immunity through mechanisms that are independent of HPA activation.

Irwin et al. (1990) have shown that CRH acts within the brain to activate the sympathetic nervous system and reduce cellular immune function. It has been shown that a reduction of cellular immune function as measured by splenic natural killer cell activity also follows the central administration of CRH. Irwin et al. microinjected synthetic rat CRH into the lateral ventricle and documented increased noradrenergic func-
tion and reduced NK activity in the rat spleen. Pretreatment of the animals by chemical sympathectomy with 6-hydroxy-dopamine produced a greater than 95% reduction of splenic norepinephrine concentration and abolished completely both the CRH-induced increase in plasma catecholamine levels and the reduction in splenic NK activity. In addition, beta-adrenergic receptor blockade by either propranolol or butoxamine antagonized the CRH-induced reduction in NK activity. Measurement of circulating levels of adrenocorticotropic hormone and corticosterone demonstrated that activation of the pituitary adrenal axis by CRF was dissociated from changes in NK activity. Those results support the hypothesis that the sympathetic nervous system mediates the suppression of splenic NK cytotoxicity caused by central CRH.

In subsequent work Irwin et al. (1992) showed that CRH-sympathetic-immune interactions are age-dependent. They microinjected rat CRH (200 pmol) into the lateral ventricle of aged (24-month-old) Fischer 344 (F344) rats and of young (4-month-old) F344 rats. Basal concentrations of plasma norepinephrine and NPY were higher in the aged than in the young animals. In addition, CRH produced a greater elevation of plasma levels of catecholamines and NPY, which persisted for a longer period of time in the aged rats compared to responses in the young animals. Splenic NK activity showed an age-related decrement at baseline, and CRH induced a further significant reduction of lytic activity in the aged rats, but did not alter cytotoxicity in the young rats. Corticosterone basal levels and responses were similar in the aged and young rats. That work showed an age-related increase in autonomic outflow and suppression of NK activity after central CRH administration. Based on those data it appears that in aged animals, the central nervous system may have a role in abnormal regulation of sympathetic activity and suppression of natural cytotoxicity in vivo.

The recent work of Okamoto et al. (1998) has shown that CRH receptor activation, not only by CRH but also by urocortin, can affect immunity through sympathetic activation. Those authors injected urocortin intracerebroventricularly and observed a marked decrease in the proliferative response of splenocytes to a mitogen. The suppressive effect of urocortin was abolished by pretreatment with a ganglionic blocking agent (chlorisondamine) or a beta-adrenergic receptor antagonist (propranolol), but not by adrenalectomy. Thus, they concluded that urocortin contributes to the central control of peripheral immune functions such as stress-induced immunosuppression, and that the immune suppressive effect of urocortin is mediated by the sympathetic nervous system.
While central CRH is directly, as described earlier, or indirectly through cortisol, an immunosuppressant, peripheral CRH has been identified as a pro-inflammatory mediator (Karalis et al., 1991). Immunocytochemistry (ICC) and in situ hybridization (ISH) have been used to identify the sources of peripheral CRH (Brouxhon et al. 1998). Immunoreactive CRH has been found in cells in the marginal zone and red pulp of the spleen, in connective tissue septa and the subcapsular region of the thymus, and in the medullary cords and sinuses of the mesenteric lymph nodes. Dual ICC/ISH for CRF and its mRNA, respectively, demonstrated CRH mRNA over CRF- immunoreactive cells, suggesting CRF synthesis. Double-label ICC for CRF and markers for specific immunocyte subsets suggest that CRF+ cells in the spleen and thymus are macrophages. CRF+ cells in primary and secondary lymphoid organs reside in compartments that are innervated by sympathetic nerves, and some cells appears to be contacted by noradrenergic sympathetic nerve fibres, suggesting that in peripheral immune organs, CRH release may be influenced by the sympathetic nervous system, as it is in the hypothalamo-pituitary-adrenal axis.

LEPTIN: AN ADIPOCYTE HORMONE THAT COUNTERREGULATES THE STRESS RESPONSE

Leptin is an adipocyte hormone that communicates a signal of nutritional status to the brain. In physiological situations weight gain leads to increased leptin synthesis and secretion. High leptin levels lead to decreases in food intake and energy expenditure, resulting in weight loss and a return of body weight to normal. In obesity there appears to be resistance to the effects of leptin as high levels of circulating plasma leptin do not seem to cause weight loss. In individuals with a mutation in the leptin gene or the leptin receptor gene there is marked obesity and endocrine abnormalities. Thus, it appears that leptin is required, but it is not sufficient, for the maintenance of normal body weight. In addition to its effects on body weight, leptin modulates various stress-related functions, including HPA activity and sympathetic tone. The administration of leptin to starved rodents normalizes the elevated levels of ACTH and cortisol that are characteristic of the fasting state (Ahima et al., 1996). In mice leptin treatment blunts induction of cortisol by immobilization stress. Moreover, in vitro studies have shown that leptin blunts the secretion of CRH by perfused rat hypothalami that have been subjected to hypoglycaemia, which is a potent stimulus to CRH secretion in that model (Heiman et al., 1997).
Our group (Licinio et al., 1997) and others (Sinha et al., 1996a; 1996b) have shown that plasma concentrations of leptin have pulsatility and diurnal variation, which seem to be of biological relevance: a blunted nocturnal rise in leptin levels correlates with weight gain (Matkovic et al., 1997). Because both cortisol and leptin exhibit statistically significant ultradian and diurnal fluctuations that are clinically relevant, it is inadequate to rely on single fasting measurements to assess a relationship between these hormones. In our own studies we have shown a highly significant inverse relationship between the variability of simultaneous 1,242 measurements of cortisol and leptin in six normoglycaemic men who were sampled every 7 minutes for 24 h (Pearson correlation: \( r = 0.764; P < 10^{-9} \)) (Licinio et al., 1997). These data are consistent with the findings that leptin acts directly in the adrenal gland to suppress cortisol production (Bornstein et al., 1997). We have proposed that the effects of leptin on hypothalamic-pituitary-adrenal function indicate a mechanism by which a pulsatile peripheral signal of nutritional status may regulate stress-related endocrine function and behaviour (Licinio, 1998b).

Leptin regulates sympathetic activity, ob/ob mice, which have a leptin gene mutation, exhibit low sympathetic tone (Bray and York, 1979). Humans with a leptin gene mutation have a similar defect, which is manifested clinically by a low blood pressure response to a cold pressor response test and to an orthostatic hypotension test, as well as by the absence of a response to median nerve and auditory stimulation in the sympathetic skin response test (Strobel et al., 1998). Leptin is thus required for the maintenance of optimal sympathetic tone.

We have recently proposed that leptin coordinates a state of satiety for which we have coined the term “feast state” (Licinio, 1998b). Cannon conceptualized a unitary state of “flight or fight,” according to which an acute emergency would activate the sympathoadrenal system globally, thereby preparing the organism to threats to homoeostasis (Goldstein, 1996). Such “flight or fight” responses include, in addition to acute activation of sympathetic responses, stimulation of the hypothalamic-pituitary-adrenal axis, and decreases in functions such as reproduction, digestion, and immunity. These physiological adaptations are exactly the opposite of the effects of circulating plasma leptin, whose concentrations increase after food intake and weight gain. Thus, the effects of leptin on the intact organism are the opposite of the manifestations of acute stress resulting in the “fight or flight” response. We proposed a role of leptin in the maintenance of homoeostasis that is the opposite of that occurring during confronta-
The autonomic nervous system

tion with predators (Licinio, 1998b). We have therefore hypothesized a “feast” state that occurs when predators or other dangers are not present, and when food is encountered and consumed in substantial amounts, causing weight gain and enhancing leptin production. In that state, increased leptin would stimulate reproduction, enhance immunity, blunt HPA activity, and maintain optimal sympathetic tone. We have proposed that the “feast” state facilitates the maintenance of vegetative functions, strengthens immunity, and optimizes reproductive success (see Table 1).

CONCLUSIONS

New developments in molecular biology, integrative physiology, and metabolism have led to considerable progress in our understanding of the neurobiology and neuroendocrinology of the stress response. The response to stress is mediated by a network of molecules that are expressed in the CNS in specific temporal and spatial patterns and are secreted into the circulation in complex rhythms. The interactions of central control systems with peripherally secreted molecules such as leptin determines the final outcome of the response to stress. Moreover, an understanding of the molecular mechanisms by which CRH elicits gene transcription has led us to develop a testable hypothesis for stress-induced alterations in disease susceptibility. Advances in the understanding of the neurobiology and neuroendocrinology of the stress response have permitted the conceptual integration of events occurring at the molecular, cellular, integrative, and clinical levels. Our ability to bridge molecular medicine and clinical investigation in this area has placed this field at the forefront of contemporary biology. To expand the frontiers of existing knowledge in stress research we now have to continue to examine at the molecular and clinical levels the role of stress mediators (that include both the autonomic nervous system and neuropeptides) in pathogenesis, pathophysiology, and therapeutics. Such work is of particular importance in the light of the recent development of non-peptide CRH antagonists, which can provide conceptually novel therapeutic strategies for human disease.
TABLE 1
Adaptive mechanisms that promote adaptation and survival

<table>
<thead>
<tr>
<th>FIGHT OR FLIGHT</th>
<th>FEAST</th>
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<tr>
<td>Occurs during confrontation with predators and acute insults.</td>
<td>Occurs during weight gain that follows consumption of large amounts of food.</td>
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ENDOCRINE-IMMUNE MANIFESTATIONS

- Increased HPA responses
- Acute sympathetic discharges
- Suppression of immunity
- Suppression of reproduction
- Decreased HPA responses
- Optimal sympathetic tone
- Promotion of immunity
- Promotion of reproductive system function

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The autonomic nervous system
THE ENDOCRINE HEART AND THE CLINICAL IMPACT OF THE NATRIURETIC PEPTIDES

Markus Meyer, Thomas Flüge and Wolf-Georg Forssmann

INTRODUCTION
In the early 1980s the natriuretic peptide family [2, 19, 51] was identified and further characterized in detail. These peptides are involved in the regulation of salt and water homeostasis [57]. The prototype of the natriuretic hormones is cardiodilatin/atrial natriuretic peptide (CDD/ANP) or A-type natriuretic peptide. CDD/ANP is primarily produced in the heart [6, 18, 20, 30]. It is synthesized as a precursor molecule (CDD/ANP-1-126) in specific granules of the atrial myoendocrine cells [22]. Upon appropriate stimuli the prohormone is cleaved into the C-terminus CDD/ANP-99-126 which is subsequently liberated and excreted into the circulation via exocytosis [22]. Following CDD/ANP, further members of the natriuretic peptide family were isolated: brain natriuretic peptide (BNP or B-type natriuretic peptide) [59] and CNP (C-type natriuretic peptide) [60]. The main biological effects of these hormones are natriuresis, diuresis, and smooth muscle relaxation [5, 6, 20, 26] but these vary among the individual peptides.

Urodilatin (URO, INN: ularitide) is a new member of the natriuretic peptide family, discovered by Schulz-Knappe et al. in 1988 [54]. This hormone which exhibits an N-terminal extension by 4 amino acids in comparison to the circulating CDD/ANP-99-126 is presumably synthesized in the kidney and exerts potent paracrine renal effects [21, 34]. Although URO is not found in human blood or lung [7, 42] this peptide reveals significant actions at the tracheobronchial tree [13, 17]. (For comparison of the amino acid sequences of the natriuretic peptides see Figure 1).

This family of regulatory peptides circulating in the bloodstream or exhibiting effects in a paracrine fashion is thought to be involved in pathophysiological mechanisms of cardiovascular, renal and pulmonary disorders. Therefore, over the last few years we were interested
in investigating the physiological and pharmacological properties of URO for therapeutic and prophylactic use in different clinical indications.

**RECEPTORS, SECOND MESSENGER SYSTEMS, AND METABOLISM OF NATRIURETIC PEPTIDES**

Cyclic guanosine 3',5'-monophosphate (cGMP) is the intracellular mediator of the biological activity induced by natriuretic peptides following the activation of particulate guanylyl cyclase [39]. cDNA analysis and cloning revealed three types of natriuretic peptide receptors (NPR): NPR-A, B and C [39]. NPR-A and NPR-B are coupled to the intracellular guanylyl cyclase catalytic domain, whereas the third member, NPR-C, is not associated with an intracellular guanylyl cyclase [36]. The physiological role of NPR-C is not clear, but convincing investigations indicate that this protein acts like a clearance receptor. Besides binding of circulating natriuretic peptides to NPR-C, enzymatic degradation in lung, liver, and kidney takes place [28, 48]. The main enzyme responsible for degradation is the metalloendoprotease E.C.3.4.24.11. One of the main location sites is the brush border of the proximal tubule and the tracheobronchial system in the lung. This enzyme cleaves the loop structure of CDD/ANP-99-126 between position Cys-105 and Phe-106, thereby reducing receptor affinity and thus biological activity [23, 58]. In contrast to CDD/ANP-99-126, results of several groups indicate a high resistance of URO to enzymatic degradation. This finding may be due to its N-terminal extension by four amino acids [12, 23, 31]. The structural difference may induce conformational changes, thereby preventing the enzyme from attacking the cleavage site. Thus, exogenously applied URO may reach the distal tubule and the collecting duct without being degraded and exerts its renal effects at this location. Furthermore its reduced rate of inactivation may facilitate prolonged binding to biological active NPR-A receptors in the lung, inducing a more pronounced bronchodilation. The different profile of metabolism appears to be of great clinical importance when comparing the potential renal and bronchial effects of intravenously administered CDD/ANP-99-126 and URO.

**RENAL PHYSIOLOGY AND PHARMACOLOGY OF URO**

Several observations suggest that URO plays a key role in the physiological regulation of renal function, especially in the control of renal sodium and water excretion [8, 11, 24, 44]. Our group investigated the effect of long-term sodium load in healthy volunteers [46]. We
found a close correlation between natriuresis and URO excretion. A stepwise increase in sodium intake induced a concomitant increase in URO excretion parallel to sodium excretion. Other groups demonstrated an increased URO and sodium excretion after acute volume load by saline infusion [9] and following balloon dilatation of the left atrium [24]. Drummer and co-workers [8] demonstrated that the circadian rhythm of urinary sodium excretion paralleled URO excretion. Compared to URO, CDD/ANP-99-126 exerts only trivial effects on renal sodium excretion. Several experiments revealed a closer correlation of natriuresis to URO excretion than to CDD/ANP-99-126 plasma levels [25]. These observations indicate that URO may be the natriuretic factor primarily responsible for sodium and water regulation.

Infusions and bolus injections of URO in rats [55], dogs with cardiomyopathy [50], and healthy volunteers [52] show the pharmacological potency of this new natriuretic peptide. Initiation of profound diuresis and natriuresis as well as slight reduction in blood pressure are the most prominent effects. However, these effects were superior to those induced by CDD/ANP-99-126 [49, 50, 52].

Haemodynamic measurements in healthy volunteers following bolus injection of URO revealed a stronger reduction of pulmonary arterial pressure and wedge pressure than after CDD/ANP-99-126 administration in equimolar doses [33]. URO administered as a bolus injection in patients with congestive heart failure showed in contrast to CDD/ANP-99-126 only a slight reduction of blood pressure followed by a reflex tachycardia, whereas cardiac index as well as stroke volume increased significantly [32] in comparison to CDD/ANP-99-126.

In summary, the pharmacological studies using URO indicated a stronger renal potency combined with fewer haemodynamic side effects as compared to CDD/ANP-99-126. For the proposed intrarenal mechanism of URO and CDD/ANP-99-126 see Figure 2.

**URO IN ACUTE RENAL FAILURE**

Acute renal failure (ARF) is a frequent post-operative complication after major surgical interventions due to haemodynamic, ischaemic or vasoconstrictive mechanisms [27, 40, 41]. The vasoconstrictive effect of cyclosporine A on arterial circulation [40] was suggested to be a particular reason for ARF in the post-operative phase after organ transplantation. Therefore, the predominant vasorelaxant effect of natriuretic peptides on vascular smooth muscle in the kidney was postulated to intervene with incipient renal failure in major surgery.
Experimental studies showed that atrial peptides like URO may exert a beneficial effect in ARF [35, 53].

Since no drug therapy is yet available for ARF, we suggested the possibility of administering URO infusions as a prophylaxis against ARF in patients following heart transplantation (HTx) [29]. This initial open and sequential trial with URO showed a significant and beneficial effect in preventing ARF. Two further double-blind, placebo-controlled studies were subsequently performed, infusing URO prophylactically in patients following HTx and liver transplantation (LTx) [3, 38].

Meanwhile, the therapeutic properties of URO were tested in patients suffering from incipient ARF following LTx and HTx. In an open study, Cedidi and co-workers [4] found that patients with postoperative ARF resistant to high doses of furosemide and conventional therapy could manage without haemodialysis/hemofiltration (HD/ HF) when URO was used as a therapeutic agent. In a subsequent double-blind, placebo-controlled study, five patients receiving URO infusion (20 ng/kg bw/min) were compared with four placebo patients suffering from ARF [37]. In this study, frequency of HD/HF was significantly reduced in the URO-treated patients and SC levels decreased consistently in the URO group. We also performed a double-blind study in patients suffering from ARF following heart operation [47]. Both groups consisted of seven patients. Summarizing the results, six patients receiving placebo needed HD/HF whereas in the URO-treated group none of the patients required HD/HF.

Following these open and non-controlled studies we initiated a pivotal phase II trial assessing the effects of URO in patients with oliguric ARF following cardiac surgery, HTx or LTx [45]. The primary objective was the avoidance of HD/HF. Summarizing, this study failed to show a significant difference in patient outcome in the URO-treated groups versus placebo. The incidence of HD/HF was similar in all groups. These results are in contrast not only with our previous results but also with a study carried out by Allgren and co-workers [1]. Here, ANP administration resulted in a significant improvement of dialysis-free survival in a subgroup analysis in patients with oliguric acute tubular necrosis. However, some other points might have contributed to these differences. During the whole study period (including follow-up until day 40) 75 patients died, indicating their severe clinical state. Furthermore, one might speculate whether a longer period from the onset of oliguria/anuria until administration of study medication would have been more adequate, particularly in order to exclude spontaneous remissions of diuresis during the first hour of infusion as documented in
the placebo group. In contrast, one might consider that this time interval was too long and contributed to further renal deterioration possibly due to peri- or post-operative renal hypoperfusion. Therefore, a prophylactic approach in the use of URO might be more promising. However, these points illustrate the difficulties in proving a therapeutic effect of URO in oliguric ARF in these patients and may also reflect the problems involved in testing any pharmacological agent in this clinical setting. Multi-morbidity, haemodynamic instability, and multiplicity of drug administration might have contributed so that not only for URO but also for other pharmacological agents a precise role for diuretic therapy in the clinical setting of potential or established ARF has not yet been clarified [56].

Summarizing, there are clinical studies with different results, significantly reducing the incidence of HD/HH using URO in some and exhibiting no beneficial effects in others. All in all, these data may reflect the problems of proving URO in this clinical setting. However, the efforts made over the last few years may have led to a better understanding of the implication of URO and the natriuretic peptides in the pathophysiological mechanisms of ARF and may ultimately result in benefit to patients.

**URO IN BRONCHIAL ASTHMA**

In *in vitro* experiments, our group demonstrated the existence of the NPR-A receptor in the tracheobronchial system and showed a potential binding site for type-A natriuretic peptides like URO and CDD/ANP-99-126. Furthermore, the intracellular increase of cGMP induced by these two natriuretic peptides in guinea pig tracheal preparations was measured [43]. Following these *in vitro* experiments pharmacological investigations with URO and CDD/ANP-99-126 were performed in animals. To compare the properties of URO and CDD/ANP-99-126 *in vivo*, we first evaluated the bronchoprotective effects against acetylcholine-induced bronchoconstriction in spontaneously breathing, halothane-anesthetized Wistar rats using whole-body plethysmography [13]. Forced parameters detect airflow changes with a greater sensitivity and were measured in hyperventilation-induced temporary apnoea after the challenge. Forced expiratory volume in 0.1 s (FEV$_{0.1}$) and all parameters of the flow-volume curve (FEF, MMER, FEF$_{75}$, FEF$_{50}$, FEF$_{25}$) showed a significant protection with i.v. URO administration but not with CDD/ANP-99-126-administration.

β$_2$-agonists are the gold standard for bronchodilator therapy in asthmatics. We therefore compared the bronchodilation induced by i.v. URO and CDD/ANP-99-126 to a subsequent inhalation of
salbutamol in 36 clinically stable patients suffering from bronchial asthma [14, 15]. Both peptides revealed significant effects on forced expiratory lung function parameters (FEV₁, PEF, MEF₅₀, MEF₇₅, MEF₂₅) during infusion and until a short time afterwards. When the improvement of lung function induced by the natriuretic peptides was standardized on the intra-individual maximum of bronchodilation after salbutamol (100%) significant differences were documented between URO and CDD/ANP-99-126. While URO caused a maximal bronchodilation of the central (FEV₁, PEF, MEF₇₅) and peripheral (MEF₅₀, MEF₂₅) airways, which was not significantly different from the β₂-agonist showing a clear dose-response relationship, CDD/ANP-99-126 reached only about 50% of the salbutamol effect.

Next, we compared the bronchodilation induced by intravenous URO (0, 10, 30 or 60 ng/kg/min, URO 00/10/30/60) in combination with 0 or 200 μg salbutamol (S0000, S0200) in a randomized, double-blind, placebo-controlled clinical phase II study with cross-over design [16]. URO alone induced an increase in the FEV₁ reaching statistical significance compared to the pre-infusion values and URO 00/S0000 from 20 to 60 min infusion in all dose groups. No significant differences between the therapeutic effects of URO 30/ S0000, URO 60/S0000 and URO 00/S0200 could be demonstrated. When 30 or 60 ng/kg/min URO were combined with 200 μg salbutamol (URO 30/S0200, URO 60/S0200) an effect not significantly different from the more than 6 times higher salbutamol dose (S1250) was reached.

In conclusion, the in vitro and in vivo studies in animals and in humans revealed bronchial effects of URO significantly superior to those of CDD/ANP-99-126. Based on these data a clinical phase II trial was initiated using URO. The results obtained demonstrate that the combination of both types of drugs (β₂-agonists and URO) should be beneficial to induce bronchodilation in asthmatics.

**URO IN CONGESTIVE HEART FAILURE**


Based on these results and similar experiments which confirmed that URO might have beneficial effects in congestive heart failure (CHF) [61], the first double-blind, placebo-controlled study with patients suffering from CHF was performed [10]. Riegger and co-
workers infused URO in a dose of 15 ng/kg bw/min for 10 hours in 12 patients with CHF. Urine flow and urinary sodium excretion were significantly increased. Furthermore, URO induced a significant reduction in systolic blood pressure and central venous pressure while diastolic blood pressure remained unchanged. The authors concluded from these results that URO lowers preload and increases diuresis and natriuresis, demonstrating a profile of effects that may be beneficial in patients with CHF.

**SUMMARY**

1) Natriuretic peptides are a peptide family modulating cardiovascular, renal and pulmonary functions.

2) URO, a member of the natriuretic peptide family and synthesized in the kidney, is thought to play an important role in the physiological regulation of fluid balance and sodium homeostasis.

3) Pharmacological studies revealed significant differences when URO and CDD/ANP-99-126 are administered intravenously. Low dose infusion of URO shows a stronger diuretic and natriuretic effect than equimolar doses of CDD/ANP-99-126, with fewer haemodynamic side effects.

4) Clinical studies indicated the prophylactic and therapeutic effect of URO in patients suffering from ARF following heart operation and organ transplantation. However, a clinical phase II trial using URO as a therapeutic drug did not confirm these previous results. Therefore, a prophylactic approach is discussed.

5) *In vitro* and *in vivo* studies as well as a study in patients suffering from bronchial asthma revealed bronchodilating effects of URO. A clinical phase II trial confirmed these results and a clinical phase III study was performed in asthmatic patients suffering an acute exacerbation. Based upon these data URO may be a tool for therapy of bronchial asthma in combination with β₂-agonists.

6) Pharmacological studies and a first clinical study showed beneficial effects in patients suffering from CHF by lowering preload and increasing diuresis and natriuresis.
FIGURE 1
Natriuretic peptides (NP) in schematic depiction. Note the prohormone of A-type NP contains the biologically active segment of the molecule, cleaved to the circulating CDD/ANP-99-125 and the renal form, urodilatin, in its C-terminus. B-type NP and C-type NP are homologous, especially showing the ring formation of the molecule by a disulfide bond of two cysteines and 15 amino acid residues.
FIGURE 2
Proposed mechanism of physiological and pharmacological effects of endogenous A-type natriuretic peptides CDD/ANP-99-126 and URO. In the proximal tubule enzymatic degradation of filtered CDD/ANP-99-126 takes place preventing an interaction of CDD/ANP-99-126 with luminal receptors located in the collecting duct. In contrast, URO is luminaly secreted from distal tubule cells and can exert its renal effects interacting with these receptors in the collecting duct.

Intrarenal Action of URODILATIN
and endogenous CDD/ANP-99-126
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The autonomic nervous system
MECHANISMS REGULATING SYMPATHETIC NERVE TRAFFIC - A FOCUS ON NORMAL SLEEP AND OBSTRUCTIVE SLEEP APNOEA

Virend K. Somers

Measurements of sympathetic nerve activity using the microneurographic technique have provided important new insights into the mechanisms regulating neural circulatory control. Microneurography involves the insertion of a tungsten microelectrode into a peripheral nerve so as to allow direct recordings of efferent sympathetic nerve traffic. Using this technique, measurements of both muscle sympathetic nerve activity (MSNA) and skin sympathetic nerve activity (SSNA) may be obtained. This discussion will focus primarily on measurements of MSNA. Attention will also be given to other methods used for measurements of autonomic responses, such as power spectral analysis of cardiovascular variability.

AUTONOMIC RESPONSES TO NORMAL SLEEP

Studies in animals have shown that non-REM sleep is associated with a fall in blood pressure, in part because of reduced cardiac output and increased peripheral resistance (1). During REM sleep there are intermittent surges in blood pressure and heart rate with occasional respiratory irregularity (2,5). Overall, during normal sleep, changes in heart rate and blood pressure are fairly modest. While heart rate and blood pressure decline progressively from stage I to stage IV of non-REM sleep, during REM, blood pressure and heart rate are similar to measurements during wakefulness.

In determining the autonomic responses to sleep and the different sleep stages, measurements of heart rate variability (6) have shown a progressive decrease in the low frequency oscillatory component during non-REM sleep and a reduction in the LF/HF of heart rate variability (7-9). During REM, there is an increase in the LF variability and an increase in the LF/HF. These findings are suggestive of a reduction in sympathetic heart rate modulation during non-REM sleep and an increase in sympathetic modulation of heart rate during REM sleep.
Spectral measures of heart rate variability appear to be influenced by cardiovascular disease. Specifically, Vanoli et al. (8) have shown that the heart rate variability patterns described above may be severely disrupted in patients after myocardial infarction. In these patients LF/HF actually increases during non-REM sleep with a further increase during REM sleep. Thus, myocardial infarction may be accompanied by a loss in the capacity for cardiac vagal activation during sleep, thus allowing unopposed sympathetic dominance even during non-REM sleep.

Microneurographic measurements of sympathetic traffic during sleep (10-12) are consistent with the paradigm that sympathetic nerve activity decreases progressively during the deepening stages of non-REM sleep. Arousal stimuli during non-REM sleep elicit K complexes on the electroencephalogram, accompanied by bursts of sympathetic nerve activity and transient increases in blood pressure (11,12). This response of MSNA to arousal is strikingly different from the MSNA arousal relationship during wakefulness. During wakefulness arousal stimuli do not increase sympathetic activity. Thus, during normal sleep there may be a change in the neural processing of auditory and possibly other arousal stimuli.

During REM sleep, MSNA increases to about twice the level seen during wakefulness and heart rate and blood pressure are similar to measurements when awake (12). There are marked fluctuations in all these measurements with sympathetic nerve activity being especially increased during phasic REM, i.e. during episodes of rapid eye movements, which are also associated with intermittent surges in blood pressure and heart rate fluctuations.

The autonomic and haemodynamic changes during sleep may have important clinical implications. There is increasing evidence of a circadian rhythm in cardiovascular events, including in sudden death (13,14). The mechanisms underlying this circadian rhythm are not known. The predominance of REM sleep in the early morning just prior to wakening and the sympathetic and haemodynamic changes during REM (15,16) may be implicated in increased platelet aggregability, plaque rupture, and coronary vasospasm, thus possibly acting as a triggering mechanism for thrombotic events that may only become clinically manifest some time after waking.

NEURAL CIRCULATORY REGULATION IN OBSTRUCTIVE SLEEP APNOEA
Several studies have demonstrated consistently that patients with obstructive sleep apnoea have very high levels of sympathetic nerve traf-
fic, even during wakefulness (17,18). These high levels of sympathetic activity are evident when patients are normoxic and in the absence of any breathing disturbance. High sympathetic activity is also independent of the presence of hypertension and is not explained by obesity (19).

Patients with sleep apnoea also have high levels of norepinephrine (20-22), faster heart rates, decreased heart rate variability and an increased low frequency (LF) oscillatory component of heart rate variability (23). This increased low frequency component is present even in normotensive otherwise healthy sleep apnoea patients and is also associated with an increase in blood pressure variability (23). Thus, otherwise healthy sleep apnoic patients who are normotensive, have faster heart rates, increased LF of RR, decreased RR variability and increased blood pressure variability. These neural circulatory control abnormalities in sleep apnoea are strikingly similar to the abnormalities in neural control evident in patients with essential hypertension, but are present in the sleep apnoea patients even in the absence of any hypertension. Thus, these abnormalities in neural control may preceed the development of sustained hypertension in sleep apnoic patients.

Abnormalities in chemoreflex function may be implicated in autonomic derangements in sleep apnoea. Chemoreflex deactivation by 100% oxygen results in heart rate slowing, blood pressure reduction and decreased MSNA. This suggests that tonic chemoreflex activity, even during normoxia, may contribute to increased sympathetic drive in sleep apnoea patients (24). Specific studies of chemoreflex function have indeed demonstrated that there is a selective potentiation of the peripheral chemoreceptor response to hypoxia in patients with sleep apnoea.

The autonomic responses to sleep apnoea are especially dramatic during sleep. During sleep, oxygen desaturation and carbon dioxide retention both contribute to peripheral and central chemoreceptor activation (25,26) with consequent increases in muscle sympathetic nerve activity. The resulting vasoconstriction elicits marked surges in blood pressure that are especially evident at the end of apnoea (18). Chemoreflex mediated sympathetic activation and vasoconstriction during sleep in patients with sleep apnoea prevent any fall in blood pressure and sympathetic activity during sleep. Thus, the normal sleep stage related changes in sympathetic traffic, blood pressure and heart rate evident in young healthy subjects are markedly deranged in sleep apnoea. These patients, who have high sympathetic activity and often high blood pressure even during normoxic wakefulness, manifest fur-
ther increases in sympathetic traffic and blood pressure during sleep (18).

The autonomic and haemodynamic abnormalities described above may be implicated in increased cardiovascular morbidity and mortality in sleep apnoea patients. Abnormalities in cardiovascular variability may precede and perhaps predispose to cardiovascular dysfunction, particularly hypertension (27). Sleep apnoea may also be an important factor contributing to increased cardiovascular risk in hypertensive non-dippers, namely those hypertensive patients whose blood pressures do not fall during sleep (28-30). It is also conceivable that since sleep apnoea and obesity frequently exist as co-morbidities, the high incidence of occult sleep apnoea in apparently asymptomatic obese people may contribute to the cardiovascular risk associated with morbid obesity (19).

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The autonomic nervous system
ANP AND OXYTOCIN AS NEUROENDOCRINE MODULATORS OF BODY FLUID HOMEOOSTASIS

J. Antunes-Rodrigues, A.L.V. Favaretto, and S.M. McCann

ABSTRACT
The role played by the central nervous system (CNS) in the control of body fluid homeostasis has been demonstrated by several authors. The anteroventral third ventricle region (AV3V) plays a key role in central control of sodium excretion since its cholinergic, adrenergic, angiotensinergic and osmotic stimulation of the AV3V enhances and its destruction blocks sodium excretion in rats and goats. Cholinergic stimulation of the AV3V induced an increase in plasma atrial natriuretic peptide (ANP) as well as a marked elevation in content of the peptide in the median basal hypothalamus, neurohypophysis and adenohypophysis. On the other hand, a decline in plasma ANP after AV3V lesions was accompanied by dramatic declines in content of ANP in these same structures. Our previous work has also indicated the essential role of the AV3V region and its ANPergic neurons in the control of ANP release in response to blood volume expansion (BVE) and indicated that α-adrenergic and muscarinic receptors are critical in mediating these responses. Lesions destroying the perikaria or caudally projecting axons of the ANP neurons in the AV3V region, the median eminence or posterior lobe of the pituitary gland blocked the increase in plasma ANP concentration in response to BVE. That this effect is related to blockade of the activity of the brain ANPergic neurons is supported by findings in sheep and in rats that the injection of antiserum directed against ANP into the AV3V region at least partially blocked the BVE-induced release of ANP. We and others have also previously shown that denervation of baroreceptors inhibits ANP release induced by BVE. Activation of the ANP neurons also causes release of ANP from the anterior and neural lobe of the pituitary gland. Since oxytocin (OT) is also released by BVE, ANP neurons may activate oxytocinergic neurons in the supraoptic and paraventricular nuclei that project to the neural
lobe. Oxytocin would circulate to the right atrium and directly activate release of ANP from the atrial myocytes, since i.v. or i.p. injection of oxytocin elevates plasma ANP and also increases sodium excretion. The ANP released from cardiac atria myocytes by OT would circulate to the kidneys and evoke natriuresis to return circulating blood volume to normal. Recently, OT-receptors were demonstrated on cardiomyocytes. When OT is directly applied to the atria in vitro or perfused through denervated heart it has an inhibitory effect on the rate and force of contraction of the heart. When OT was incubated in vitro with rat atria it induced a dose-related increase in the ANP release from the incubated atrial quarters. This OT-induced release of ANP from the rat atria was blocked by an OT antagonist. The negative ino- and chronotropic effects of OT via ANP would produce a rapid compensation for increased blood volume by decreasing cardiac output. The released ANP would have a rapid compensatory effect by evoking vasodilatation. We have also shown that OT acts on its renal receptors on NOergic cells (macula densa and proximal tubule cells) to stimulate release of NO that activates guanylyl cyclase releasing cGMP that probably activates protein kinase g that closes Na+ and K+ channels, limiting Na+ and K+ reabsorption and evoking increase in salt excretion, these effects being almost completely inhibited by blocking NO synthesis. Thus, both ANP and OT-induced natriuresis and kaliuresis appear to be mediated by cGMP.

INTRODUCTION
The role of the central nervous system (CNS) in the control of sodium intake/excretion and body fluid homoeostasis has been known since Fisher et al (1935) drew attention to the role of the hypothalamus in the regulation of water metabolism. Since then many other groups have studied the effects induced by chemical or electrical stimulation of several areas in the CNS in the control of hydromineral balance. We undertook systematic studies to determine the effects of bilateral localized lesions of the rat hypothalamus on the free choice ingestion of tap water and 2% NaCl solution. Our group has mapped the various pathways within the CNS controlling salt intake. Subsequent experiments delineated a neural circuit that controls sodium intake and/or excretion. This circuit involves the septal area, the anteroventral part of the third ventricle region (AV3V), the amygdaloid complex, the hypothalamus and the olfactory bulbs (Covian et al 1975).

The role played by the central nervous system in the control of sodium excretion has been demonstrated by several authors (Andersson et al, 1966; Anderson, 1977; Brody and Johnson, 1983;

With Dorn we have shown that intraventricular injection of carbachol evoked a natriuretic response which mimicked the response to intraventricular injection of hypertonic saline (Dorn et al, 1970). Our group in Brazil and McCann with Orias and Mariana Morris in the United States carried out independent studies to investigate the effects of injection of cholinergic, adrenergic and hypertonic saline injections into the third ventricle of conscious male rats made diuretic by intragastric water loads. Both natriuretic and kaliuretic responses and an increase in the Na*/K* ratio were induced by intraventricular injection of noradrenaline and carbachol. The beta-receptor stimulator isoproterenol induced an antiglomerular and antikaliuretic effect. The alpha-adrenergic blocker phentolamine abolished the natriuretic response to intraventricular injection of hypertonic saline, noradrenaline or carbachol. In contrast, the beta-adrenergic blocker propranolol induced a natriuresis and kaliuresis when injected alone. Propranolol also potentiated the natriuretic response to carbachol. Cholinergic blockade with atropine diminished the response to norepinephrine and blocked the natriuretic response to hypertonic saline.

Silva-Netto et al (1986) have shown the natriuretic effect of carbachol stimulation of the lateral hypothalamic area (LHA) in rats with normal or denervated kidneys. As expected, the control values of Na* excretion were significantly higher in rats with denervated kidneys, consistent with the already known "denervation diuresis". Injection of carbachol into the AV3V led to a highly significant increase in urine volume and sodium excretion from both innervated and denervated kidneys. These results clearly show that the natriuresis induced by intrahypothalamic injection of carbachol occurs completely independently of the alterations in renal efferent nerve activity induced by intrahypothalamic actions of carbachol.

We hypothesized that a hypothalamic natriuretic hormone released by the neurohypophysis might mediate the neural control of electrolyte excretion. Therefore, we evaluated the effect of median eminence (ME) lesions. ME lesions temporarily blocked the natriuresis, kaliuresis and antidiuresis which followed the injection of hypertonic saline, carbachol or noradrenaline into the third ventricle (3V).
Hypophysectomy did not block but delayed the responses, which ruled out the obligatory participation of anterior pituitary hormones (Morris et al, 1976). The responses still occurred in rats with diabetes insipidus, which lacked arginine vasopressin (AVP) (Orias and McCann, 1971). This ruled out AVP as the natriuretic hormone, but left oxytocin as a possibility.

**THE NATRIURETIC HORMONE**

Cort and his coworkers (1969) reported the purification of a hypothalamic natriuretic factor. At this time Orias and McCann demonstrated that AVP, OT and alpha-MSH had natriuretic activity in water-loaded rats (Orias and McCann 1970; 1972a; 1972b). Until now it has not been clarified whether these hormones have a physiological role in the induction of natriuresis. Orias and McCann (1972b) obtained natriuretic activity in ME extracts and, at this time, they ruled out the possible participation of AVP and OT in this activity since the treatment of the extracts with thioglycollate, an agent which opens the disulfide bridge in the neurohypophysial hormones, thereby inactivating them, did not interfere with the natriuretic activity. Since they did not assay for residual AVP activity after thyoglycollate treatment, in retrospect, the thyoglycollate probably did not inactivate the neurohypophysial hormones in the extract or the activity could have been caused by the peptide ANP not then discovered but now known to reside in the ME.

De Wardener and Clarkson (reviewed in 1985) presented evidence showing that natriuresis could occur following blood volume expansion even though factors such as increased glomerular filtration rate or changes in aldosterone secretion were eliminated. Davis and Freeman (reviewed in 1976) obtained evidence for a circulating natriuretic factor in volume-expanded dogs by cross-circulation experiments.

Even earlier, Gauer and Henry (1963) showed that dilation of the right atrium by a balloon could induce diuresis. At that time it was thought that distension of the atria activated impulses which travelled up the vagus to inhibit the release of AVP. Immersion in thermal baths had been known to evoke diuresis since the mid 19th century. Immersion probably increased venous return to the heart and dilated the atria.

With the discovery of ANP by De Bold’s group (1981) it became important to know whether the diuresis which follows distension of the atria was due to secretion of ANP from the atria which circulated to the kidneys and evoked natriuresis.
THE BRAIN ANPERGIC NEURONS

The major brain peptides involved in the control of body fluid balance are angiotensin II (AII) and ANP. ANP, AII and osmoreceptors are located in circumventricular organs, such as the subfornical organ (SFO), the organum vasculosum lamina terminalis (OVLT), and in other brain structures involved in the regulation of body fluid and arterial blood pressure (Brody and Johnson, 1983). In 1985 Samson demonstrated the presence of ANP in extracts from various hypothalamic regions (Samson et al, 1985). Several laboratories have shown the presence of receptors for ANP and the presence of ANPergic neurons in the brain. They were located in the same regions of the CNS which contained ANG-II and were related to blood pressure regulation. The ANPergic neurons are localized in the region extending from the paraventricular nucleus rostrally to the SFO and ventrally to the OVLT, areas known to be involved in thirst. Their axons project caudally to the median eminence and neural lobe, terminating in proximity to the long or short portal vessels, respectively, so that the peptide could be released into the anterior pituitary and also into the general circulation (Palkovits et al, 1987; for review see Phillips 1987).

These findings suggested to us that ANP secreting neurons could be involved in control of natriuresis, as well as water and salt intake.

THE ANPERGIC NEURONS IN WATER AND SALT INTAKE

We performed experiments showing that injection of ANP into the 3V induces a dose-related block of dehydration, carbachol- and ANGII-induced drinking. This inhibitory response was present at doses of intraventricularly injected ANGII ranging from 4.8 to 25 pmol (Antunes-Rodrigues et al, 1985). Masotto and Negro-Vilar (1985) and Nakamura et al (1986) described similar results.

Since ANGII also increases salt intake, we speculated that ANP should have the opposite effect and could inhibit saline intake when injected into the 3V of conscious salt-depleted rats. The animals were salt-depleted by 4 days of salt restriction followed by intraperitoneal dialysis with 5% glucose solution to produce hyponatraemia. Intake of 1.5% sodium chloride solution was reduced dramatically by a minimal effective dose of 0.2 nmol of ANP (Antunes-Rodrigues et al, 1986), five times lower than the dose that reduces water intake (Antunes-Rodrigues et al, 1985).

To evaluate the physiological significance of various peptides in the control of water intake, Franci et al (1983) injected into the AV3V region antibodies against peptides thought to be involved in water
intake. Antiserum against AII when injected into the AV3V of overnight water-deprived rats decreased water intake significantly if the water was offered 1 h after the ICV injections. However, when the water was offered 3 h after injection of antiserum, drinking was completely abolished. Control injections of normal rabbit serum were ineffective. These results indicate that AII is required to induce the drinking that follows dehydration.

**BRAIN ANPERGIC NEURONAL SYSTEM AND RELEASE OF ANP**

The role of the central nervous system (CNS) in the control of renal sodium excretion has been demonstrated by several studies (Anderson, 1977; Anderson et al, 1966; Brody and Johnson, 1983; Dorn and Porter, 1970; Dorn et al, 1970; Fitts et al, 1985). We evaluated the possible role of the brain ANPerergic system in evoking the changes in renal sodium excretion that followed stimulation (osmotic, cholinergic or adrenergic) or lesions of the AV3V, a region that has been implicated in the control of sodium excretion (Brody and Johnson, 1983).

Injection of carbachol into the third ventricle produced natriuresis as expected on the basis of our earlier experiments, accompanied by a dramatic increase in the plasma ANP concentration and an increase in ANP content in the medial basal hypothalamus (MBH), the neurohypophysis (NH) and particularly in the anterior pituitary gland (AP), but without alterations in the content of ANP in the lungs or the right or left atrium (Baldissera et al, 1989). These data suggest that the natriuresis resulting from this stimulation is brought about, at least in part, by the release of ANP from the brain.

Conversely, there was a dramatic decline in plasma ANP at both 24 and 120 h after the AV3V lesions had been placed. There was also a dramatic decline in the ANP content in the MBH, NH, AP, choroid plexus and olfactory bulbs (OB). These reductions persisted or became more evident at 120 h, except in the OB, where the decline was no longer significant. ANP content did not change in the right atrium at 24 h after lesions, but there was a significant increase at 120 h, probably caused by decreased release of the peptide in the face of increased synthesis (Antunes-Rodrigues et al, 1991).

The dramatic increase in plasma ANP after cholinergic stimulation of the AV3V that was accompanied by a marked elevation in content of the peptide in the basal hypothalamus and the neuro- and adenohypophysis, suggested that the natriuresis resulting from this
stimulation is brought about, at least in part, by the release of ANP from the brain. Similarly, there was a decline in plasma ANP concentration and content in the MBH, OB, choroid plexus, NH and AP which was accompanied by decreased sodium excretion following AV3V lesions that may be explained, at least in part, by decreased release of ANP from the brain. The increase in plasma concentration that follows cholinergic stimulation of the AV3V region is fully mediated by a central site of action, via muscarinic receptors, and the receptor subtype involved in the peptide release is the M1 receptor (Massi et al., 1991). We also observed similar increases in plasma ANP after osmotic or adrenergic stimulation of the AV3V region.

However, in view of the much larger quantities of the peptide stored in the atria (more than 1000-fold greater), it is probable that changes in atrial release are largely responsible for the alterations in plasma ANP observed after the stimulation or ablation of the AV3V region (Baldissera et al., 1989).

**ROLE OF THE ANPERGIC NEURONS IN VOLUME EXPANSION-INDUCED RELEASE OF ANP**

It was well known at this time that expansion of the blood volume (BVE) causes a release of ANP that plays an important role in the induction of the subsequent natriuresis and diuresis. Since atrial stretch can cause ANP release, this was believed to be the mechanism of ANP release induced by volume expansion. Since stimulation of the AV3V region induced a rapid rise in plasma ANP, whereas lesions of the AV3V were followed by a marked decrease in plasma concentrations of the peptide, we hypothesized that release of ANP from the brain ANP neuronal system might be important in the control of plasma ANP. To test this hypothesis, we destroyed the AV3V, the site of the perikarya, in male rats by electrolytic lesions. Other lesions were made in the median eminence and posterior pituitary, sites of termination of the axons of these neurons, and also hypophysectomy was performed in other animals to evaluate the role of anterior pituitary hormones (Antunes-Rodrigues et al., 1991).

In conscious, freely moving rats, volume expansion and stimulation of postulated sodium receptors in the hypothalamus were induced by intra-atrial injection of hypertonic saline (0.3M NaCl, 2ml/100 g body weight, over 1 min). Volume expansion was also induced with the same volume of isotonic saline or glucose. In the sham-operated rats, BVE with hypertonic or isotonic solution induced equivalent rapid increases in plasma ANP that peaked at 5 min and returned to control values by 15 min. These lesions (AV3V, ME, NH or hypox)
caused a decrease in the basal plasma levels of ANP in comparison with those of the sham-operated rats. Each type of lesion resulted in a marked decrease in the response to BVE on testing 1 or 5 days after lesions were made. Because a common denominator of the lesions was the elimination of the brain ANP neuronal system, these results suggest that brain ANP plays a role in the mediation of the release of ANP that occurs with volume expansion (Antunes-Rodrigues et al., 1991). Thus, the results at this point indicated the crucial participation of the CNS in the response of ANP and natriuresis to volume expansion.

We considered the possibility that the baroreceptors, when they were stretched by BVE, would activate the brain ANP neurons, which would then produce the release of ANP and the ensuing natriuresis. We therefore performed experiments to determine the role of baroreceptors in the increase in plasma ANP from BVE induced by i.v. (intra-atrial) injection of hypertonic saline solution (0.3 M NaCl, 2 ml/100 g body weight, over 1 min) in conscious freely moving rats. In sham-operated rats BVE induced a rapid increase in plasma ANP as before. The concentration peaked at 5 min and was still elevated at 15 min after saline injection.

One week after deafferentation of the carotid-aortic baroreceptors, basal plasma ANP concentrations were significantly decreased compared with values in sham-operated rats. Plasma ANP levels at 5 min after BVE in the deafferented rats were greatly reduced (Morris and Alexander, 1988; Antunes-Rodrigues et al., 1992). Unilateral right vagotomy reduced resting levels of plasma ANP but not the response to BVE. The resting levels and BVE response were normal in bilaterally vagotomized rats. In the rats that had undergone renal deafferentation the resting levels were normal but the response to BVE was significantly reduced, presumably because of elimination of input from renal baroreceptors (Davis and Freeman, 1976). These data indicate that afferent impulses via the right vagus nerve may be important under basal conditions, but they are not required for the ANP release induced by BVE. In contrast, baroreceptor impulses from the carotid-aortic sinus regions and the kidney are important pathways involved in the neuroendocrine control of ANP release.

In another group of experiments we assessed the requirement of the brain ANP neuronal system for BVE-induced ANP release by injecting antiserum directed against ANP into the 3V prior to inducing BVE. The antiserum had no effect on resting levels of ANP; however, it partially blocked the increase in ANP and natriuresis which
followed BVE (Antunes-Rodrigues et al, 1993 a). Other experiments in sheep had given similar results (Charles et al, 1993).

Finally, we investigated whether cholinergic and adrenergic synapses within the brain are essential to the response of ANP to BVE by injecting the receptor-blocking agents into the third ventricle 30 min before the BVE. These blockers had no effect on resting levels of hormone; however, a significant blockade of the response was induced by prior injection of the muscarinic cholinergic receptor blocker atropine sulphate (5 nmol in 2 µl 0.9% NaCl). Similar results were obtained with micro-injection of the alpha receptor blocker phentolamine (5 nmol in 2 µl saline) (Antunes-Rodrigues et al, 1993 b).

The putative pathway of activation of ANP release and natriuresis via BVE involves distension of baroreceptors in the right atria, carotid and aortic sinususes and in the kidney, which alters their afferent input to the brain stem in the nucleus tractus solitarius (NTS). Impulses generated from NTS presumably activate the locus coerules (LC), a major source of noradrenergic axons to the hypothalamus. The axons of these noradrenergic neurons projecting to the AV3V region activate the cholinergic interneurons there, which in turn stimulate the hypothalamic ANPergic neurons. These neurons would activate efferent neurohumoral or neuronal pathways, which induce the release of ANP from the brain or the atria (Antunes-Rodrigues et al, 1992).

An afferent pathway to the AV3V region via serotonergic (5-HTergic) neurons with cell bodies in the raphé nuclei has been demonstrated. Earlier studies had shown that injection of 5-HT agonists into the third or lateral ventricle influences renal electrolyte and water excretion. To determine the effect of loss of 5-HT input into the AV3V region, bilateral lesions were made in the dorsal raphé nuclei (DRN), a major source of 5-HT neurons that project to the AV3V region, and in other rats, depletion of 5-HT from the 5-HTergic neurons was accomplished by systemic administration of parachlorophenylalanine (PCPA), an amino acid that competes with tryptophan, the substrate of tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of indolamines.

The DRN lesion produced a diabetes insipidus-like state in which there was a highly significant increase in water intake and urine volume beginning on the first day after the lesions were made, reaching a peak of water intake at 3 days, followed by a gradual decline in water intake and urine volume to control levels a week after the lesions were made. During the diuresis, the osmolality of the urine was
dramatically reduced, as were sodium excretion and plasma ANP levels. The rats with PCPA lesions showed a similar reduction in natriuresis to that in rats with DRN lesions. The plasma ANP levels were significantly lowered, which led us to conclude that the serotonergic system has a tonic stimulatory drive on the release of ANP. We also observed that the response of plasma ANP to BVE was significantly reduced.

These data suggest that there is a tonic stimulatory input from the 5-HT neurons to the AV3V region, the removal of which results in an increase of AII release, causing increase in the water intake and decreased ANP release into the systemic circulation and sodium retention. The raphé nuclei may be stimulated by afferent inputs from the baroreceptors via the NTS and may be involved in the BVE-induced ANP release.

**WHAT ARE THE EFFERENT PATHWAYS INVOLVED IN THE BRAIN CONTROL OF ANP RELEASE?**

The efferent pathway to the heart could be completely neural. However, it cannot be cholinergic since bilateral section of both vagi does not block the response to BVE. It is unlikely that it is a sympathetic efferent pathway, since BVE by elevating blood pressure should if anything diminish sympathetic outflow. Thus it seems to us that an unknown neuroendocrine efferent pathway reaching the atria may release ANP. It could be peptidergic in nature by release of one or more other brain peptides induced by the ANP neurons such as endothelin (Antunes-Rodrigues et al, 1993c), alpha-MSH, AVP or OT (Haanwinckel et al, 1995).

Some of the ANP neurons terminate in the median eminence and neural lobe of the hypophysis. It is probable that their activation leads to release of the peptide into the vasculature draining the median eminence or the neural lobe. Since the quantity of the peptide is 1000-fold less in these structures than in the atria (Baldissera et al, 1989), we believe that ANP released from the brain plays a minor role in the response. Rather, we would suggest that these ANPergic neurons activate descending pathways, which then activate efferent pathways to the heart with consequent release of ANP from the cardiac myocytes.

**OXYTOCIN (OT) AND VASOPRESSIN (AVP)**

Both vasopressin and oxytocin are stored in large amounts in the neural lobe of the pituitary and were the substances considered most likely to be released into the venous drainage of the neurohypophysis by
BVE. These peptides could circulate to the atria and release ANP. Decreased blood volume, such as occurs with haemorrhage, stimulates AVP release via baroreceptor input to the brain stem. Therefore, one would predict that BVE would not elevate, and perhaps would suppress, AVP release. Therefore, we hypothesized that hypothalamic ANP neurons cause release of OT, which triggers the release of ANP from the atria. Indeed, we found that isotonic BVE induced ANP release, accompanied by OT release and decreased AVP secretion, and was followed by natriuresis. Our data supported the hypothesis that volume expansion-induced ANP release and natriuresis is caused by release of OT, which stimulates ANP release from the right atrium, which in turn induces natriuresis.

In water-loaded rats undergoing diuresis, i.p. or i.v. oxytocin induced a significant (P<0.01), dose-related increase in sodium and potassium excretion, as well as in urine osmolality. A dose-response relationship was evident by 20 min with sodium and by 40 min with potassium. The natriuresis was maximal at 40 min. The kaliuretic response was smaller than the natriuretic response. There was also a dose-related decrease in urine volume which was maximal at 40 min with the 1 nmole (1.0 μg) dose and at 80 min for the 10.0 nmole dose. Control injections of isotonic saline did not induce any significant changes in the values for urine volume, sodium and potassium excretion and urine osmolality.

Effect of OT on plasma ANP
In hydrated rats there was a dose-related increase (P<0.001) in plasma ANP concentrations at 5 min after i.v. injection of OT. The minimally effective dose was 1 nmole. The plasma ANP levels remained elevated for 15 min only with the highest dose (10.0 nmole) of OT. No changes in plasma ANP levels were observed in the control rats (Haanwinckel et al, 1995).

Effect of BVE on plasma OT concentration
BVE is a well-known stimulus for ANP release. In this experiment we wished to determine the effect of BVE on release of OT, the putative stimulator of ANP release. BVE with either isotonic or hypertonic saline induced a rapid increase in plasma OT concentration that was highly significant at 5 min. Although the initial release of OT was higher with the hypertonic saline solution, it was not significantly greater than that caused by isotonic saline. By 15 min OT levels returned to the basal levels in the case of isotonic saline but were still significantly elevated after hypertonic
saline-induced BVE. Isotonic or hypertonic saline-induced BVE were associated with equivalent increases in plasma ANP with a similar time-course as that found for plasma OT concentration observed in this experiment. The magnitude of the release of OT following BVE was even greater than that which followed suckling in lactating rats, the classical stimulus for OT release. The OT release by suckling also was associated with an increase in plasma ANP.

Our hypothesis is that BVE induces stretching of the sino-aortic and renal baroreceptors, which increases afferent baroreceptor input to the brain stem in the NTS. These neurons send projections to the LC, which activates the noradrenergic neurons located there and to the DRN, which activate the serotonergic neurons located there. These neurons send axons to the AV3V region and stimulate cholinergic interneurons, which activate the ANPergic neurons. These neurons stimulate the release of OT from the neurohypophysis. The released OT circulates to the atria and triggers ANP release, which then induces the natriuresis (Figure 1).

Others have shown receptors for OT, ANP and AVP in the kidney, so part of the natriuretic response may be due to the direct action of oxytocin on the kidneys. Studies in our laboratory with AVP V2-receptor blockers have shown that they block the antidiuretic but not the natriuretic OT-induced response. Since our previous experiments showed that neural lobectomy, which would largely abolish the release of OT and AVP following BVE, nearly completely blocked the BVE-induced release of ANP, we believe that OT is the major mediator of CNS-induced release of ANP.

The evidence is clear that direct release of ANP can occur by atrial stretch, but this does not contribute appreciably to the response to blood volume expansion under our conditions since it can be almost completely blocked by denervation of baroreceptors or lesions of the AV3V and caudal projections of ANP, oxytocinergic and vasopressinergic neurons to the neurohypophysis. Another possibility which should be carefully evaluated is that not only does OT causes a release of ANP, which thereby mediates the natriuresis, but OT also acts independently at the kidney level to induce natriuresis and there may be synergistic effects at kidney level between the natriuretic actions of ANP, OT and even AVP. The function of OT in males is largely unknown. Therefore its principal function in both sexes may be in the control of sodium and potassium excretion and thereby body fluid homoeostasis.
Direct chrono- and inotropic effects of OT in the atria coupled with release of ANP

Experiments were undertaken to investigate the direct effects of OT on the atria. Besides the well-known effects of OT on the uterus and mammary glands, it was reported that OT also decreased blood pressure. In a series of experiments right and left atria were mounted in tissue baths and the effects of OT to modify the force or rate of spontaneously beating right atria were examined by cumulative addition. At a concentration of $3 \times 10^{-7}$ to $10^{-5}$ M OT decreased the force and rate of atria contraction (Favaretto et al, 1997). Since the concentration of ANP in the atria is much higher than in the vascular system our results suggest that ANP may also act directly on the heart. The effects of OT on the rate and force of contraction of the heart were independent of cholinergic vagal inhibition since these effects were not blocked by previously administered atropine.

When ANP was applied directly to the atria it exerted a more potent inhibitory action on the rate and force of contraction than OT. Since these effects were mimicked by 8-monobutyryl cGMP, it appears that ANP acts on the heart through the stimulation of its membrane-bound guanylate cyclase receptors, which convert GTP to cGMP. A relatively high concentration of the analog ($10^{-2}$ M) was required, probably due to its low permeability through the cell membranes. We hypothesize that the inhibitory action of OT on atrial function is mediated by ANP released directly from the atria by combination of OT with its receptors on the atrial myocytes. Oxytocin was incubated *in vitro* with rat atria. It induced a highly significant, dose-related increase in the ANP release from the incubated atrial quarters. Further support for this hypothesis comes from additional experiments in which basal ANP release was blocked by a 1000-fold lesser concentration of an oxytocin antagonist.

Mechanism of action of ANP and OT at kidney level

In recent experiments we tested the possibility of NO participation in OT- and ANP-induced natriuresis. Our hypothesis is that oxytocin (OT) causes natriuresis by activation of renal nitric oxide synthase (NOS) that releases NO followed by cyclic guanosine monophosphate (cGMP) that mediates the natriuresis. To test this hypothesis, an inhibitor of NOS, L-nitroarginine methyl ester (NAME) was injected into male rats. Blockade of NO release by NAME had no effect on natriuresis induced by atrial natriuretic peptide (ANP). This natriuresis is presumably caused by cGMP since ANP also activates guanylyl cyclase that synthesizes cGMP from guanosine triphosphate (GTP).
The 18-fold increase in sodium (Na\textsuperscript{+}) excretion induced by OT (1 μg) was accompanied by an increase in urinary cGMP and, preceded by 20 min, a 20-fold increase in NO\textsubscript{3} excretion. NAME almost completely inhibited OT-induced natriuresis and increased NO\textsubscript{3} excretion; however, when the dose of OT was increased 10-fold, a dose that markedly increases plasma ANP concentrations, NAME only partly inhibited the natriuresis. We conclude that the natriuretic action of OT is caused by a dual action: generation of NO leading to increased cGMP and at higher dose release of ANP that also releases cGMP. OT-induced natriuresis is mainly caused by decreased tubular Na\textsuperscript{+} reabsorption mediated by cGMP. In contrast to ANP that releases cGMP in the renal vessels as well as in the tubules, OT acts on its receptors on NOergic cells demonstrated in the macula densa and proximal tubules to release cGMP that closes Na\textsuperscript{+} channels. Both ANP and OT-induced kaliuresis also appears to be mediated by cGMP. We conclude that cGMP mediates natriuresis and kaliuresis induced by both ANP and OT (Soares et al, 1998).

Our hypothesis is that oxytocin (OT) causes natriuresis by activation of renal nitric oxide synthase (NOS) localized mainly in the macula densa and in the renal proximal tubules that releases NO followed by cyclic guanosine monophosphate (cGMP) that mediates the natriuresis. In these structures we and others have demonstrated the presence of nNOS (Bachman et al, 1995; Soares et al, 1998). A specific inhibitor of nNOS, 7-nitroindazole, was injected i.p. into male rats. The OT-induced natriuresis was only partly inhibited by the nNOS inhibitor. These data suggest that OT induces natriuresis at kidney level by the activation of cGMP production, but since this effect is only partially blocked by the nNOS inhibitor, the principal form of NOS present in the macula densa and in the proximal tubular cells, the residual natriuresis may be caused by the ANP released by OT that also releases cGMP.

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FIGURE 1
ANP neuronal control of ANP release

H₂O intake → Achn → ANPn → On → 5HTn → NTS → LC → NEn

OC → A → PV → AL → NTS → IC → CS → AS → A → rBr → RA → V → K

↑ANP, ↑OXY

Saline Infusion → RA