TRANSMITTER INTERACTIONS IN THE CENTRAL CHOLINERGIC CONTROL OF BLOOD PRESSURE REGULATION

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KEY WORDS

Physostigmine; transmitter interactions; blood pressure

SUMMARY

There are at least five mechanisms by which the central nervous system regulates neural and humoral systems that control the blood pressure (BP). Particular attention has been paid to central cholinergic-adrenergic interactions in the regulation of BP. Physostigmine and other anticholinesterases which penetrate the blood-brain barrier, both carbamates and organophosphates, produce an increase of BP. This effect can be abolished by atropine, but not by methylatropine. The available evidence indicates that physostigmine and other AChE inhibitors initially

produce an activation of central muscarinic receptors, which subsequently leads to an increase of the peripheral adrenergic activity. The hypertensive response to physostigmine is possible only if a functionally competent ChE is present in the brain. This effect of physostigmine is regularly associated with a dose-related increase in the neural activity in the preganglionic fibers of the cervical sympathetic nerve. BP rise after physostigmine is significantly less in immunosympathectomized animals and almost completely abolished after chemical sympathectomy.

Physostigmine significantly increased the plasma concentration of catecholamines. After electrocoagulation of the *locus coeruleus*, not only did a significant decrease occur in the basic level of noradrenaline in plasma, but there was also a strong depression of the noradrenaline plasma response to physostigmine and immobilization. Physostigmine increased lipolysis and glycogenolysis, whereas neostigmine did not produce any change.

Several directly acting cholinergic agonists alter the functions of the cardiovascular system when injected directly into the cerebral ventricular system, or directly into various brain regions. The most probable sites of action of AChE inhibitors and directly acting cholinergic agonists are the *locus coeruleus*, the *nucleus tractus solitarii* and the rostral ventrolateral medulla (RVLM). The primary activation of the cholinergic synapse is believed to take place in RVLM.

Met-enkephalin, Leu-enkephalin and β-endorphin, when applied exogenously, depress or even abolish the hypertensive effect of physostigmine. The same type of response was obtained after application of substances which inhibit the enkephalin-degrading enzymes (bestatin, phosphoramidon). Thus, the exogenous or endogenous enkephalins activate the opioid receptors in the brain and at the same time produce a depression of the cholinergic-adrenergic interaction in the central nervous system, which is a prerequisite for the hypertensive response to physostigmine.

The functional role of the central cholinergic mechanisms in BP control under physiological conditions has not been established with certainty. These mechanisms might have a more significant role under pathological or homeostatic disturbances. For example, physostigmine showed a life-saving effect in acute hypovolemic shock in rabbits.

I. INTRODUCTION

The role of the central nervous system in neural and humoral control of the cardiovascular system, particularly of blood pressure (BP), has been firmly established. There are at least five mechanisms by which the central nervous system affects blood pressure /88/. There are strong indications that the central cholinergic muscarinic mechanisms are linked to arterial blood pressure regulation /33,110,111/. There is a compelling association of brain acetylcholine with hypertension /110/. Physostigmine, as well as the other acetylcholinesterase (AChE) inhibitors which penetrate the blood-brain barrier, produces hypertensive effects associated with augmented sympathetic activity /4,6,7,33,111/. The action of anticholinesterase agents is produced by inhibition of brain acetylcholinesterase.

The pressor response to physostigmine and other AChE inhibitors is due to a central cholinergic-adrenergic interaction. This interaction takes place in structures located in the medulla and/or pons, most probably in the *nucleus tractus solitarii*, the *locus coeruleus* and the rostral ventrolateral medulla (RVLM) /41,43,44/. The primary activation of the cholinergic synapse takes place in the rostral ventrolateral medulla /104-107/. The pressor effects of AChE inhibitors are considered to be, at least partly, mediated through stimulation of the M_2 muscarinic receptor subtype /46,108/. These findings imply that the functional interaction of brainstem acetylcholine with muscarinic M_2 receptors is involved in the tonic regulation of central sympathetic outflow and arterial blood pressure /108/. The role of this cholinergic-adrenergic interaction may be particularly important in pathological states and in disturbances of homeostatic mechanisms. It is suggested that changes in the central presynaptic cholinergic mechanisms and/or changes in muscarinic receptor function may represent the main brain neurochemical abnormalities which may be causally related to the development of hypertension in spontaneously hypertensive rats /108,112/.

The central cholinergic-adrenergic interaction may be very important in hemorrhagic shock /86,87/. Physostigmine produces a life-saving effect in this type of shock /87/.

The congeners of oxotremorine behave as centrally selective antimuscarinics at doses which have low peripheral antimuscarinic activity. The unique pharmacological profile of these substances makes them novel probes to study central cholinergic muscarinic

mechanisms participating in cardiovascular regulation, particularly in hypertension. It is possible that some of these substances might become useful drugs in the treatment of high blood pressure /108/.

It has been a challenge to us to collect and present all these data about transmitter interactions in the central cholinergic control of blood pressure. This review covers the work not only from our own laboratory, but also from laboratories all over the world. Due to space and time limitations, it has proved impossible to mention all the work that has been carried out.

II. CENTRAL NERVOUS SYSTEM MECHANISMS FOR REGULATING THE NEURAL AND HUMORAL SYSTEMS THAT CONTROL BLOOD PRESSURE

The traditional focus of research has been on the cardiovascular control systems that are critical for the tonic maintenance of arterial pressure and sympathetic nervous system activity whose functional neuroanatomical basis resides in the *medulla oblongata*. Recent studies have provided new evidence that organization of central cardiovascular control is far more complex. Numerous interactions between the brain stem and rostral portions of the neuroaxis have been demonstrated that involve not only reciprocal neuronal connections, but a central effect of humoral factors as well /88/.

2.1 Baroreflex mechanisms

Stretch receptors in the major arteries, cardiac atria and ventricles detect changes in arterial pressure, central venous pressure and left ventricular pressure, respectively. The afferents from these receptors, known to project through the ninth and tenth cranial nerves, terminate in the region of the *nucleus tractus solitarii* (NTS) /89/.

Neurochemical and immunochemical investigations have identified numerous aminergic and peptidergic transmitters within the NTS /90/. These include acetylcholine, 5-hydroxytryptamine, noradrenaline, vasopressin and oxytocin. All these transmitters appear to arise from other central structures and not from baroreceptor terminals themselves.

Glutamate and **substance P** are prominent candidates for a

primary neurotransmitter role in the baroreceptor reflex. Local microinjection of glutamate into the NTS mimics the baroreceptor reflex, whereas injection of glutamate antagonist (glutamate diethylester) abolishes the baroreceptor reflex and produces an effect similar to deafferentation /91,92/. These and other data are consistent with the hypothesis that glutamate may be the primary neurotransmitter of the baroreceptor reflex arc. Substance P has been localized to baroreceptor terminals in the NTS. It is a candidate for the sensory neurotransmitter and for the baroreceptor mechanisms.

Despite extensive knowledge about the basic function of the baroreflex in buffering against acute changes in arterial blood pressure, the role of these receptor mechanisms in the chronic regulation of arterial pressure remains controversial.

2.2 **Origins of vasomotor activity**

Earlier notions that a specific, well-circumscribed center accounts for tonic sympathetic activity are widely documented /88/. In recent years a new concept has been proposed about a group or groups of neurons as the origin of vasomotor tonic activity. Such cells are present in the rostral ventrolateral medulla (RVLM) that incorporates the adrenaline-containing C_1 cell group, noradrenergic projections from the A_5 pontine cell group, tryptaminergic projections from the raphe system and projections from the NTS /90/. Interruption of neuronal transmission in the RVLM by chemical or electrolytic lesion reduced arterial pressure to near spinal levels /93,94/.

Lesions of dorsomedial medulla in the region of the *nucleus reticularis parvocellularis* also produce a lowering of arterial blood pressure /94/, suggesting that both areas have the potential to exert tonic control over vasomotor function.

The bilateral microinjection of tetrodotoxin, some GABA analogs or clonidine into the lateral reticular nucleus produces hypotension /109/.

Given the large number of putative excitatory and inhibitory neurotransmitters, including vasopressin, oxytocin, adrenaline, noradrenaline, substance P, enkephalins and 5-hydroxytryptamine, that connect synaptically with preganglionic sympathetic cell bodies, the specific neurotransmitter mechanism(s) and sites of origin of central projections to the spinal cord required for tonic

sympathetic activity, remain largely unknown /88/.

2.3 Functional interactions between forebrain and brain stem

As already pointed out, all visceral afferents project almost exclusively to the NTS. From this primary termination site there is an extensive radiation of secondary and tertiary projections to the forebrain. Reciprocal connections between forebrain structures and the brain stem have also been described /90/. There are many examples of this reciprocal arrangement, e.g., catecholaminergic projections to the parvocellular paraventricular nucleus originate from the A_1 cell group of the ventrolateral medulla, from the A_2 region of the dorsomedial medulla and from the *locus coeruleus* /95/.

The forebrain structures, although perhaps not significant for the tonic maintenance of sympathetic discharge, are capable of strongly influencing the neurogenic and humoral control of arterial blood pressure. The neuroanatomical substrates for this role are interconnections between brain stem and forebrain.

2.4 Neural-humoral coupling

Certain substances carried in the blood can influence central neural and humoral regulation of the circulation by direct action on selected central structures. The circumventricular organs, which lack a blood-brain barrier, provide the sites through which humorally derived substances can penetrate into the brain and activate both neuronal mechanisms and the release of humoral factors, such as **vasopressin**. It is now appreciated that several of these circumventricular organs not only lie in close apposition to areas important for cardiovascular and autonomic control, but also send projections to other critical brain sites /96/.

Angiotensin has been widely studied with respect to a central mechanism of cardiovascular control. The receptive regions in the *area postrema* are activated by angiotensin carried in the bloodstream to increase sympathetic activity and the release of vasopressin /97/. Certain central pathways may employ angiotensin as a neurotransmitter /98/. The brain renin-angiotensin system may participate in cardiovascular, neuroendocrine and fluid-electrolyte homeostasis /99/.

2.5 Visceral and somatic afferents

Afferents originating from arterial and cardiopulmonary baroreceptors and from chemoreceptors are the major visceral sensory systems that control cardiovascular function on a moment-to-moment basis. Still, the afferents from other viscera can not only contribute significantly to physiological regulation of the cardiovascular system, but also play an important role in abnormalities of circulatory regulation, such as hypertension /88/.

The renal afferent system is of special interest. Sensory fibers from renal parenchyma project through dorsal root ganglia to the dorsal horn of the spinal cord /100/, whereas the secondary projections relay from the dorsal horn to the NTS. The renal sensory system is critical for the expression of renal and other forms of experimental hypertension /101/. Activation of renal afferents through central relays increases sympathetic nervous system activity and elevates arterial blood pressure /102/.

The contribution of somato-sensory afferents to cardiovascular control has received considerable attention, especially in cardiovascular adjustments associated with exercise /103/.

III. THE EFFECTS OF ANTICHOLINESTERASES ON BLOOD PRESSURE

According to classical textbooks, the cardiovascular actions of AChE inhibitors are complex. On the periphery they reflect both ganglionic and postganglionic effects of accumulated acetylcholine (ACh) in blood vessels and the heart. Some AChE inhibitors penetrate the blood-brain barrier and the effects of these substances are characterized by facilitation or stimulation at various sites, followed by inhibition or paralysis at higher concentrations /1/. This is further complicated by the finding that the vasodilation of vascular beds by cholinesterases is due to the action on muscarinic receptors located on endothelial cells of the vasculature. When these receptors are stimulated, the endothelial cells release the endothelium-derived relaxing factor (EDRF) that diffuses to the adjacent smooth muscle cells and causes them to relax /2,3/. Vasodilation may also be due to inhibition by

acetylcholine of noradrenaline release from adrenergic nerve endings.

It was discovered in 1955 that intravenous injection of physostigmine produced an increase of blood pressure in the rat under urethane anesthesia /4/. That was the starting point for investigation of the role of the central cholinergic-adrenergic interaction in the central cholinergic control of blood pressure. The same type of response to physostigmine was also found in the conscious rat /5/.

The other AChE inhibitors, including both carbamates and organophosphates, also increase the arterial BP in the rat, but also in the cat and the dog.

The hypertensive response to AChE inhibitors was observed only with those substances which penetrate the blood-brain barrier after intravenous injection /6-13/. The quaternary ammonium compounds, which do not penetrate the blood-brain barrier, do not produce a BP rise at all, or elicit only a small rise, after intravenous injection. In contrast to this, even these substances produce a BP rise if injected directly into the central nervous system /8,9,14,15/. The same effect was obtained if AChE inhibitors were injected directly into the cerebral ventricles, into the carotid or vertebral artery, or into the *cisterna magna*.

The hypertensive response to AChE inhibitors of the carbamate type is dose-dependent and can be obtained many times without any sign of tolerance or tachyphylaxis. In contrast, tachyphylaxis was regularly observed after intravenous injection of organophosphate AChE inhibitors /16,17/.

It was shown that even in man physostigmine produced a BP rise, together with an increase of the heart rate /18/. In another study, acute effects of physostigmine and neostigmine in man were compared /22/. Physostigmine – in contrast to neostigmine – caused statistically significant decreases in behavioral inhibition and negative effect. Similarly, physostigmine, to a significantly greater extent than neostigmine, caused systolic blood pressure rise and increase in pulse rate in the subjects. It was also found that physostigmine caused relatively greater increases in serum cortisol, serum prolactin, ACTH and beta-endorphin than neostigmine. Furthermore, physostigmine, but not neostigmine, dramatically increased serum adrenaline levels.

3.1 Activation of muscarinic receptors

The pressor responses to AChE inhibitors can be blocked by atropine, or by depleting the brain acetylcholine with hemicholinium /17/. The quaternary anticholinergics (e.g. methylatropine), that do not cross the blood-brain barrier, do not block the hypertensive response to physostigmine /12,19/.

In the hypertensive effect of physostigmine, both systolic and diastolic pressures are increased to the same extent, whereas pulse pressure is unaffected. The heart rate is only slightly increased, but the peripheral vascular resistance is significantly increased. During physostigmine hypertension the sinocarotid baroreflex is significantly potentiated, whereas the cardiac output remains unchanged. In practice, all these effects are almost identical to general sympathetic activation. Thus, the available evidence indicates that physostigmine and other AChE inhibitors reaching the central nervous system initially produce an activation of muscarinic receptors, which subsequently leads to an increase of the peripheral adrenergic activity /7/.

3.2 Dependence of blood pressure changes on cholinesterase (ChE) inhibition

The hypertensive response to physostigmine can be correlated with ChE inhibition in the whole brain. Both BP rise and ChE inhibition are dose-dependent. The tachyphylactic BP response to di-isopropyl-fluorophosphate (DFP) and paraoxon is most probably due to additive inhibition of brain ChE. This may also be the reason that in animals pretreated with large doses of DFP, physostigmine does not produce a BP rise any more, but rather a BP fall or no change. It is evident that the hypertensive response to physostigmine is possible only if a functionally competent ChE is present in the brain. These findings indicate that the phenomenon of peripheral adrenergic activation is preceded by activation of the central cholinergic mechanisms. Evidently, this cholinergic-adrenergic interaction is the basic mechanism for BP rise after injection of ChE inhibitors /16/.

3.3 Preganglionic sympathetic activity

Physostigmine regularly produced a significant and dose-related increase in the neuronal activity in the preganglionic fibers of the cervical sympathetic nerve of the rat. Good correlation was found between the hypertensive response to physostigmine and the

increase of neuronal activity in the preganglionic fibers of the cervical sympathetic /20/. Atropine completely blocked the increased activity in the cervical sympathetic. In contrast to this, pretreatment with methylatropine, hexamethonium or phentolamine did not alter the increased neuronal activity produced by physostigmine. Once again, these experiments strongly suggest that the increased adrenergic activity at the periphery is preceded by activation of the cholinergic processes in the brain /10,20/. Similarly, paraoxon facilitates the preganglionic action potentials in the rat /21/.

3.4 Immunosympathectomy and chemical sympathectomy

Immunosympathectomy was produced in the rat by pretreatment with an anti-Nerve-Growth-Factor (NGF) serum, whereas chemical sympathectomy was produced by pretreatment with 6-hydroxydopamine. In immunosympathectomized animals, physostigmine produced a significantly lower BP rise than in the control animals. Immunosympathectomy caused serious damage to the sympathetic ganglia and by this mechanism produced a significant decrease in the tonic sympathetic influence. Immunosympathectomy produced not only a decreased response to physostigmine on BP, but also an increase in the concentration of glycogen in the liver. It was also found that in immunosympathectomized animals physostigmine produced a significantly smaller glycogenolytic effect than in the control /23/.

Chemical sympathectomy almost completely abolished the hypertensive response to physostigmine. A complete block of the hypertensive response to tyramine was also obtained. Among research workers in this field, this finding has been taken as a crucial proof of functional elimination of sympathetic activity /17/.

IV. THE EFFECT OF PHYSOSTIGMINE ON CATECHOLAMINE METABOLISM

Physostigmine produced a dose-dependent reduction in the noradrenaline content of the brain stem and hypothalamus /24/. This effect was correlated in time with the BP rise produced by intravenous injection of physostigmine. Both atropine and propranolol easily and completely blocked the effect of physo-

stigmine on catecholamine metabolism. It is of particular interest that physostigmine, in parallel with an increase of blood pressure at the periphery, simultaneously produced an increase in synthesis of noradrenaline in the aforementioned brain areas.

4.1 The effect of physostigmine on plasma catecholamines

The concentrations of noradrenaline and adrenaline in plasma were significantly increased by intraperitoneal injection of physostigmine. The plasma levels of catecholamines reached the maximum 15 minutes after injection of physostigmine. This roughly corresponds to the time course and the peak of the hypertensive response to physostigmine in the rat /25/. Immobilization stress has been known to produce a highly significant increase in plasma catecholamines /26/. The stressogenic effect of forced immobilization was significantly potentiated by pretreatment with physostigmine /25/.

After electrocoagulation of the *locus coeruleus*, a significant decrease occurred not only in the basic level of noradrenaline, but also there was a strong depression of the noradrenaline plasma response to physostigmine and immobilization /27/. The lesioned animals still respond to physostigmine and immobilization, but to a significantly lower degree. Most probably, this still remaining central noradrenergic effect after electrocoagulation of the *locus coeruleus* might be due to an activation of noradrenergic neurons outside the *locus coeruleus*, particularly the lateral tegmental noradrenergic neurons. These results are taken to indicate that the *locus coeruleus* might be of fundamental importance for regulating the noradrenergic status at the periphery, both at rest and in stress situations /27/.

4.2 The effect of physostigmine on glycogenolysis and lipolysis

The adrenergic nervous system has been known to participate in mobilization of the metabolic fuel. Catecholamines have also been known to activate lipolysis and glycogenolysis /28/. If the hypertensive response to physostigmine is presumed to be due to a cholinergically-mediated general sympathetic activation, then this effect might be expected to affect glycogen and fat metabolism. In fact, it was found that physostigmine increased lipolysis /29/ and

glycogenolysis /30,31/. Contrary to physostigmine, the intravenous injection of neostigmine did not produce any change of BP and at the same time did not affect glycogen and fat metabolism.

The glycogenolytic and lipolytic effects of physostigmine can be blocked by the centrally acting antihypertensive agent mebutamate, by pretreatment with reserpine, or with guanethidine. This effect of physostigmine can also be blocked by propranolol, a finding indicating the implication of beta-adrenoceptors in glycogenolysis and lipolysis.

The action of physostigmine on glycogen and fat stores is accomplished via the mediation of the nervous system, because physostigmine does not produce glycogenolysis *in vitro*. It was therefore concluded that, similar to the BP rise, the metabolic effects of physostigmine are evoked by a central cholinergic activation of the sympathetic outflow.

4.3 The effect of physostigmine on thermoregulation

The intravenous injection of physostigmine in rats kept under ordinary laboratory conditions regularly produces a dose-dependent hypothermia /32/. This effect of physostigmine can be blocked by atropine, indicating the involvement of the central muscarinic receptors. In contrast to this, the hypothermic effect of physostigmine is unaffected by methylatropine. The hypothermic response to physostigmine is associated with a dose-dependent decrease in oxygen consumption. It should be pointed out that physostigmine produced hypothermia even at an ambient temperature close to thermal neutrality. These data indicate that physostigmine produces a central cholinergic activation, thereby leading to an inhibition of thermogenesis.

V. THE EFFECT OF DIRECTLY ACTING CHOLINERGIC AGENTS

The basic mechanism of action of physostigmine, as well as of all the other AChE inhibitors, is the accumulation of endogenous acetylcholine. If the AChE inhibitors act through liberation of acetylcholine, then it may be expected that other directly acting cholinergic substances produce similar effects on BP.

5.1 Directly acting cholinergic agonists

Several cholinergic agonists have been shown to alter the functions of the cardiovascular system when injected directly into the cerebral ventricular system, or directly into various brain regions of the rat /14,33/.

It was found that acetylcholine, injected intracysternally or into the lateral cerebral ventricles of the rat, produced an immediate rise of BP, which it was possible to abolish by atropine or by spinal cord transection /34,35/. Pretreatment of the animals with physostigmine changes this response to acetylcholine into a short-lasting depressor response, with or without a secondary rise of BP /36/. An extensive study of cardiovascular responses to centrally administered acetylcholine in rats revealed that both muscarinic receptors mediating the pressor response and the muscarinic receptors mediating the depressor response do exist at the sensitive sites of the brain, but not in an equal proportion. The muscarinic cholinoceptors responsible for the pressor effect of acetylcholine are probably more sensitive and more competent than the cholinoceptors for the depressor effect of acetylcholine, but the latter are present in a markedly greater number. Therefore, acetylcholine most probably participates in the physiological BP control in the rat, both as an excitatory and as an inhibitory regulator /37,38/.

5.2 Implications of M_1 muscarinic receptors

It has been found that infusion of pilocarpine, acetylcholine, bradykinin and the selective M_1 muscarinic agonist McNeil-A-343 into the lateral septal area of rats produces a dose-dependent increase of arterial BP and heart rate /39/. This effect is associated with an increased sympathetic outflow /40/. It is suggested that the action of bradykinin involves the release of acetylcholine. Carbamylcholine, an essentially M_2 muscarinic agonist, causes a rise in arterial BP when injected into the anterior lateral ventricles, but it does not produce any cardiovascular effects when infused into the lateral septal area. Chronic treatment with atropine induced supersensitivity to the muscarinic agonists and a significant increase in the number of muscarinic receptors.

Pirenzepine, an M_1 muscarinic blocking agent, completely inhibited the cardiovascular effects of both muscarinic agonists

(acetylcholine, pilocarpine) and of bradykinin. These radio-ligand binding experiments suggest that the M_1 subtype of muscarinic receptor is present in the lateral septal area and that it plays a central role in the cardiovascular effects of cholinergic agonists infused into this area /39/.

VI. SITE OF CENTRAL ACTION OF CHOLINERGIC AGENTS

It was observed that the BP rise after injection of physostigmine into the jugular vein is higher than after injection of this substance into the left or right carotid artery /41/. These and other data obtained after a detailed study suggest that in the rat the brain regions supplied by the vertebral arteries (e.g. medulla oblongata and pons) are of greater functional importance for the hypertensive response to physostigmine than the regions supplied by the common carotid artery (e.g. mesencephalon, diencephalon, cerebral cortex).

Injection of cholinergic agonists close to or directly into the hypothalamic areas produces cardiovascular changes. A rise of BP is consistently evoked after injection of these substances into the posterior hypothalamic nucleus and the ventromedial nucleus /14,15,42/.

6.1 The *locus coeruleus* and the plasma catecholamine response

Serial transections of brain suggest that regions in or caudal to the midbrain mediate the hypertensive response to physostigmine /43/. Microinjections of physostigmine into the *locus coeruleus* (pons) or into the *nucleus tractus solitarii* (medulla) have been shown to increase the arterial BP or to increase the firing rate of nervous structures /44/. It was therefore suggested that the most probable sites of the cholinergic-adrenergic interaction, responsible for the hypertensive response to physostigmine, are these two structures in the posterior region of the brain.

It has been found that the *locus coeruleus* might have a crucial role in regulation of the noradrenergic status at the periphery, both at rest and in stress situations. After electrocoagulation of the

locus, not only did a significant decrease occur in the basic plasma level of noradrenaline, but also there was a strong depression of the noradrenaline plasma response to physostigmine and immobilization stress /27/. The lesioned animals still responded to physostigmine and to immobilization, but to a significantly lower degree. As already mentioned, this remaining noradrenaline effect after electrocoagulation of the locus might be due to activation of noradrenergic neurons outside the *locus coeruleus*. The plasma level of adrenaline was not affected by electrocoagulation of the locus, but this procedure depressed the plasma adrenaline response to immobilization stress. All these results are in accordance with the well known fact that the *locus coeruleus* is one of two major clusterings of noradrenaline cell bodies in the central nervous system (caudal pontine grey matter). In the rat, the locus itself contains about 1500 neurons on each side of the brain /45/.

6.2 Muscarinic receptor agonists and the M_2-receptor subtype

The central pressor effects induced by muscarinic receptor agonists is considered to be, at least partly, mediated through stimulation of M_2 muscarinic receptor subtype in a cholinergic synapse in the rostroventral lateral medulla (RVLM) /46,104-107/. When peripheral muscarinic activity was blocked by administration of methylscopolamine, a dose-dependent hypertension was obtained following the injection of oxotremorine, arecoline and aceclidine, by both intraperitoneal and intracerebroventricular routes. On the other hand, the muscarinic receptor agonists pilocarpine, AF-30 and McN-A-343, considered as partially M_1-selective compounds, did not produce any effect on blood pressure. Moreover, the hypertension induced by oxotremorine was completely blocked by intracerebroventricular administration of the non-subtype-selective muscarinic receptor antagonist scopolamine, but was unaffected by the M_1-selective pirenzepine. All these findings indicate the important role of central M_2 muscarinic receptors in the central hypertensive action of muscarinic agonists /46/.

The congeners of oxotremorine exhibit profound selectivity as indicated by their ability to depress hypertension caused by stimulation of brain cholinergic neurons, at doses which minimally affect peripheral cholinergic function. In contrast to atropine and scopolamine, the oxotremorine analogs possessed selective central

muscarinic effects in blocking the hypertensive effect of physostigmine, but they were completely unable to antagonize the peripheral depressor response to acetylcholine /108/.

The central selectivity makes the family of oxotremorine analogs potentially useful probes for studying the central muscarinic mechanisms involved in cardiovascular regulation and hypertension and in future may be therapeutic agents in the treatment of hypertension.

6.3 The role of the *nucleus tractus solitarii*

It has already been established that the intermediate portion of the *nucleus tractus solitarii* receives baroreceptor and chemoreceptor afferents from the carotid sinus and aortic arch and is important in regulation of cardiovascular function /47/. It has also been demonstrated that this nucleus contains acetylcholine esterase /48/, choline acetyltransferase /49/, acetylcholine /50/ and muscarinic receptors /51/. All these data suggest that the cholinergic mechanisms in the nucleus may be involved in cardiovascular regulation, but their precise mode of action has not been identified /52/. The possible role of the *nucleus tractus solitarii* is supported by reports that microinjections of non-subtype selective cholinergic agonists (e.g. carbachol and acetylcholine) into the nucleus induce dose-dependent hypotension and bradycardia /53/.

The M_2 muscarinic receptor agonists produce hypotension and bradycardia when injected into the *nucleus tractus solitarii* /54/. Thus, a decrease in blood pressure and in the heart rate can be obtained after microinjection into the intermediate portion of the *nucleus tractus solitarii* of a potent M_2 receptor agonist (cis-methyldioxolane, CD), but not after injection of a relatively selective M_1 receptor agonist (McN-A-343). The effect of CD can be blocked by a selective M_2 receptor antagonist (AFDX-116), but not by a potent and selective M_1 receptor antagonist (pirenzepine). Quantitative autoradiographic studies have also indicated that the muscarinic receptors in the *nucleus tractus solitarii* are predominantly of the M_2 subtype /54/. Still, the role of these receptors remains to be established.

VII. MODULATION OF THE CENTRAL CHOLINERGIC-ADRENERGIC INTERACTION

The endogenous opioid peptides have been shown to affect cardiovascular function *via* both central and peripheral mechanisms /55/. Enkephalins modify neurotransmitter release in many regions of the central nervous system. Thus, enkephalins have been shown to inhibit *in vitro* release of noradrenaline /56/, dopamine /57/ and of substance P /58/. Both Met-enkephalin and Leu-enkephalin, as well as some of their derivatives, have been found to depress the *in vivo* release of cortical acetylcholine /59/. It has been known for a long time that morphine and related opioids inhibit acetylcholine release at the peripheral cholinergic synapses /60/.

7.1 The effect of opioids on the hypertensive response to physostigmine

Met-enkephalina, Leu-enkephalin, β-endorphin and morphine significantly depress or even abolish the hypertensive effect of intravenously injected physostigmine. This depressive action of opioids is easily reversed by naloxone. It is assumed that opioids most probably depress the central cholinergic link implicated in the hypertensive response to physostigmine. This effect might be accomplished through inhibition of acetylcholine and/or noradrenaline release in the structures of the central nervous system relevant for BP rise after injection of physostigmine. It should be particularly pointed out that this interaction is carried out through mediation of opioid receptors in the central nervous system /61,114/. This assumption is supported by the finding that the BP responses to exogenous catecholamines are unaffected by opioids, thus indicating that the peripheral adrenoceptors are not influenced by opioids injected into the carotid artery.

The pressor response to intracerebroventricular administration of physostigmine was found to be inhibited in both acutely adrenalectomized and sham-operated rats, but not in animals adrenalectomized 24 hours earlier /62/. Laparotomy performed just before the experiment also inhibited the pressor effect of physostigmine. It is assumed that the endogenous opioid peptides, released as a response to the surgical manipulation stress, could inhibit the pressor effect of centrally administered physostigmine.

This inhibition was completely abolished by naloxone /63/.

7.2 Substances inhibiting the enkephalin-degrading enzymes and the hypertensive response to physostigmine

Many pharmacological studies with inhibitors of enkephalin-degrading peptidases have shown that these substances afford protection for both exogenous and endogenous opioids, and thereby mimic a large number of the effects of opioids /64-70/. This seems understandable if one assumes that the enkephalins are short-lived biological messengers that are rapidly cleaved by peptidases. Most important among these enzymes are amino-peptidase and dipeptidilcarboxypeptidase (usually termed enkephalinase). The most potent inhibitor of aminopeptidase is bestatin, whereas the action of enkephalinase can be inhibited by phosphoramidon.

In accordance with the previous data, it was not surprising to find that both bestatin and phosphoramidon inhibit the hypertensive effect of physostigmine in the same way as the opioids themselves /71/. The finding that opioids and the substances inhibiting the enkephalin-degrading enzymes produce qualitatively the same effects, indicates that the action of the enzyme inhibitors was realized indirectly, through enkephalins accumulated in the brain at critical sites. The accumulated enkephalins activate the opioid receptors in the brain, but at the same time produce depression of the cholinergic-adrenergic interaction in the central nervous system, which is known to be a prerequisite for the hypertensive response to physostigmine /71/.

7.3 The effect of adenosine on the hypertensive response to physostigmine

Adenosine has been found to produce an inhibitory action on neuronal firing rates /72/, on synaptic transmission /73/, on the release of neurotransmitters /74/, and on a variety of behavioral responses /75/. Some of these effects of adenosine can be blocked by methylxanthines.

It has also been found that intracerebroventricular injection of adenosine and some of its derivatives (L-phenylisopropyladenosine, L-PIA; 5'-N-ethylcarboxamideadenosine, NECA) produces a

dose-related reduction in the blood pressure and the heart rate of the rat. Injections of these substances directly into the *area postrema* or the *nucleus tractus solitarii* produce a decrease in BP and heart rate. All these data are taken to indicate a neuromodulatory role of adenosine in central cardiovascular control /76-78/.

In a separate study on the interaction between adenosine and the cholinergic-adrenergic interaction in the central nervous system during the hypertensive response to physostigmine, it was found that adenosine potentiates the hypertensive effect of physostigmine. If injected intravenously – but immediately before physostigmine – adenosine produced a potentiation of the BP response to physostigmine. This effect is even more evident when adenosine was injected into the carotid artery, also immediately before intravenous injection of physostigmine. Neither aminophylline nor 8-phenyltheophylline affected the potentiating action of adenosine on the hypertensive action of physostigmine. This may indicate that the potentiating action of adenosine is mediated through some other type(s) of adenosine receptor which presumably are not affected by aminophylline and 8-phenyltheophylline. Thus, this modulating effect of adenosine is different from the other already known modulating actions of adenosine /79/.

VIII. FUNCTIONAL ROLE OF THE CHOLINERGIC-ADRENERGIC INTERACTION

8.1 The role in BP control

All the available evidence indicates the existence of an interaction between the central muscarinic cholinergic system and the peripheral adrenergic system in BP control. It is quite possible that a tonically inactive cholinergic system can become activated and in this way produce cardiovascular changes. If this is the case, then acetylcholine in the brain may play a role in the development and maintenance of specific forms of hypertension. In this form of hypertension both hemicholinium and atropine lower BP /33/.

Spontaneous hypertension in the rat is usually considered as a good model for human essential hypertension. The origin of this type of hypertension has not yet been elucidated, but it may be associated with an increased sympathetic activity /82-85/. The intravenous injection of physostigmine in the spontaneously hypertensive rat produces significantly higher pressor responses

than in normotensive controls. This potentiated response to physostigmine appears to involve an increased release of acetylcholine at functionally critical site(s) /33/. It may well be that the central cholinergic mechanisms might have a physiological relevance. These mechanisms might have an even more significant role under specific pathological or homeostatic disturbances /33/.

8.2 **The life-saving effect of physostigmine in hypovolemic shock**

In lethal hemorrhagic shock in rats, physostigmine has been found to produce a reversal /86/. In hemorrhagic shock of non-anesthetized rabbits, physostigmine produces a life-saving effect /87/. In this type of hypovolemic shock, 71% of the control animals died within 1 hour after bleeding, while all the physostigmine-treated animals survived this period. In all the physostigmine-treated animals the mean arterial blood pressure was significantly increased in comparison to the saline-treated animals. All the physostigmine-treated animals showed a significant increase of plasma volume, in comparison to the postbleeding predicted values. In physostigmine-treated and surviving animals, the hematocrit values were at very low levels. In contrast to hematocrit, the plasma proteins in physostigmine-treated surviving animals were at almost physiological levels. The total blood volume was increased in the surviving animals almost entirely due to the increased plasma volume.

At least three lines of defense are known in hemorrhagic shock: keeping the blood pressure up, normalization of blood volume and antagonism of humoral factors that may aggravate the shock. It is quite possible that physostigmine is usefully active in all three lines of defense against shock, thus producing a life-saving effect /87/.

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