0031-6997/96/4802-0179$03.00/0
PHARMACOLOGICAL REVIEWS
Vol. 48, No. 2
Printed in U.S.A.

The Role of Central Cholinergic Neurons in the Regulation of Blood Pressure and in Experimental Hypertension

JERRY J. BUCCAFUSCO[a]

Department of Pharmacology and Toxicology, Medical College of Georgia and Department of Veterans Affairs Medical Center, Medical Research Service, Augusta, Georgia

I. Introduction

A. Cardiovascular Consequences of Dysregulation of Central Sympathetic Tone

Essential hypertension continues to be a contributing factor to the morbidity and mortality associated with cardiovascular disease in the United States. Despite a large armamentaria of drugs for the treatment of hypertension, and often in the face of pharmacologically controlled arterial pressure, heart disease and peripheral and cerebral vascular complications continue to occur (Cruickshank et al., 1989; Cutler et al., 1989; Collins et al., 1990). At least part of the cause of this scenario is that the effects of enhanced central sympathetic drive to peripheral organs and the vasculature are not always

[a] Address for correspondence: Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, Georgia 30912-2300.

abated by antihypertensive therapy. In certain instances, antihypertensive medication may actually facilitate sympathetic activity. Various cardiotoxic sequelae have been attributed to exaggerated sympathetic activity, including chronic heart failure (Bristow et al., 1990; Daly and Sole, 1990; Cohn, 1990), atherosclerosis (Pauletto et al., 1991), angina (Oishi et al., 1991), and cardiac arrhythmias (Oppenheimer et al., 1990; Podrid et al., 1990). Although efferent sympathetic tone is constantly under baroreceptor control, hypertensive disease is often associated with a resetting of the sensitivity of this reflex around the higher arterial pressure. Baroreceptor control does not, however, operate in isolation. Many other brain regions can alter the sensitivity of the reflex according to ongoing demands on the organism. For example, heightened mental stress, particularly on a chronic basis, can lead to or exacerbate cardiovascular toxicity (Pagani et al., 1991; Verrier and Dickerson, 1991; Weiner, 1991). This brief description of the role of the sympathetic nervous system in human hypertension may also be applied to one of the most widely used genetically induced animal models of this disease, the spontaneously hypertensive rat (SHR). As with the human disease, this strain is essentially normotensive at birth, but gradually develops hypertension with adulthood (see Birkenhager and Reid, 1984). During the developmental phase of hypertension and at early stable stages, elevated blood pressure is maintained in large part by enhanced central sympathetic outflow (Hallbäck, 1975; Judy et al., 1976, 1979; McCarty et al., 1978, 1987; Sakaguchi et al., 1983; Yamori, 1984). Since the first report of this strain in 1963, literally thousands of studies have addressed the cause of the development of hypertension in this model. Many of these have studied and implicated a role for a wide variety of neurotransmitters, neuromodulators, and neurohormones. Because it is beyond the scope of this article to review this vast work, the focus here will be the role of central cholinergic neurons in cardiovascular regulation and in the development and maintenance of hypertension in the SHR and other animal models of the disease. Admittedly, the relative importance of cholinergic neuronal control of the circulation has not been as well recognized as compared with those of the other, perhaps more well studied, brain neurotransmitter systems. Even more tenuous is the possibility that central cholinergic neurons play a role in hypertensive disease in animal models, let alone in humans. In view of the complexity of the central nervous system (CNS), it is even more daunting a task to consider the myriad of interactions between cholinergic and other neurotransmitter systems in cardiovascular regulation. The following, however, is an attempt to provide a review of the work (accomplished mostly over the past 20 years) put forward to support the concept of the cholinergic hypothesis.

Abbreviations: SHR, spontaneously hypertensive rat; CNS, central nervous system; HC-3, hemicholinium-3; mRNA, messenger ribonucleic acid; i.c.v., intracerebroventricular; PG, prostaglandin; Tx, thromboxane; ACTH, adrenocorticotropic hormone; CSF, cerebrospinal fluid; i.t., intrathecal; RVL, rostral ventrolateral medulla; NTS, nucleus of the tractus solitarius; CVL, caudal ventrolateral medulla; PNMT, phenylethanolamine-N-methyltransferase; IML, intermediolateral; GABA, γ-aminobutyric acid; cDNA, complementary deoxyribonucleic acid; NMDA, N-methyl-D-aspartate; d-AP7, D(−)-2-amino-7-phosphonohepatanoic acid; MK801, dizocilipine maleate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; NO, nitric oxide; NOS, nitric oxide synthase; WKY, Wistar/Kyoto (rat); DOCA, desoxycorticosterone; 4-DAMP, 4-diphenylacetoxy-N-methylpiperidine; K_m, substrate concentration at half-maximal velocity; V_{max}, maximal velocity; [^{3}H]QNB, [^{3}H]quinuclidin-3-yl benzilate; PCR, polymerase chain reaction; RT-PCR, polymerase chain reaction-reverse transcriptase; DKJ-21, N-(4-diethylamino-2-butynyl)-succinimide; G3PDH, glyceraldehyde-3-phosphate dehydrogenase.

B. Early Evidence for the Role of Central Cholinergic Neurons in the Regulation of Sympathetic Tone and Blood Pressure

The first in-depth study of central cholinergic involvement in cardiovascular regulation was reported by Suh and his colleagues in 1936. In the anesthetized dog, they essentially mapped sites in the medulla that were sensitive to either topical or iontophoretic application of acetylcholine (Suh et al., 1936). Their findings included the observations that, in contrast to the depressor response to intravenous acetylcholine, central administration of the neurotransmitter produced a reliable pressor response. The pressor response to intracisternal injection of acetylcholine (of 1 to 10 μg) was immediate and brief, but subject to rapid tolerance; the latter effect is now known to be caused by rapid down-regulation of muscarinic receptors (Aronstam et al., 1987). This group also reported that sectioning of the cervical spinal cord, but not sectioning of the vagus nerve, eliminated the pressor response to intracisternal injection of acetylcholine. Again, this finding has been corroborated in that the pressor response to central cholinergic neurons is mediated mainly through the sympathoadrenal system. Finally, the investigators reported that pressor-sensitive areas were localized to the ventromedial area of the rostral medulla—possibly within or near the vestibular complex. Thirty-four years later, Brezenoff and Jenden (1970) essentially confirmed this site of cholinergic sensitivity in the anesthetized rat using microinjection of the acetylcholine analog carbachol.

Although these early studies using acetylcholine pointed to a role for cholinoceptive sites in the brain in cardiovascular regulation, the involvement of neurally released acetylcholine initially was inferred from the action of certain inhibitors of acetylcholinesterase. The vast majority of this early work with cholinesterase inhibitors was performed by Varagić and his coworkers beginning in 1955. The reader is referred to several excellent, relevant reviews (Philippu, 1981; Brezenoff and Giuliano, 1982; Brezenoff, 1984; Buccafusco and Brezenoff, 1986; Varagić et al., 1991). The centrally acting cholinesterase inhibitor physostigmine (eserine) was

initially used to test the original hypothesis that the release or mobilization of endogenous brain acetylcholine resulted in a hypertensive response. Physostigmine is a carbamate cholinesterase inhibitor that is not highly charged at physiological pH. Intravenous administration of low μg/kg amounts of physostigmine in the rat results in a reliable increase in arterial blood pressure. In contrast to the pressor action of physostigmine, the quaternary cholinesterase inhibitor neostigmine does not elevate arterial pressure over a similar dose range. However, if neostigmine is introduced directly into the CNS, a pressor response is observed. Atropine sulfate (but not the quaternary derivative, methylatropine) both prevents and reverses the pressor response to intravenous injection of physostigmine. The pressor response to physostigmine is also prevented by surgical or pharmacological interruption of the sympathetic nervous system; conversely, physostigmine was shown to enhance cervical sympathetic neural tone. In confirmation of the role of the sympathetic nervous system in mediating physostigmine's pressor response, Varagić and coworkers (1970) later reported that adult rats, which had previously been treated with antisera to nerve growth factor, were much less sensitive to the pressor action of physostigmine. Moreover, acute bilateral adrenalectomy or vagotomy did not affect the expression of the pressor response to centrally acting cholinesterase inhibitors. Cervical spinal transection, but not decerebration, appeared to block the pressor response to intravenous injection of physostigmine in anesthetized rats (Varagić, 1955), suggesting that the site of action was relegated to the lower brain stem, although, preliminary data from this group (Varagić and Krstić, 1966) were also consistent with a hypothalamic site of action for physostigmine. As a tribute to these pioneering investigators, virtually all of these early findings were confirmed in subsequent studies reported by several laboratories.

C. Acetylcholine Receptors in the Central Nervous System

Between the mid-1960s and mid-1970s, there was a dearth of published reports regarding cholinergic involvement in cardiovascular regulation. Part of this trend may have reflected the rising interest in the role of central catecholaminergic pathways in blood pressure control. Interest in central adrenergic mechanisms was fueled in large part by the discovery of the central mechanism of action of a new class of antihypertensive drugs. The α-adrenergic agonists clonidine and α-methyl norepinephrine (the active metabolite of α-methyldopamine) were demonstrated to produce clinically effective antihypertensive responses through stimulation of "adrenergic" vasomotor centers in the CNS (Schmitt, 1977; Kobinger, 1978; Buccafusco, 1992).

Although the concept that central cholinergic neurons play a role in cardiovascular regulation was supported by the results of studies which used physostigmine and related drugs, the obvious corollary to this hypothesis—that drugs which interfere with central cholinergic function lower arterial blood pressure—was not apparent either from the clinical or experimental literature of the time. Muscarinic receptor antagonists like atropine and scopolamine as used clinically do not generally lower blood pressure. Thus, we and others (Buccafusco and Spector, 1980a; Brezenoff and Giuliano, 1982) suggested the possibility that central cholinergic neurons involved in blood pressure regulation were not tonically active, but were called into play to modulate ongoing sympathetic activity under specific physiological conditions. Perhaps the best example of this situation is the hypertensive state itself. Drugs that interfere with central cholinergic function, receptor antagonists such as atropine and acetylcholine depeletors such as hemicholinium-3 (HC-3), can produce dramatic reductions in blood pressure in hypertensive rats, but are minimally effective in this regard in normotensive animals (table 1). In some respects, this situation is similar to that of the centrally acting adrenergic receptor agonists such as clonidine and methyldopa, which are more effective in producing an antihypertensive response than they are a hypotensive response (see Buccafusco, 1992).

Perhaps the single most important discovery to aid in the interpretation of the complex cholinergic system(s) involved in cardiovascular regulation was that of the identification of muscarinic receptor subtypes. The existence of subtypes of the muscarinic receptor originally was inferred from experiments that examined the pharmacological specificity of muscarinic receptor antagonists such as pirenzepine in various peripheral organs. Subsequently, five muscarinic receptor genes (m1-m5) were cloned that encode distinct muscarinic cholinergic receptors (Kubo et al., 1986; Bonner et al., 1987, 1988; Peralta et al., 1987; see McKinney and Coyle, 1991). Gene products of the m1, m3, and m5 transcripts correspond to respective receptors that activate phospholipase C via a pertussis toxin-insensitive G-protein. These subtypes are thought to mediate primarily excitatory synaptic transmission; m2 and m4 receptors inhibit adenylate cyclase activity via a pertussis toxin-sensitive G-protein and mediate primarily inhibitory synaptic transmission (McKinney and Coyle, 1991). Studies in which molecular probes were used that are selective for conserved sequences of messenger ribonucleic acid (mRNA) encoding each subtype have provided evidence of a heterogenous distribution of the subtypes throughout the brain. The m1 mRNA is enriched in the cortex and hippocampus but drops off dramatically in more caudal brain regions. On the other hand, there is a relatively even distribution of m3 and m5 receptors throughout the CNS. The m2 subtype is found to be enriched in caudal brain regions, where it has been reported to be the predominant subtype (Buckley et al., 1988; Levey et al., 1991). Finally, m4 mRNA has been

TABLE 1

Effect of pharmacologic inhibition of central cholinergic neuronal function on resting blood pressure

Reference	Route	Age (weeks)	Drug	Strain	Change in Blood Pressure From Pre-injection Levels
Brezenoff and Caputi, 1980	i.c.v.	12–16	HC-3	SHR	↓
			HC-3	WKY	–
			HC-3	SD_{NT}	–
Buccafusco and Spector, 1980a	i.c.v.	12–14	HC-3	SHR	↓ ↓
			HC-3	WKY	↓
Coram and Brezenoff, 1983	i.v., i.c.v.	22–36	DKJ-21	SHR	↓
			DKJ-21	WKY	–
Buccafusco, 1984c	i.c.v.	15–18	HC-3	SHR	↓
Brezenoff and Guliano, 1987	i.c.v.	18–60	HC-3	SHR	↓ ↓
			HC-3	WKY	↓
			HC-3	SD_{NT}	–
Brezenoff et al., 1988	i.c.v.	18	4-DAMP	SHR	↓
			4-DAMP	WKY	–
			pirenzepine	SHR	–
Vargas and Brezenoff, 1988	i.c.v. (chronic)	4	HC-3	SHR	↓
Brezenoff and Xiao, 1989	i.c.v., PH	17	HC-3	SHR	↓
Brezenoff et al., 1990	i.c.v., PH	18–22	4-DAMP mustard	SHR	↓
	i.c.v.		4-DAMP mustard	SD_{NT}	–
Kubo et al., 1995	RVL	12–16	scopolamine	SHR	↓ ↓
			scopolamine	WKY	↓

i.v., intravenous; PH, posterior hypothalamus; SD_{NT}, Sprague-Dawley (normotensive).
↓, decrease; –, no change; ↓ ↓, effect in SHR was greater in magnitude than the effect in the normotensive strain.

demonstrated consistently to be enriched in the striatum (Levey et al., 1991; Yasuda et al., 1993; Wei et al., 1994). Although quantification of mRNA does not provide direct information regarding the expression of receptor protein, it does provide a powerful tool for assessing whether changes in gene regulation are correlated with expression of the phenotype, for example, hypertension. In the case of muscarinic receptors, it has been demonstrated that a wide variety of chemical stimuli and disease states that are known to affect the cholinergic system, particularly at the receptor level, produce changes in cholinergic function that are reflected at the level of transcription of the receptor genes (Asanuma et al., 1992; Eva et al., 1992; Wang et al., 1992a, b; Ogawa et al., 1992). Also, the change in receptor number in response to prolonged stimulation or blockade (down-regulation and up-regulation) has been demonstrated to occur as a result of changes at the transcription level of the genes encoding specific muscarinic receptor subtypes (Wall et al., 1992; Lee and Frazer, 1993; Longone et al., 1993). The mechanism for such changes in mRNA levels has been attributed both to altered transcription rates (Longone et al., 1993) and altered stability of transcribed mRNA (Lee and Frazer, 1993), depending upon the subtype being studied.

Alterations in the expression of muscarinic receptor levels as described in the preceeding paragraph may involve the synthesis or release of noncholinergic mediators. For example, Hays and coworkers (1989) examined the effect of inhibition of brain prostaglandin synthesis with indomethacin or meclofenamate on the pressor response to central cholinergic stimulation. In these experiments, the intracerebroventricular (i.c.v.) injection of carbachol into normotensive, freely moving rats produced an immediate pressor response that could be eliminated by blockade of brain prostaglandin synthesis under certain conditions. If an initial i.c.v. dose of carbachol was administered 2 hours before a second, similar injection, the pressor response to the second dose was almost abolished if an i.c.v. injection of indomethacin was administered 20 min before the second carbachol treatment. In contrast, pretreatment with the same dose of indomethacin in naive rats did not effect the pressor response to carbachol given 20 min later. These initial studies were extended (Buccafusco et al., 1993) to show that if the time interval between the two carbachol injections was extended to permit return of the full second carbachol response (i.e., 24 hr later), indomethacin no longer inhibited the second pressor response. In support of the pharmacological results, in vivo (i.c.v.) administration of carbachol was demonstrated to reduce the number of [^{3}H]N-methyl scopolamine binding sites (down-regulation) measured in vitro. However, post-treatment with indomethacin produced a further enhancement of the carbachol-induced down-regulation. These findings suggested that prostaglandins play a role in the development of tachyphylaxis to brain muscarinic receptor stimulation: activation of prostaglandin synthesis in vivo may decelerate the development of desensitization to muscarinic agonists. Thus, an alteration in the processing or metabolism of mediators via the arachidonic acid cascade could conceivably alter the expression of muscarinic receptors involved in central cardiovascular regulation. In fact, it has been reported that

endogenous prostanoid biosynthesis in SHR was elevated 2 months before hypertension became established and that much higher levels of prostaglandin E_2 (PGE_2) and 6-keto-$PGF_1\alpha$ (a stable PGI_2 metabolite) were found in SHR in hypothalamus and pons-medulla oblongata; thromboxane B_2 (TxB_2), the stable metabolite of TxA_2, was higher in the pons-medulla but lower in the hypothalamus (Ohtsu and Matsuzawa, 1980). A slightly elevated PG synthetic rate could amplify the effects of neurally released acetylcholine by inhibiting the process of receptor down-regulation. Thus, eicosanoids may play a role in maintaining the sensitivity of cholinergic signaling mechanisms in the CNS.

Most of the earlier studies of central cholinergic cardiovascular regulation used either indirect cholinergic agonists such as physostigmine, or nonselective receptor agonists such as acetylcholine or carbachol. Although in the vast majority of cases, the pressor responses to these agents could be completely blocked by muscarinic receptor antagonists, selective stimulation of central nicotinic receptors could also alter resting blood pressure (see Brezenoff and Giuliano, 1982). An example of the earlier work in this field was the study of Feldberg and Guertzenstein (1976), who studied the effects of application of nicotine to the ventral surface of the medulla of anesthetized cats. Nicotine evoked a significant depressor response when applied to the 'nicotine-sensitive' area just caudal to the trapezoid bodies. Nicotine's actions were sensitive to blockade by the nicotinic receptor antagonist hexamethonium, but were resistant to atropine. Of particular interest was their observation that physostigmine and carbachol applied to the same area also evoked depressor responses; however, these were sensitive to atropine and resistant to hexamethonium. Part of the explanation for this apparent discrepancy may reside in the more recent appreciation for the selectivity of nicotine as a ligand for subtypes or specific affinity states of the receptor.

Both central and peripheral nicotinic cholinergic receptors are ligand-gated ion channel receptors composed of five subunits. In the CNS, distinct genes encode for at least 11 such transmembrane subunits, eight α ($\alpha 2$ to $\alpha 9$) subunits and three β ($\beta 2$ to $\beta 4$) subunits. The stoichiometry or composition of each receptor subtype made up of different combinations of α and β subunits will dictate the various properties of each nicotinic receptor subtype, including ligand selectivity, the type of ion channel opened, channel open times, and the rate of desensitization of the receptor to agonists (for review, see Arneric et al., 1995a, b; Bannon et al., 1995). For example, the presence of either $\alpha 2$ or $\alpha 3$ subunits in a receptor will result in very different levels of sensitivity to cholinergic receptor agonists and antagonists. Although several chimeric forms of the nicotinic receptor have been studied in artificial membranes, in the CNS, only three native receptors have been pharmacologically characterized by using labeled nicotinic probes. These include (*a*) a high affinity site that can be labeled with (−)-nicotine, acetylcholine or cytisine ($\alpha 4\beta 2$), (*b*) a site that binds labeled α-bungarotoxin ($\alpha 7$), and (*c*) a site that can be labeled with neuronal bungarotoxin ($\alpha 3$). These three subtypes of nicotinic receptors have distinct distribution patterns throughout the CNS, although this can be species-specific.

As indicated in the discussion to follow, knowledge of the brain and spinal distribution of muscarinic and nicotinic receptor subtypes has allowed for a more realistic interpretation of the anatomical and neurochemical interactions taking place in cardiovascular regulatory centers. Questions regarding the role of cholinergic receptor regulation in the development and maintenance of hypertension could now be posed experimentally.

II. The Effect of Central Cholinergic Stimulation on Systemic Blood Pressure in Normotensive Animals and Humans

A. Acetylcholinesterase Inhibitors

There is no specific cardiovascular response to central cholinergic receptor stimulation. Increases, decreases, and biphasic changes in blood pressure and heart rate have been reported. This diversity of response to central cholinergic stimulation is related to several factors, including species specificity, drug selectivity, anatomical location, and the level of anesthesia. However, when normotensive rats, rabbits, dogs, and sheep are unanesthetized and cholinesterase inhibitors are administered peripherally (centrally acting) or centrally, a hypertensive response is invariably observed (see, Philippu, 1981). One notable exception has been the cat. In this species, cholinesterase inhibitors generally elicit a profound fall in blood pressure (Edery et al., 1986; Gillis et al., 1988; Hara et al., 1992; Ally et al., 1993). Many of the same pharmacological features described above for the physostigmine-induced pressor response in rats hold true for the physostigmine-induced depressor response in cats. For example, the depressor response appears to be mediated by muscarinic receptors, and inhibition of central sympathetic tone underlies the hypotension. The majority of the work performed in cats, however, has been performed under general anesthesia. One possibility is that central cholinergic pressor systems are more sensitive to the effects of anesthetics in the cat than they are in the rat. In support of this supposition are the data provided by Ally and his coworkers (Ally et al., 1995). This group clearly demonstrated that physostigmine produced a dose-dependent pressor response after i.c.v. administration in the conscious cat. The response was mediated by muscarinic receptors, reportedly of the M_2 subtype. When the same cats were anesthetized with pentobarbital, physostigmine produced a decrease in arterial pressure. A likely candidate for the site of action mediating the pressor response to i.c.v. injection of physostigmine in conscious cats is the hypothalamic defense

area. This area is readily accessed via i.c.v. administration, and direct microinjection of carbachol into this region elicits a 'defense reaction' accompanied by a sustained pressor response (Wu and Wei, 1982). Thus, the anesthetized cat model may have a significant role in the continuing study of central inhibitory cholinergic cardiovascular centers.

The relevance of the various animal models discussed in the preceeding paragraph to the situation in humans is dependent upon the information that can be derived from the clinical literature regarding the administration of cholinergic agonists. Unfortunately, the potentially serious side effects associated with cholinergic drugs in humans has limited the therapeutic potential of these drugs. When cholinergic agonists are used clinically, the doses administered are exceedingly low (to limit side effects) or are used in therapeutic situations of cholinergic hypofunction. The latter situation is encountered in situations of drug overdose causing antimuscarinic activity or decreasing cholinergic function. Nevertheless, centrally acting cholinergic agonists, particularly the cholinesterase inhibitor physostigmine, have occasionally been used experimentally to enhance central cholinergic function in humans. One of the first such studies (Freeman and Carmichael, 1936) actually predates the first studies of physostigmine in rats. In a group of 24 normotensive male volunteers, intravenous administration of 1 mg of physostigmine produced an increase in both systolic and diastolic pressures of 13 mmHg. The response began immediately after injection, peaked about 15 min later, and gradually declined to preinjection levels. More recently, physostigmine has been used as a general analeptic in the treatment of drug overdose to restore consciousness. In such cases, physostigmine has been shown to restore depressed arterial pressure caused by the overdose of certain tricyclic antidepressants, diazepam, secobarbital, propoxyphene or certain phenothiazine antipsychotic agents. In certain overdose situations, physostigmine induced a short-lasting hypertensive state (Nattel et al., 1979; Stewart, 1979; Nilsson et al., 1983). In an attempt to reverse some of the symptoms of Huntington's chorea, 40 mg of physostigmine was administered intravenously to four patients (Aquilonius and Sjöström, 1971). The patients were pretreated with methylscopolamine to obviate peripheral cholinergic side effects. Under these conditions, physostigmine produced short-lasting (less than 30 min) but dramatic increases in blood pressure. In one case, systolic blood pressure increased by 40 mmHg. In concert with the findings in the rat and other animal models discussed in the first paragraph of this section, administration of the quaternary cholinesterase inhibitor neostigmine to the same patients failed to significantly elevate blood pressure.

The most systematic study of the psychological and physiological effects of physostigmine in humans was carried out by Janowsky and his coworkers (1973), who demonstrated in psychiatric patients that centrally acting cholinomimetics decrease the symptoms of mania and can induce a depressive syndrome. This pharmacological treatment also elicited the release of certain stress hormones—cortisol, adrenocorticotropic hormone (ACTH), and β-endorphin (Risch et al., 1981; Janowsky et al., 1986). Consistent with the findings in animals, physostigmine was shown to elicit epinephrine release (Kennedy et al., 1984). In a study involving 7 normals and 23 psychiatric patients, this group reported that when subjects were pretreated with a selective peripheral antimuscarinic agent, intravenous infusion of physostigmine produced an increase in systolic and diastolic blood pressure that was significantly greater than that produced by an equivalent dose of neostigmine. Moreover, pretreatment with scopolamine (but not methylscopolamine) blocked all the behavioral and cardiovascular effects of physostigmine (Janowsky et al., 1986). Notwithstanding the obvious limitations imposed in these clinical studies, the results are consistent with the presence of a central muscarinic pressor system in humans that shares many pharmacological features with those in experimental animals, particularly those reported for the rat.

B. Centrally Acting Cholinergic Receptor Agonists

As a pharmacological probe to activate central muscarinic receptors, physostigmine has proven to be a useful tool. Although the drug has the capacity to evoke severe peripheral side effects mediated through both muscarinic and nicotinic receptors, at doses that evoke a centrally mediated pressor response (low μg/kg range), animal subjects do not usually exhibit obvious signs of distress. Cholinesterase inhibitors, however, are nonselective with respect to their central cholinergic receptor activation. To address this limitation, various direct-acting muscarinic receptor agonists that are known to enter the CNS have been studied for potential cardiovascular actions. Although centrally acting, such agents can have profound peripheral vasodilatory activity that could essentially obviate the central pressor action. Therefore, centrally acting muscarinic agonists such as arecoline and oxotremorine have been reported to evoke significant pressor responses in experimental animals when the subject is pretreated with a selective peripheral antimuscarinic agent such as methylatropine (Walker and Weetman, 1970; Buccafusco, and Spector, 1980b; Buccafusco and Aronstam, 1988; Makari et al., 1989; Barnes and Roberts, 1991). Two drugs that are possible exceptions are the centrally acting muscarinic receptor agonists pilocarpine and McN-A-343. These drugs are much less effective than either arecoline or oxotremorine in evoking a pressor response (Pazos et al., 1986; Barnes and Roberts, 1991) or may actually produce a fall in blood pressure (Dage, 1979). The apparent discrepancy may be related to the partial selectivity of pilocarpine and McN-A-343 for the M_1 subtype of the

muscarinic receptor (see Pazos et al., 1986). Finally, the results of one clinical study indicate that intravenous administration of arecoline to normal subjects pretreated with the selective muscarinic receptor antagonist glycopyrrolate evokes a hypertensive response accompanied by adrenal catecholamine release (Polinsky et al., 1991). The authors attributed this action of arecoline to nicotinic ganglionic stimulation. However, they could not rule out the possibility that at least part of the response was mediated centrally. Unfortunately, a centrally acting antimuscarinic compound was not used to test this possibility.

C. Direct Central Injection of Cholinergic Agonists

The results from experiments in which muscarinic receptor agonists and antagonists were introduced into the cerebrospinal fluid (CSF) of experimental animals, particularly rats, have in general confirmed the conclusions derived from those using centrally acting drugs. The advantages of direct CSF administration include avoidance of peripheral drug actions and the ability to use drugs regardless of chemical structure. Thus, acetylcholine itself as well as the analog carbachol each elevate blood pressure after cerebroventricular administration. Aronstam and coworkers (1988) demonstrated that the ability of acetylcholine and three structural analogs to increase blood pressure after i.c.v. injection in conscious rats was correlated with their binding affinities for brain muscarinic receptors in vitro. In the rat, the pressor response to i.c.v. administration of acetylcholine was atropine-sensitive and mediated through enhanced central sympathetic outflow (Krstić et al., 1978; Krstić, 1982). Pressor responses to muscarinic receptor stimulation have also been observed after injection of muscarinic receptor agonists into the fourth cerebral ventricle (Krstić, 1982), the cisterna magna (Buccafusco et al., 1990), and the spinal cord intrathecal (i.t.) space (Marshall and Buccafusco, 1987; Magri and Buccafusco, 1988). Although the possibility exists that introduction of drug directly into the CSF from any brain vantage point would eventually lead the compound to a site of action, it is clear that there exists some degree of site specificity. For example, using tissue cholinesterase inhibition as a marker for neostigmine distribution, Takahashi and Buccafusco (1991a) demonstrated that lower thoracic i.t. injection of the drug resulted in significant cholinesterase inhibition only within the thoracic and lumbar spinal cord. There was no inhibition of medullary cholinesterase. Likewise, Xiao and Brezenoff (1988) demonstrated in rats that the pressor response to i.c.v. injection of neostigmine could be inhibited by more than 50% after bilateral microinjection of a muscarinic receptor antagonist into the posterior hypothalamus. Moreover, significantly lower doses of physostigmine were required to elevate arterial pressure in conscious normotensive rats by the i.c.v. route compared with the intracisternal route (Buccafusco et al., 1990). Even intravenous administration of physostigmine appears to have a specified site of action within the medulla, at least for anesthetized rats (Punnen et al., 1986; Giuliano et al., 1989). Despite the limitations of cerebroventricular or i.t. injections in terms of site specificity, the value of such experiments is related to the ability to use conscious, freely moving animals. It is quite difficult experimentally to use modern microinjection techniques in hindbrain and spinal sites in unanesthetized animals. As indicated below, much of the pharmacology evinced regarding cholinergic regulation of central cardiovascular function that was obtained via the CSF route of administration has been confirmed using discreet microinjection techniques.

III. Anatomical Substrates for the Cholinergic Regulation of Blood Pressure

A. Forebrain Pathways—The Posterior Hypothalamus

The caudal and lateral regions of the hypothalamus have been examined for several decades using electrophysiological techniques. Electrical and chemical stimulation of the posterior hypothalamus in the cat can elicit a full rage and defense reaction (Myers, 1964). Moreover, prolonged intermittent stimulation of the defense area of the hypothalamus has been reported to lead to a sustained hypertensive condition (Folkow and Rubinstein, 1966). Chemical stimulation of the posterior hypothalamus with cholinergic agonists mimics quite well the response to electrical stimulation (Brezenoff and Jenden, 1969). In the conscious rat, microinjection of carbachol or various cholinesterase inhibitors all led to marked and sustained pressor responses (Buccafusco and Brezenoff, 1979). The power of this cholinergic hypothalamic pressor system was underscored by the fact that microinjection of as little as 30 nmol of echothiophate, an irreversible cholinesterase inhibitor, resulted in a fulminant type of hypertension accompanied by pulmonary edema and death. The pressor response to intrahypothalamic injection of cholinesterase inhibitors was both atropine- and HC-3 sensitive. Thus, local release of acetylcholine with subsequent muscarinic receptor stimulation within the posterior hypothalamic nucleus was a requirement for the expression of the pressor response to inhibition of hypothalamic cholinesterase. This pressor response is mediated primarily via the sympathetic nervous system, although some evidence exists to suggest a role for the release of vasopressin into the systemic circulation (Hoffman and Phillips, 1976). Areas within the caudal hypothalamus sensitive to muscarinic receptor stimulation were highly localized to just a few regions—the posterior hypothalamic nucleus, the supramammillary nucleus, and the medial mammillary nucleus. Even in the cat, hypothalamic chemical stimulation with carbachol evokes an atropine-sensitive hypertensive response that outlasts the behavioral manifestations of the rage or attack response (Karmos-

Várszegi and Karmos, 1977). Some years later, Eckersdorf and coworkers (1987) reported that the behavioral response to intrahypothalamic injection of carbachol in cats was not sensitive to pretreatment with the neurotoxin kainic acid. Because kainic acid destroys perikarya, but not nerve endings, one possibility is that carbachol's actions might be mediated through the release of a second neurotransmitter. This concept fits with an earlier observation in the rat that the excitatory behavioral response produced by injection of cholinergic agonists into the posterior hypothalamic nucleus is mediated through nicotinic cholinergic receptors (Buccafusco and Brezenoff, 1980). Moreover, local pretreatment with HC-3, at doses known to deplete hypothalamic levels of acetylcholine, blocked the effects of carbachol. The inference is that carbachol, acting upon nicotinic receptors located on cholinergic nerve endings, enhanced the release of acetylcholine. This scenario is in keeping with the kainic acid experiments cited above (Eckersdorf et al., 1987). It is also consistent with more recent findings that demonstrate that the cardiovascular response to nicotine administered by the i.c.v. route in conscious rats is mediated through the release of endogenous acetylcholine, which then acts upon muscarinic receptors (Buccafusco and Yang, 1993).

The role of hypothalamic cholinergic pressor systems in mediating the hypertensive response to systemically administered physostigmine (or other centrally acting muscarinic receptor agonist) is not yet apparent. The work of Janowsky and colleagues (1973, 1985, 1986) in humans suggests that higher brain functions are engaged concomitantly with the cardiovascular changes elicited after intravenous injection of physostigmine. However, at least in the anesthetized rat, the pressor response to intravenous injection of physostigmine is purported to be mediated almost exclusively within the medulla (see Brezenoff and Giuliano, 1982), particularly within the area termed the rostral ventrolateral medulla (RVL) (Punnen et al., 1986; Giuliano et al., 1989), whereas the posterior hypothalamus appears to play a dominant role in mediating the pressor response to i.c.v. injection of cholinesterase inhibitors (Xiao and Brezenoff, 1988). This latter finding was supported by a recent preliminary study in which it was demonstrated that i.c.v. injection of a pressor dose of neostigmine in rats produced an anatomically selective increase in the expression of immunoreactive *c-fos*, a neuronal early response gene product (Brezenoff et al., 1994). Increases in *c-fos* immunoreactivity were relegated to periventricular and posterior hypothalamic nuclei, the lateral septum, and the amygdala. Previous i.c.v. administration of atropine blocked both the subsequent neostigmine-induced pressor response and the increased *c-fos* activity.

Citing the known autonomic dysfunction observed to be associated with Parkinson's disease, Pazo and Medina (1983) suggested that visceral disturbances, including arterial hypotension, might derive from the nigrostriatal degeneration thought to give rise to the characteristic motor disturbances. They undertook a study in anesthetized cats in which the caudate nucleus was mapped through microinjection of carbachol or dopamine (representing the primary neurotransmitters known at that time to be affected in Parkinson's disease). Most of the striatal regions examined were responsive in terms of blood pressure changes, although pressor responses were obtained primarily from rostral sites, whereas depressor responses were obtained from caudal sites. Both actions were blocked by previous local muscarinic, but not nicotinic receptor blockade.

Owing in large part to the pioneering work of Brody and his colleagues (see Brody et al., 1978; Hartle and Brody, 1984), perhaps the most comprehensive pharmacological study of a forebrain system involved in cardiovascular regulation includes the periventricular structures of the third cerebral ventricle. The continuum of structures from the organum vasculosum of the lamina terminalis through the anteroventral third ventricle to the subfornical organ represent a series of interconnecting centers that have a profound role in the regulation of fluid balance and vascular homeostasis. Because part of this region is devoid of a functional blood-brain barrier, sensory mechanisms exist in these regions that respond to humoral factors such as hyperosmolarity, sodium concentration, and angiotensin II. Also, the subfornical organ was shown to be a site of action for the central pressor response to angiotensin II. Specific lesions placed within these periventricular structures have been demonstrated to prevent the development of several types of renal-induced hypertension, both angiotensin II-dependent and angiotensin II-independent forms; as well as steroid/salt-induced hypertension. Efferent projections from these regions innervate various hypothalamic areas as well as more caudal cardiovascular centers. One pharmacological relationship between the hypothalamus and the periventricular structures is their sensitivity to the dipsogenic and pressor properties of i.c.v.-injected carbachol. As indicated above, only about 50% of the pressor response to i.c.v. injection of a cholinergic agonist was shown to be mediated within the posterior hypothalamus. Electrolytic lesion of the anteroventral third ventricle region also reduced, but did not eliminate, the pressor and dipsogenic responses to i.c.v. injection of carbachol (Menani et al., 1990). Therefore, it is possible that both regions contribute to the pressor response evoked by i.c.v. administration of carbachol, although no experiment has been attempted using lesions combining both structures.

B. Hindbrain Pathways—The Ventrolateral Medulla

Dampney (1994) has summarized the evidence that five primary groups of sympathetic premotor neurons exist in the rat that originate from the paraventricular nucleus of the hypothalamus, the pontine A5 catecholamine cell group, the rostral ventrolateral medulla, the

ventromedial medulla, and the caudal raphe complex. These regions receive baro- and chemoreceptor fibers and also cardiovascular afferents from widespread areas of the CNS. Of these, three regions of the medullary reticular formation have been neurochemically, electrophysiologically, and pharmacologically characterized for their role in the central cholinergic regulation of blood pressure: the nucleus of the tractus solitarius (NTS), the RVL, and the caudal ventrolateral medulla (CVL). Neuronal interactions among these groups, as well as from higher centers, are known to exist, and each of these regions contain neuronal markers for the presence of cholinergic cell bodies and/or terminals (Ruggiero et al., 1990). The NTS is the primary site of termination of afferent fibers from arterial baro- and chemoreceptors; electrical stimulation or chemical stimulation with glutamate results in a marked fall in blood pressure (Sundaram et al., 1989b), whereas electrolytic lesion of the region results in a neurogenic form of hypertension in the rat (Reis et al., 1976). The development of this form of hypertension is dependent upon the integrity of higher brain centers. In the first study in which microinjections of cholinergic drugs were discreet enough to be limited to the NTS, carbachol, acetylcholine, and physostigmine each evoked depressor responses after injection into the nucleus (Criscione et al., 1983). Local pretreatment with atropine reversed the depressor responses to the cholinergic agonists, but not that to glutamate. Alone, atropine produced a modest increase in blood pressure and significantly reduced the reflex bradycardia evoked by systemic injection of a pressor agent. Muscarinic receptor blockade in the NTS did not mimic the effects of electrolytic lesion (e.g., the development of neurogenic hypertension) of the nucleus. Thus, although inhibitory cholinergic neurons may contribute to the regulation of cardiovascular reflex activity within the NTS, they do not appear to be the only factor. Using partially selective muscarinic receptor agonists and antagonists, Sundaram and coworkers (1989b) demonstrated that the muscarinic receptor subtype mediating the inhibitory actions of acetylcholine in the NTS was a non-M_1, possibly the M_2 subtype. One efferent projection from the NTS (which may in part comprise a contingent of cholinergic neurons) terminates in the RVL (fig. 1) (Ruggiero et al., 1990).

The RVL is essentially a subdivision of the nucleus paragigantocellularis lateralis, which includes in its rostral boundaries cells containing phenylethanolamine-N-methyltransferase (PNMT) activity, the so-called C-1 region (Reis et al., 1987). The RVL provides direct tonic vasoconstrictor activity to spinal preganglionic neurons and plays a primary role in mediating the reflex hypotensive response to baroreceptor activation (Granata et al., 1985). The RVL also has been demonstrated to receive innervation from cholinergic fibers, and stimulation of muscarinic receptors in this region evokes a hypertensive response; alternatively, muscarinic blockade

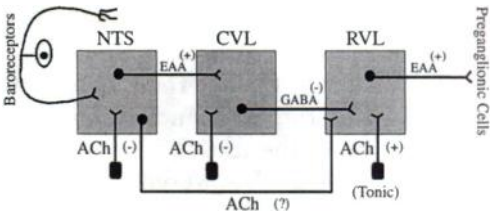

FIG. 1. Schematic representation of the proposed primary interactions between cardiovascular afferent (NTS and CVL) and premotor (RVL) nuclei in the medulla. Plus (+) and minus (−) signs refer soley to the effect on blood pressure produced by the application of respective agonists of excitatory amino acid EAA, GABA, and acetylcholine (ACh) neurons to the terminal fields in each region. Direct neurotransmitter-neurotransmitter interactions should not be implied. A more precise representation of neurotransmitter interactions within the RVL is depicted in figure 2. "(Tonic)" refers to the concept put forth (see Section III.B.) that cholinergic innervation to the RVL is tonic in nature and helps maintain sympathetic vasomotor tone. The direct cholinergic connection between the NTS and the RVL has been identified through anatomical studies (Ruggiero et al., 1990).

results in a fall in blood pressure, even in normotensive animals (Willette et al., 1984; Punnen et al., 1986; Ernsberger et al., 1988; Morrison et al., 1988; Giuliano et al., 1989). The fall in blood pressure to muscarinic receptor blockade or acetylcholine depletion in the RVL is equivalent to that produced by spinal transection. In view of the inability of global central muscarinic receptor blockade to lower blood pressure in conscious normotensive animals (table 1), the finding that microinjection of atropine into the RVL lowered blood pressure to the extent of spinal section is somewhat perplexing. Partly confounding the results of these experiments are (*a*) the potential local anesthetic action of atropine and (*b*) the use of general anesthesia. The first concern may be partly abrogated by the fact that local microinjection of HC-3 to deplete endogenous stores of acetylcholine essentially achieved the same results as those with atropine (Giuliano et al., 1989). Moreover, microinjection into the RVL of the muscarinic receptor antagonists scopolamine and AF-DX 116 (partially M_2 selective), but not pirenzepine (partially M_1 selective) produced similar hypotensive actions compared with atropine (Sundaram and Sapru, 1988; Sundaram et al., 1988; Giuliano et al., 1989). To further ascertain the selectivity of muscarinic receptor blockade, after microinjection of the antagonists into the RVL, the pressor response to glutamate was shown to be unaffected. Also, the presynaptic nature of the blockade produced within the RVL by HC-3 was demonstrated by the failure of the depleting agent to inhibit to pressor responses to local microinjection of direct receptor agonists, while still inhibiting the pressor response to local cholinesterase inhibition (Giuliano et al., 1989). The limitations inherent in the use of general anesthesia are more difficult to address. At least one study has suggested that even the fall in blood pressure to bilateral electrolytic lesion of the RVL is anesthetic-dependent (Cochrane et al., 1988). Under

urethane anesthesia, the magnitude and duration of the hypotensive effect of RVL lesions is greater than when rats are anesthetized with either pentobarbital or α-chloralose. In one study in which pentobarbital was used, microinjection of the muscarinic receptor antagonist AF-DX116 in the RVL lowered blood pressure by approximately 20 mmHg for 60 to 150 min (Sundaram et al., 1988). The results of these studies suggest in the least that cholinergic input to the RVL represents an important component of the regulation of sympathetic excitatory tone from this nucleus.

The source of the cholinergic input to the RVL is currently unknown. However, stimulation of the hypothalamic defense area was shown to increase the content of acetylcholine in push-pull perfusates from the RVL (Lin and Li, 1990). Although interactions between diencephalic structures and the RVL have been demonstrated electrophysiologically, this was the first study to suggest a neurochemical link between the posterior hypothalamus and RVL involving acetylcholine. There is also some evidence that cholinergic neurons arising from spinal sites interact at medullary levels, possibly within the RVL (see Section III.C. and fig. 2).

Muscarinic receptors within the RVL that regulate arterial blood pressure appear to comprise mainly the nonpirenzepine-sensitive, possibly M_2-selective subtype (Pazos et al., 1986; Murugaian et al., 1989). The M_2 and M_4 subtypes appear to inhibit adenylate cyclase activity via a pertussis toxin-sensitive G-protein and are generally ascribed to mediating inhibitory synaptic responses (see McKinney and Coyle, 1991). The inhibitory nature of the M_2 and M_4 receptor subtypes fits with the physiological characterization of the neurotransmitter interactions in the RVL that suggest that cholinergic input to this region inhibits a γ-aminobutyric acid (GABA)ergic system that is inhibitory to efferent spinobulbar glutamatergic neurons (Arneric et al., 1990). Thus, cholinergic M_2 stimulation of the RVL essentially disinhibits the glutamate pathway (fig. 2). One additional feature of note for the RVL is that chemical lesions, or bilateral injection of cholinergic-depleting agents or muscarinic antagonists in anesthetized rats essentially eliminates the pressor response to intravenous injection of physostigmine (Giuliano et al., 1989).

The CVL, which has been less well characterized than either the NTS or the RVL, is essentially a caudal extension of the reticular nuclear group of the RVL, which originally was thought to include the A1 norepinephrine-containing cell group (Blessing and Reis, 1982). More recently, it has been determined that the CVL sympathoinhibitory neurons are functionally and anatomically distinct from the A1 cell group. Functionally, the CVL appears to be the opposite of the RVL. Electrical or chemical stimulation of the area with glutamate results in a vasodepressor response, whereas microinjection of GABA results in a pressor response (Willette et al., 1983, 1984; Murugaian et al., 1989). The latter finding suggests that GABAergic sympathoinhibitory neurons within the CVL are tonically active. As recently summarized by Dampney (1994), the CVL appears to play an important role in baroreceptor function, although many of the neurotransmitter interactions suggested to occur within this region are not yet completely understood. The interactions between the NTS, CVL, and RVL, insofar as these regions modulate the expression of the baroreceptor reflex, are schematically represented in figure 1. Stimulation of baroreceptor afferents is thought to activate excitatory amino acid receptors within the NTS. In turn, the NTS sends excitatory amino acid projections to the CVL that stimulate a GABAergic inhibitory pathway to the RVL. Thus, stimulation of baroreceptor afferent fibers ultimately results in the inhibition of tonically active sympathetic vasomotor efferents from the RVL.

Using partially selective muscarinic receptor agonists and antagonists, Murugaian and coworkers (1989) dem-

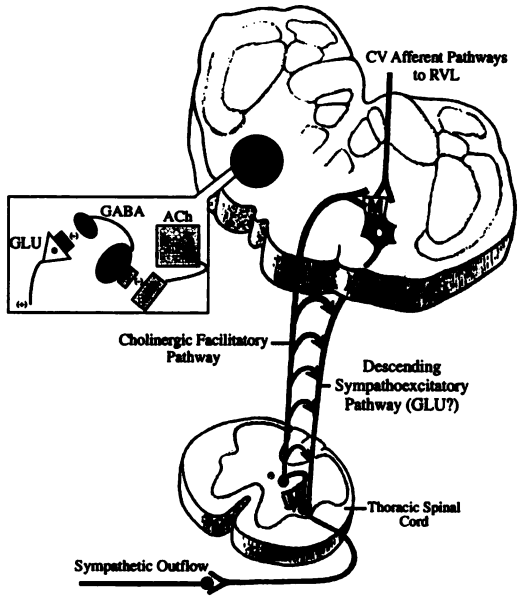

FIG. 2. Schematic representation of the proposed interactions that mediate the pressor responses to pharmacological activation of muscarinic cholinergic (M) sites in the rostral ventrolateral medulla (RVL) and thoracic spinal cord. The neurochemical nature of the pathway indicated as descending from the RVL and activating sympathetic preganglionic sympathetic cells is mediated by an excitatory amino acid, most likely glutamate. The pathway indicated by the arrows is probably intrinsic to the spinal cord, but may ascend to the medulla and provide, along with cholinergic pathways from other cardiovascular (CV) afferents, the cholinergic innervation to the RVL pressor pathway. The spinal cholinergic pathway does not directly innervate preganglionic cells, but provides an excitatory modulatory component via the bulbo-spinal pathway. Neurochemical interactions within the cholinergic facilitatory pathway involves the participation of spinal $GABA_B$ and glutamate NMDA receptors and may be similar to that already proposed for the RVL. Within the RVL, the release of acetylcholine activates muscarinic M_2 inhibitory (−) receptors that in turn inhibit the activity of GABAergic interneurons. The loss of inhibitory GABAergic tone leads to facilitation of descending vasomotor tone mediated by glutamatergic neurons.

onstrated in anesthetized rats, that non-M_1, possibly M_2 muscarinic receptors mediate the depressor response to receptor stimulation of the CVL. Bilateral microinjection of the partially selective M_2 receptor antagonist AFDX-116 into the CVL produced a modest increase in blood pressure, suggesting the presence of some degree of tonic cholinergic sympathoinhibitory tone. Finally, the depressor response to muscarinic receptor stimulation in the CVL was prevented in animals pretreated with the GABA antagonist bicuculline microinjected into the RVL. Therefore, the neuronal circuitry of the CVL involves, at least in part, a cholinoceptive (muscarinic) link to GABAergic neurons that relay inhibitory tone to the RVL.

C. Spinal Pathways

Before the mid-1980s, little was known regarding the role of spinal cholinergic systems in cardiovascular regulation other than perhaps the function of preganglionic cells located in the intermediolateral (IML) cell column. Autoradiographic and immunohistochemical studies have demonstrated that muscarinic cholinergic receptors and choline acetyltransferase-immunoreactive neurons exist in several regions of the spinal cord, including the superficial lamina, central autonomic cell column dorsal to the central canal, and the intermediolateral column (Yamamura et al., 1983; Barber et al., 1984; Borges and Iverson, 1986; Ribeiro-da-Silva and Cuello, 1990). Whereas the cholinergic neurons that exist in the superficial lamina were suggested to mediate antinociception (Yaksh et al., 1985; Smith et al., 1989), the functional role of the other spinal cholinergic cell groups has not been fully elucidated with regard to cardiovascular and respiratory functions. The results of studies using systemic administration of centrally acting muscarinic or nicotinic agonists in high spinal-transected animals proved to be equivocal (e.g., Dage, 1979; Bhargava et al., 1982). Also, direct microinjection of cholinergic agonists into spinal sites generally elicited heart rate, but not blood pressure responses (Sundaram et al., 1989a). The failure to observe pressor responses after spinal muscarinic receptor stimulation may have been attributable to the following factors: (*a*) Injections were made into high thoracic levels of the spinal cord, whereas spinal cholinergic pressor neurons are somatotopically organized, with tachycardic responses evoked predominantly from high thoracic and cervical levels and pressor responses elicited from lower thoracic and high lumbar levels (Magri and Buccafusco, 1988; Takahashi and Buccafusco, 1992). (*b*) The primary site of injection in the Sundaram study (Sundaram et al., 1989a) was the IML, whereas spinal sites responsive to muscarinic receptor stimulation are located outside of the IML (Takahashi and Buccafusco, 1992). (*c*) The density of muscarinic receptors becomes sparse proceeding caudally from the medulla, and the highly localized volumes of drug injectate (20 nl) used in the Sundaram study (Sundaram et al., 1989a) may not have activated a sufficient number of receptors to elicit a pressor response (Takahashi and Buccafusco, 1992). Using the techniques of i.t. injection, topical spinal cord application of drugs, and direct microinjection into the spinal cord parenchyma, studies from this laboratory over the past decade have been directed at the pharmacological characterization of a newly described spinal muscarinic pressor pathway. The following discussion summarizes some of the more pertinent findings (see Marshall and Buccafusco, 1985; Magri and Buccafusco, 1988; Buccafusco and Magri, 1990; Takahashi and Buccafusco, 1991a, b, 1992; Holland et al., 1993; Feldman and Buccafusco, 1993a, b). Intrathecal injection of neostigmine produced a marked pressor response in conscious and anesthetized rats. This response was inhibited by previous spinal acetylcholine depletion with i.t. pretreatment of HC-3 and by spinal muscarinic receptor blockade with atropine. When neostigmine was applied to the surface of the entire spinal cord, pressor and tachycardic responses could be observed that were similar to those seen after i.t. injection. Topical application of neostigmine to upper thoracic segments (Th1 to Th6) resulted in a tachycardic response of similar magnitude to that after i.t. injection, but with a pressor response that was significantly smaller in magnitude than that to i.t. injection. On the other hand, the topical application of neostigmine to lower thoracic segments (Th7 to Th11) resulted in a pressor response having similar magnitude to that after i.t. injection, with no apparent increase in heart rate. The application of neostigmine below Th12 evoked little change in blood pressure or heart rate.

To determine the role of each spinal segment in mediating pressor and tachycardic responses, experiments were performed in immobilized and artificially ventilated rats. Mapping of cholinoceptive sympathoexcitatory sites within the spinal cord was performed by microinjecting 0.54 nmol of carbachol into various regions within the Th2 and Th11 levels. Marked tachycardic responses and smaller pressor responses were observed after microinjection of carbachol into several sites, including the IML (in confirmation of the results obtained by Sundaram and coworkers (1989a)), a site between the IML and the central canal, the right lower dorsal horn, and the ventral horn. In contrast, when injected into lower thoracic levels (Th11), carbachol elicited a pressor response (but no heart rate response) from a site between IML and central canal. However, direct injection of the agonist into the IML failed to evoke a significant pressor or tachycardic response. Our finding of the inability of carbachol to increase blood pressure after direct microinjection into the IML is in concert with the more recent report of Gibson and Logan (1995) that demonstrated that superfusion of spinal sympathetic preganglionic neurons with carbachol resulted only in hyperpolarization of the cells.

Because it did not appear likely that stimulation of spinal muscarinic receptors were located on preganglion cells within the IML, the possibility that i.t. injection of cholinergic agonists facilitated tonic descending sympathetic activity was next examined. Stimulation of a descending sympathoexcitatory pathway was produced through electrical stimulation of the lateral funiculus between C1 and C2 after transection of the spinal cord above C1. Pressor and tachycardic responses were elicited by electrical stimulation of the lateral funiculus of the spinal cord, and the magnitude of each response increased in proportion to stimulus strength. Although i.t. administration of neostigmine (5 μg) did not alter resting blood pressure in the spinal transected animals, the drug produced a marked potentiation of the pressor responses to electrical stimulation. Atropine pretreatment did not alter resting blood pressure, but did block the facilitating action of neostigmine on the electrically evoked sympathetic activity. The pressor and tachycardic responses to electrical stimulation also were eliminated by placing an ipsilateral lesion in the segment below the stimulation site, a finding that confirmed the descending nature of the sympathetic pathway. Surgical denervation of the IX and X nerves had no effect on the responses to electrical stimulation of the lateral funiculus before or after neostigmine or atropine. Finally, approximately 50% of the pressor response to spinal cholinergic receptor stimulation was shown to be dependent on higher centers, because selective depletion of medullary acetylcholine with HC-3 or blockade of medullary muscarinic receptors with atropine significantly reduced the magnitude of the pressor response to i.t. injection of carbachol.

In summary, it appears that spinal cholinergic receptor activation produces a potentiation of descending sympathoexcitatory tone. Although it is not likely that this interaction is mediated directly at the level of the preganglionic cell bodies, descending bulbospinal pressor neurons may provide the substrate through which spinal administration of cholinergic agonists increases blood pressure and heart rate. The origin of the cardiac response is clustered within the upper thoracic region, whereas the pressor response is distributed throughout the thoracic cord. Thus, the spinal cord contains a powerful cholinergic, muscarinic modulatory system (possibly interneurons) that is not tonically active, but which can modify bulbospinal neuronal influences to sympathetic preganglionic neurons. A schematic representation of this ascending cholinergic pressor pathway is depicted in figure 2.

IV. Interaction Between Cholinergic Neurons and Other Neurotransmitters

A. Biogenic Amines and Clonidine

The mutually antagonistic roles for peripheral autonomic adrenergic and cholinergic neurons have been well studied. In dually innervated organs, activation of the release of norepinephrine from sympathetic neurons impairs the release of acetylcholine from parasympathetic neurons, and vice versa. This relationship between the transmitters is known to exist in the CNS; however, the phenomenon has not been as well characterized. The issue arose early on with regard to the investigation of the mechanisms involved in the pressor response to physostigmine and other centrally acting cholinergic agonists. Biochemically, there exists significant evidence that in certain brain regions, including those involved in cardiovascular regulation, the hypothalamus and medulla, cholinergic muscarinic receptor agonists inhibit the release of norepinephrine (see Philippu, 1981; Brezenoff and Giuliano, 1982), and adrenergic agonists inhibit the release of acetylcholine (see Buccafusco, 1992). Therefore, it was possible that the pressor response to intravenous injection of physostigmine was mediated not solely through the stimulation of central muscarinic receptors, but also through the subsequent release of brain catecholamines. The results of several studies performed before 1980 demonstrated that depletion of brain catecholamines or blockade of central α-adrenergic receptors could inhibit the expression of the pressor response to central cholinergic stimulation (see Philippu, 1981; Brezenoff and Giuliano, 1982). One inherent weakness with many of the early studies was related to the use of cerebroventricular administration of sympatholytic agents. The doses used were usually quite high, often as high as 100 $\mu g/kg$, and rarely was there a control experiment provided to account for redistribution of the blocking agent to the peripheral circulation. Even drugs that do not readily penetrate the CNS from peripheral administration, such as norepinephrine and phentolamine, are rapidly transported out of the CNS, where they can have peripheral vascular actions (Buccafusco and Brezenoff, 1977). In a more recent study in which selective α-adrenergic blocking drugs were used by i.c.v. injection, the doses that appeared to block the pressor response to subsequent i.c.v. injection of neostigmine ranged from 40 to 120 $\mu g/kg$ (Taira and Enero, 1994). Under these experimental conditions, the α_1-selective antagonist prazosin, but not the α_2-selective antagonist yohimbine, blocked the pressor response to neostigmine. The authors interpreted the data to indicate that there exists an adrenergic link via central α_1-adrenergic receptors mediating the response to neostigmine. An alternate explanation is that some of the antagonists may have been redistributed to the peripheral circulation, where prazosin is the more effective sympatholytic agent.

Of particular relevance is a study by Stamenović and Varagić (1970), in which they measured preganglionic nerve activity in anesthetized rats treated intravenously with physostigmine. Under these circumstances, physostigmine produced a dose-related increase in arterial blood pressure and concomitant increase in cervical

sympathetic nerve activity. In reserpinized rats, there was no alteration of the increase in sympathetic nerve activity compared with controls. In contrast, the same reserpine treatment abolished the pressor response to physostigmine (Lešić and Varagić, 1961). Thus, under conditions of combined central and peripheral catecholamine depletion, the sympathoexcitatory response but not the pressor response to central cholinesterase inhibition remained intact. This would be the result expected if peripheral sympathetic, but not central adrenergic pathways, mediate the pressor response to physostigmine.

The confusion of this issue is perhaps underscored by the results of two studies reported by the same research group. In the first study, i.c.v. administration of the adrenergic neurotoxin 6-hydroxydopamine was used in rats to cause the depletion of brain catecholamines (Gordon et al., 1979). In central amine-depleted animals, the pressor response to central cholinergic receptor stimulation was blocked. In a subsequent study (Gordon et al., 1985), specific ascending and descending noradrenergic and dopaminergic pathways were depleted of transmitter through selective microinjection of 6-hydroxydopamine into respective areas of cell origin for each pathway. In this situation, there was no significant effect of brain catecholamine depletion on the pressor response to i.c.v. injection of carbachol, even though the extent of depletion of catecholamines in their terminal fields was similar to that for the earlier i.c.v. study. The authors suggested that the apparent discrepancy was related to the possibility that, with i.c.v. injection of the neurotoxin, a site of catecholamine depletion not affected by the methods used in the second study contained the crucial adrenergic link involved in the pressor response to carbachol. Alternatively, they suggested that the neurotoxin may have produced a nonspecific toxic action on periventricular structures necessary for the cardiovascular action of i.c.v.-injected carbachol.

In one respect, the independence (rather than the dependence) of the pressor response to physostigmine from central catecholaminergic systems is more consistent with current findings. Accepting the evidence cited above that the site of action of intravenously administered physostigmine is the RVL, the majority of cholinergic terminals in this brain region does not form a significant number of synaptic contacts with the catecholamine/PNMT-containing cells of the C1 region (Milner et al., 1989). PNMT cells arising from the C1 region descend to the spinal cord along the same trajectory as the bulbospinal pathway that provides tonic sympathetic nervous activity to sympathetic preganglionic cells in the ILM. Although the concept of an adrenergic link in the cholinergic pressor system is far from settled, future studies must account for the many subtypes of adrenergic receptors known to exist in the brain, as well as the nature of the cellular response mediated by each subtype. Also, care must be taken to insure that the antagonists being studied have actions only within the CNS.

In 1977, the currently recognized pharmacological properties of clonidine included the peripheral actions, hypertension, vasoconstriction, contraction of the nictitating membrane, and hyperglycemia (see Schmitt, 1977). In addition, clonidine possessed a number of pharmacological properties that were attributable to an action on the CNS. These included hypotension, inhibition of sympathetic tone, activation of vagal tone, bradycardia, sedation, antinociception, hypothermia, inhibition of water intake, inhibition of food intake and aggressive behavior. Direct evidence for the central site of clonidine's blood pressure-lowering ability was first provided by Kobinger and colleagues in the mid-1960s and by Schmitt and colleagues in the early 1970s. The details of these experiments have been extensively reviewed (Schmitt, 1977; Kobinger, 1978). Although clonidine has been reported to directly inhibit the release of norepinephrine from sympathetic fibers, this is usually achieved by high, supraclinical doses or at low, nonphysiological stimulation frequencies (Schmitt, 1977). In contrast, the release of acetylcholine from parasympathetic nerve terminals in several tissues is quite sensitive to inhibition by clonidine and other α_2-adrenergic agonists, including norepinephrine (Deck, et al., 1971; Werner et al., 1972; Drew, 1978). This parasympatholytic action of clonidine is often encountered as side effects associated with therapy. For example, patients often complain of dry mouth, constipation, and visual disturbances.

Perhaps the earliest study to indicate an interaction between clonidine and central cholinergic neurons involved in cardiovascular regulation was reported by Bently and Li (1968), who demonstrated that clonidine pretreatment in rats prevented the centrally mediated hypertensive response to the cholinesterase inhibitor physostigmine. Subsequently, it was demonstrated that pretreatments that increased central cholinergic activity reduced the hypotensive actions of clonidine (Laubie, 1975). Characterization of the clonidine/physostigmine interaction was then carried out by Buccafusco and colleagues (Buccafusco and Spector, 1980a), who confirmed the marked inhibitory action of clonidine on the hypertensive response to physostigmine. They demonstrated that the ability of clonidine to inhibit the pressor response to cholinergic stimulation was selective for indirect-acting agonists such as physostigmine. The pressor response produced by arecoline, a direct muscarinic receptor agonist, was not sensitive to clonidine pretreatment (Buccafusco and Spector, 1980b). This observation was consistent with the finding that clonidine produced a significant reduction in the biosynthesis of acetylcholine in several regions of rat brain, particularly in regions important for cardiovascular regulation, the hypothalamus and medulla (Buccafusco and Spector, 1980a; Buccafusco, 1982, 1984a). The latter action of clonidine

was demonstrated to be mediated through stimulation of central α-adrenergic receptors. Also, both clonidine and the related drug α-methyldopa produced decreases in blood pressure and inhibition of brain acetylcholine biosynthesis in hypertensive animals at respective clinically relevant doses (Buccafusco 1984a, b). The proposed site for clonidine's antihypertensive action, the RVL, is also the site that mediates the hypertensive response after systemic injection of physostigmine (Punnen et al., 1986; Giuliano et al., 1989). Moreover, the RVL is the only brain region demonstrated thus far to mediate a significant fall in blood pressure in normotensive rats after acetylcholine depletion or muscarinic blockade (Sundaram and Sapru, 1988; Giuliano et al., 1989). Therefore, the cholinergic pressor neurons within the RVL may represent a potential site for some of the cardiovascular actions of clonidine and related antihypertensive agents.

Although clonidine is a potent agonist at central and peripheral α_2-adrenergic receptors, Bousquet and colleagues (1984) reported that the hypotensive action of clonidine may be more related to its chemical structure as an imidazole than to its ability to act as an α_2-agonist. Subsequently, Reis and colleagues (Ernsberger et al., 1987) determined that norepinephrine was not able to completely inhibit [^{3}H]-*p*-aminoclonidine binding from a membrane preparation derived from the ventrolateral medulla. This 'imidazole' binding site was then characterized and demonstrated to exhibit affinity for clonidine equal to the classical α_2-adrenergic receptors in the RVL. Also, the relative potencies of a series of imidazoline and non-imidazoline clonidine-like antihypertensive drugs to lower blood pressure after intracisternal injection in SHR were strongly correlated with their ability to interact with imidazoline receptors, but not with α_2-adrenergic receptors, in vitro (Buccafusco et al., 1995a).

To complicate the picture further, based upon homology screening of complementary deoxyribonucleic acid (cDNA) libraries from several species, three subtypes of the α_1-adrenergic receptor ($\alpha_{1a/d}$, α_{1b}, α_{1c}) and four subtypes of the α_2-adrenergic receptor (α_{2a}, α_{2b}, α_{2c}, α_{2d}) have been cloned and partially pharmacologically and functionally characterized (see Bylund et al., 1994). Other subtypes are likely to be forthcoming. Although the catecholamines norepinephrine and epinephrine have generally been characterized as inhibitory neurotransmitters in the CNS, it is clear that this simplistic view can no longer be maintained. The cellular response to norepinephrine in a particular brain region will depend upon the relative proportions of these receptor subtypes being stimulated, and whether the receptors are located on pre- or postsynaptic aspects of the nerve ending. This concept is underscored by the finding that microinjection or superfusion of epinephrine onto identified spinal preganglionic neurons in a slice preparation from neonatal rat spinal cord evokes both depolarization and hyperpolarization of single units (Miyazaki et al., 1989). Depolarization of preganglionic cells was mediated through α_1-receptors, and hyperpolarization was mediated through α_2-receptors. That α_1-receptors mediate the pressor response to local microinjection of epinephrine into the IML was confirmed in intact adult rats (Malhotra et al., 1993). As more specific pharmacological probes become available, or perhaps with the development of transgenic knockouts, as has been reported for the α_{2a} receptor (Surprenant et al., 1992), experiments to elucidate the role of each subtype in central cardiovascular regulation and the nature of each one's interaction with cholinergic neurons will be possible.

In many respects, knowledge of the pharmacology of central serotonin systems has advanced faster than that for central adrenergic systems, particularly with regard to the development of subtype selective ligands. In terms of the development of clinically useful centrally acting drugs modifying arterial pressure, the adrenergic system has the advantage. Nevertheless, there are perhaps many parallels between central adrenergic and serotonergic neuronal systems involved in cardiovascular regulation. Both adrenergic and serotonergic pathways include many areas known to play a role in central cardiovascular regulation, including the anterior hypothalamus, the NTS and RVL, and the spinal cord. As discussed with the adrenergic system, serotonin acts upon multiple subtypes of pre- and postsynaptic receptors, and pressor or depressor responses appear to be mediated by different subtypes (see McCall and Clement, 1994). In fact, 5-HT$_{1A}$ and 5-HT$_2$ receptors located within the RVL mediate respectively a sympathoinhibitory and a sympathoexcitatory response. Not unexpectedly, the administration of serotonin into the CSF or directly microinjected into sensitive brain regions has been reported to elicit a variety of cardiovascular changes. Unfortunately, the role of central cholinergic neurons in the expression of cardiovascular responses to central serotonin receptor stimulation or blockade has not been attempted using serotonin receptor subtype-selective drugs. However, using serotonin itself, the results of microinjection studies have indicated that a cholinergic link exists within the forebrain pathway mediating the pressor response to serotonin (Robinson, 1982; Kristić and Djurković, 1987).

Within the concept of the "cholinergic link," it may be of interest to underscore the considerable number of brain neurotransmitters for which a cholinergic link has been suggested to participate in the expression of the cardiovascular response to that neurotransmitter. Table 2 lists 12 such substances. The interactions between the biogenic amines and cholinergic neurons already have been described earlier in this section. The neuropeptides listed all evoke pressor responses after central administration and all require either the release of endogenous acetylcholine and/or functioning brain cholinergic receptors to elicit their respective responses. Both muscarinic

TABLE 2

The role of brain cholinergic neurons in mediating the changes in systemic blood pressure to the central actions of several neuromediators

Neuromediator	Blood Pressure Response to Neuromediator	Representative References
Serotonin	Increase	Robinson, 1982; Krstić and Djurković, 1987
Angiotensin II	Increase	Moore and Drexler, 1982; Buccafusco and Serra, 1985
Substance P	Increase	Trimarchi et al., 1986; Lin et al., 1990
Bradykinin	Increase	Buccafusco and Serra, 1985
Calcium channel antagonist (viz. nicardipine)	Increase	Montastruc et al., 1987
Thyrotropine releasing hormone	Increase	Okuda et al., 1987
Nicotine	Increase	Buccafusco and Yang, 1993
Naloxone (morphine-dependent)	Increase	Buccafusco, 1992
Norepinephrine (viz. clonidine)	Decrease	Buccafusco, 1992
Glycine	Decrease	Talman et al., 1991
Prostaglandin I_2	Decrease	Saito et al., 1985
Opiates	Decrease	Willette et al., 1987

and nicotinic receptors have been shown to participate in the expression of the pressor response to substance P, angiotensin II, and bradykinin (Buccafusco and Serra, 1985; Trimarchi et al., 1986). It was, therefore, of interest to characterize the nature of the pressor response to central administration of nicotine itself. In conscious rats, i.c.v. injection of nicotine produced a dose-dependent increase in blood pressure. As expected, the pressor response to nicotine was abolished by previous i.c.v. injection of the nicotinic receptor antagonist hexamethonium. Of greater relevance, however, was the observation that depletion of brain acetylcholine levels with HC-3 or blockade of brain muscarinic receptors with atropine each inhibited the expression of the nicotine-induced pressor response (Buccafusco and Yang, 1993). The inference was that nicotine's actions were mediated through stimulation of presynaptic excitatory nicotinic receptors. The evoked release of acetylcholine (which was confirmed experimentally, in vitro) then stimulated atropine-sensitive (muscarinic) receptors to elicit the hypertensive response to nicotine.

The apparently ubiquitous role for cholinergic neurons and perhaps muscarinic receptors in central cardiovascular regulation suggests a central integrative/modulatory role for the transmitter. Nevertheless, it should be considered that the brain appears able to regulate the cardiovascular system after significant disruption (inhibition) of cholinergic neurons. This may be true for most neurotransmitters. Thus, the relative importance of central cholinergic neurons with regard to other transmitter systems in ongoing blood pressure regulation has not been established. However, it is clear that excitation of central cholinergic neurons can severely disrupt cardiovascular function with potentially dire consequences.

B. Glutamate and γ-Aminobutyric Acid

The interactions between cholinergic, GABAergic, and glutamatergic neurons in central cardiovascular regulation have been characterized most thoroughly for sites within the CVL and the RVL. Using a combination of microinjection, anatomical, immunohistochemical, and electrophysiological techniques, the results from several laboratories have provided a working model for the neural interactions within the baroreceptor reflex arc of the medulla and spinal cord (see Lorenz et al., 1985; Guyenet et al., 1987; Gordon, 1987; Giuliano et al., 1989; Sapru, 1989; Arneric et al., 1990; Ruggiero et al., 1990; Dampney, 1994). Although cholinergic neurons have pharmacological actions within the NTS, CVL, and RVL, all of which participate in modifying baroreceptor reflex function (fig. 1), elimination of cholinergic neuronal activity within the NTS or CVL does not dramatically alter the normal function of the reflex. Within the RVL, local cholinergic neurons inhibit (via stimulation of M_2 muscarinic receptors) the release of GABA onto reticulospinal sympathoexcitatory neurons. Therefore, cholinergic agonists acting within the RVL essentially disinhibit descending tonic sympathetic activity (fig. 2). Cholinergic inhibitory activity to neurons within the RVL appears to be tonic in nature because muscarinic receptor antagonists or acetylcholine-depleting agents microinjected into the area elicit a marked fall in arterial pressure. The origin of these cholinergic RVL neurons has not been determined, although it has been suggested that they may arise from cells intrinsic to the RVL, as well as from other areas that project to the nucleus (see Arneric et al., 1990).

Although no major cholinergic pathways arising from the lateral medulla appear to descend to spinal sympathetic cells, afferent cholinergic projections from ILM to RVL may exist (Ruggiero et al., 1986, 1990). This projection may form the anatomical construct for the spinobulbar cholinergic pressor pathway that was pharmacologically characterized by Buccafusco and colleagues as described above (see Section III.C.). The results of preliminary studies from this laboratory suggest that the pharmacological interactions reported to exist within the RVL may also occur within the spinal cord (Feldman and Buccafusco, 1992, 1993b). For example, i.t. or intracisternal pretreatment with the N-methyl-D-aspartate (NMDA) receptor antagonists D(−)-2-amino-7-phosphonohepatanoic acid (D-AP7) or i.t. administration

of dizocilipine maleate (MK801) attenuated the pressor response to i.t. administration of the carbachol in anesthetized rats. In contrast, i.t. pretreatment with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA antagonist, was not effective in this regard, indicating that the carbachol-evoked pressor response was mediated specifically by the NMDA-subtype of glutamate receptors. Intrathecal pretreatment with the $GABA_B$ receptor agonist baclofen also inhibited the pressor response to i.t. carbachol at doses that did not alter motor function. Thus, cholinergic neurons may indeed inhibit the function of spinal GABAergic neurons that have been shown to provide inhibitory tone to sympathetic preganglionic neurons (Gordon, 1985). These findings parallel the results described in section III.B. for the RVL in that cholinergic activation of the spinal pressor system requires the participation of both spinal glutamate-NMDA and $GABA_B$ receptor systems. As depicted in figure 2, this spinal cholinergic pressor system may represent a continuum of pressor pathways ascending to the medulla that use a similar neurochemical relationship—cholinergic (M_2 receptor) disinhibition of descending glutamatergic (NMDA) sympathetic tone through inhibition of GABAergic inhibitory interneurons.

C. Nitric Oxide

It has recently been established that cholinergic agonists can cause relaxation of peripheral blood vessels through stimulation of endothelial muscarinic receptors. Receptor activation in turn leads to the production and release of endothelium-derived relaxing factor, now known to be nitric oxide (NO) (Shibuki and Okada, 1991; Bredt and Snyder, 1992). NO synthetic pathways have also been discovered in the CNS. Influx of Ca^{2+} during depolarization of the postsynaptic cell activates a calmodulin-dependent enzyme, nitric oxide synthase (NOS). In the CNS, NO was first linked to glutamatergic synapses rather than cholinergic synapses. In fact, NO may underlie the phenomenon of long-term potentiation exhibited by certain hippocampal cells possibly involved in learning and memory (Schuman and Madison, 1991). More recently, it has been recognized that at least two isozymes of the constitutive form of the enzyme exist that have different gene products (Sessa et al., 1993). Although both the endothelial form and brain form exist in the CNS, the brain form is more prevalent. The brain form of NOS has been linked to the actions of several neurotransmitters, including acetylcholine (Rettori et al., 1992; Bickford et al., 1993; Castoldi et al., 1993; Price et al., 1993), and is found in high concentrations in both the hypothalamus (Rettori et al., 1992) and the thoracic spinal cord, particularly in the dorsal horn and intermediolateral column (Lin et al., 1993; Liuzzi et al., 1993; Terenghi et al., 1993). Gebhart and his colleagues (reported in Meller et al., 1992) have reported that agonists of the NMDA subtype of the glutamate receptor facilitated a thermal nociceptive spinal reflex. This facilitation was mediated by the NOS system within the spinal cord. As with the hippocampal system, NO activated a soluble guanylate cyclase system in the spinal cord. In a more recent study (Zhuo et al., 1993), they demonstrated that spinal NO release was under tonic control by a cholinergic (muscarinic) system.

Of particular relevance is the report that systemic administration of the centrally acting cholinesterase inhibitor tacrine produced an atropine- and L-N^G-nitro-L-arginine methyl ester-reversible five-fold increase in the level of hippocampal NOS activity measured in vitro 15 min after tacrine administration (Bagetta et al., 1993a). Although the brain form of NOS is generally considered a constitutive enzyme, muscarinic receptor stimulation has been shown to both activate protein kinase C and increase intracellular Ca^{2+}. Either effect can enhance both the mRNA encoding the brain form of NOS as well as the NOS protein itself (Bagetta et al., 1993b).

Systemic administration of NOS inhibitors recently has been demonstrated to interfere with the expression of morphine withdrawal symptoms (Adams et al., 1993; Cappendijk et al., 1993). We have suggested that an intrinsic cholinergic muscarinic system within the spinal cord initiates the expression of many of the autonomic and behavioral symptoms of precipitated morphine withdrawal (see Buccafusco, 1992). The cholinergic spinobulbar pressor pathway discussed above most likely mediates the sympathoexcitatory aspects of spinal cord morphine withdrawal. The results of our more recent studies suggest that stimulation of spinal muscarinic receptors, directly or indirectly, activates an NMDA receptor-linked NOS system in which the symptoms of naloxone-precipitated morphine withdrawal are mediated by local NO production (Buccafusco et al., 1995b). Thus, i.t. pretreatment with either muscarinic receptor antagonists (non-M, possibly M_2 subtype), with glutamate NMDA receptor antagonists, or with NOS inhibitors blocked the pressor response to naloxone in morphine-dependent rats (Holland et al., 1993; Buccafusco et al., 1995b). Although at present it is not clear which of these spinal transmitter systems is directly linked to NO production, we have reported that inhibition of spinal NOS prevents the expression of the pressor response to spinal muscarinic receptor stimulation in nondependent rats (Feldman and Buccafusco, 1993b).

V. Role of Central Cholinergic Neurons in Animal Models of Hypertension

A. Central Cholinergic Activation

Developed by selective inbreeding of the Wistar/Kyoto strain, the SHR has become one of the most widely used models for the human disease. The reader is referred to a number of excellent reviews regarding the development and relevance of the SHR and other genetic models

of hypertensive disease (Okamoto and Aoki, 1963; Yamori, 1977, 1984; Dietz et al., 1978) as well as some limitations inherent in the model (McGriff and Quilley, 1981; Trippodo and Frohlich, 1981). Some features of interest regarding spontaneous hypertension should be pointed out. SHR at birth exhibit arterial pressures that are not considered hypertensive; however, as they become adults, blood pressure gradually increases until it is maintained at a markedly elevated level after approximately 12 weeks of age. In young animals, elevated blood pressure is generally maintained by enhanced central sympathetic outflow. After several months of hypertension, however, vascular, cardiac, and perhaps hormonal adaptation takes place that helps maintain the hypertensive state, although enhanced sympathetic function always appears to be present. This concept of enhanced sympathetic function in the SHR is important both with regard to the discussion in the Section I.A. of this review regarding the role of the sympathetic nervous system in mediating the morbidity and mortality associated with the human disease and with regard to the fact that central sympathetic activity mediates the hypertensive response to cholinergic receptor stimulation. Indeed, in one of the earliest reports of a potential neurochemical derangement in the SHR, Yamori and colleagues (Yamori, 1976) provided evidence that the activities of both acetylcholinesterase and choline acetyltransferase, respectively the degredative and synthetic enzymes for acetylcholine, were each elevated in the brainstem of SHR compared with two normotensive strains of rats. One of these was the ancestor strain of the SHR, the Wistar/Kyoto (WKY) rat. Unlike the rate-limiting enzymes of the biogenic amine biosynthetic pathways, neither cholinergic enzyme is very responsive to changes in cholinergic neuronal activity. Increases in the levels of these enzymes might signify an increased density of cholinergic cells or fibers in the brainstem of the SHR. The finding of elevated brain cholinergic enzyme levels in SHR was subsequently confirmed (Bagjar et al., 1979).

Helke and colleagues (1980) measured choline acetyltransferase activity in micropunches taken from over 40 brain nuclei from age-matched SHR and WKY. Only two brain regions exhibited increases in choline acetyltransferase: the nucleus gigantocellularis and the locus ceruleus. The finding that choline acetyltransferase activity is increased in the nucleus gigantocellularis of prehypertensive SHR is at least consistent with the possibility that this brain region participates in the initiation of spontaneous hypertension. That the RVL is essentially a subregion of the nucleus paragigantocellularis lateralis is perhaps suggestive of such a role for the cholinergic (muscarinic) pathway of the RVL. The fact that no change in choline acetyltransferase activity was found in the nucleus gigantocellularis for desoxycorticosterone (DOCA)-salt-induced hypertensive rats (Helke et al., 1980) indicates that the increase found for SHR was not caused by the hemodynamic changes associated with early phase hypertension. This apparently was not the case for certain hypothalamic nuclei that exhibited late stage decreases in enzyme activity, because similar decreases were observed in the DOCA-salt rats. In fact, the decrease in paraventricular nucleus hexokinase activity—measured in vitro as an index of neuronal activity—was observed in both SHR and renal hypertensive rats compared with age-matched normotensive controls (Krukoff, 1988). As with the choline acetyltransferase activity, the author of the latter study concluded that the apparent decrease in hypothalamic metabolic activity in SHR was most likely not related to the genetic makeup of SHR, but possibly caused by a reflexive reduction in excitatory tone within this region developed to oppose the elevated blood pressure.

Based upon their findings of elevated cholinergic enzymes, Yamori and colleagues (1976) were the first to suggest that this apparent derangement of cholinergic neurochemistry in SHR might result in some altered pharmacological manifestation. In support of this contention was their experiment demonstrating the ability of i.c.v. injection of carbachol to evoke an exaggerated pressor response in SHR compared with normotensive controls. Subsequently, Hoffman and coworkers (1978) demonstrated that low doses of carbachol (ng range) given by the i.c.v. route were more potent in evoking pressor responses in SHR with established hypertension compared with WKY normotensive controls. The authors suggested that both heightened vascular responsivity to circulating vasopressin, as well as enhanced sympathetic nervous activity, mediated the heightened pressor response to i.c.v. injection of carbachol in SHR. Somewhat similar results were obtained by Takahashi and coworkers (1984). They found that i.c.v. injection of carbachol in urethane-anesthetized rats elicited a hypertensive response that was significantly greater in magnitude in SHR compared with that measured for either an out-bred normotensive Wistar strain, or for the WKY strain. Of particular interest was their observation that carbachol elicited a biphasic response in the out-bred Wistar group—an initial fall, followed by the characteristic pressor response. This initial hypotensive effect was not observed in either WKY or SHR, even though the pressor response to carbachol was greater in SHR. These results could reflect the first evidence of a phenotypical difference between WKY and other out-bred normotensive strains in the central regulation of cholinergic cardiovascular function.

Because carbachol generally acts as a cholinergic receptor agonist, the results of the above studies did not clarify whether presynaptic aspects of cholinergic neurotransmission contributed to the alterations of central cholinergic function in the hypertensive strain. Confirmation of this possibility was provided by studies in which inhibitors of brain cholinesterase were used (Kubo and Tatsumi, 1979; Buccafusco and Spector,

1980a). Kubo and Tatsumi (1979) reported that intravenous injection of physostigmine evoked an exaggerated pressor response in SHR compared with WKY controls. In contrast, the pressor response to physostigmine was not enhanced in either two-kidney Goldblatt hypertensive rats or DOCA-salt hypertensive rats compared with their respective normotensive controls. Thus, central cholinergic mechanisms involved in generating the enhanced pressor response to cholinergic stimulation are not dependent upon the level of elevated blood pressure per se and appear to be selective for the genetic model of hypertension. The pressor response to physostigmine was abolished by intravenous injection of atropine but not the quaternary derivative methylatropine, confirming the central muscarinic nature of the response. In an attempt to determine whether enhancement of central muscarinic receptor function was responsible for the enhanced pressor response to physostigmine in SHR, the authors used the direct-acting muscarinic receptor agonist oxotremorine. Like physostigmine, oxotremorine was shown to elicit an atropine-sensitive pressor response in normotensive rats. However, in contrast to the effect of physostigmine, intravenous injection of oxotremorine did not elicit an enhanced pressor response in SHR. From these results, it was concluded that hyperresponsiveness to central cholinergic stimulation was not dependent upon the status of central muscarinic receptors, but possibly upon presynaptic mechanisms. Also, this was the first study to reveal a dichotomy between the effects of direct- and indirect-acting cholinergic agonists in SHR. The possibility that this dichotomy was because of different sites of action was suggested also by the finding that the exaggerated pressor response to physostigmine in SHR was observed when the inhibitor was administered by the intravenous or brain intracisternal routes of administration (Buccafusco et al., 1990), whereas the pressor response to i.c.v. administration of physostigmine was only slightly potentiated in SHR. Because physostigmine given intravenously or intracisternally is more likely to affect medullary cholinergic sites and i.c.v. injection of carbachol appears to be mediated primarily within the hypothalamus, the possibility exists that different mechanisms underlie the heightened pressor response to muscarinic receptor stimulation within these two brain regions. This possibility is further suggested by the observation that the heightened pressor response to intravenous injection of physostigmine in SHR, unlike the response to i.c.v. injection of carbachol, does not involve the release of vasopressin (Kawashima et al., 1986, 1987).

Although structural changes have been reported to occur in certain blood vessels, and enhanced vascular resistance within perfused limbs in situ is well known to occur in young, even prehypertensive SHR (e.g., Cheng and Shibata, 1980; Yamori, 1984), it is the enhanced centrally mediated neurogenic tone that appears to maintain the hypertensive state during the first 3 or 4 months of life (Yamori, 1976, 1984). Moreover, the exaggerated pressor responses elicited by central cholinergic stimulation in this strain appear to be primarily neurogenic in nature and not caused by heightened peripheral vascular reactivity. This contention is supported by the results of the studies listed in table 3. Clearly, only certain pharmacological classes of cholin-

TABLE 3

Effect of pharmacological stimulation of central cholinergic neuronal function on blood pressure in SHR

	Route	Age Weeks	Drug	Change in Blood Pressure Ratio: SHR/WKY
Yamori, 1976	i.c.v.	adult	carbachol, physostigmine	↑
	i.c.v.	adult	angiotensin II	–
Hoffman et al., 1978	i.v.	adult	ADH	–
	i.c.v.		carbachol	↑
Kubo and Tatsumi, 1979	i.v.	10	physostigmine	↑
	i.v.		oxotremorine	–
Makari et al., 1989	i.v.	18–20	physostigmine	↑
			arecoline	–
Buccafusco and Spector, 1980a	i.v.	12–14	DMPP	–
	i.v.		physostigmine	↑
Takahashi et al., 1984	i.c.v.	16	carbachol	↑
	i.v.		ADH	–
Kawashima et al., 1986	i.a.	15	physostigmine	↑
Kawashima et al., 1987	i.a.	15	physostigmine	↑
Buccafusco et al., 1990	i.c.v.	16–20	arecoline, physostigmine	–
	i.c.		physostigmine	↑
Buccafusco and Magri, 1990	i.t.	16–20	neostigmine, carbachol	↑
Kubo et al., 1995	RVL	12–16	physostigmine	↑
			glutamate, ACh, carbachol	–
	i.v.		norepinephrine	–

ADH, antidiuretic hormone; DMPP, dimethylphenylpiperizinium.
i.a., intra-arterial; i.c., intracisternal; i.v., intravenous.
↑, increase; ↓, decrease; –, no change between strains.

ergic agonists, and only specific routes of administration, were associated with the eliciting of exaggerated pressor responses in SHR. Also, in four of the studies, intravenous administration of peripheral vasoconstrictor agents were shown to evoke pressor responses similar in magnitude to those obtained from the normotensive controls.

B. Central Cholinergic Inhibition

Two laboratories reported simultaneously that depletion of brain acetylcholine by using i.c.v. injection of HC-3 resulted in a marked fall in blood pressure in SHR (Brezenoff and Caputi, 1980; Buccafusco and Spector, 1980a). The results of both studies were consistent in that HC-3 was not very effective in lowering blood pressure in WKY. The time course of the fall in blood pressure and its recovery appeared to be correlated with changes in the levels of acetylcholine in the hypothalamus, more so than with those in the midbrain-pons or medulla (Buccafusco and Spector, 1980a). Because HC-3 is quite selective in its action to inhibit cholinergic function, the results of these initial studies with the depleting agent demonstrated for the first time that cholinergic neuronal activity played a role in maintaining the heightened sympathetic activity associated with hypertension. It is worth mentioning that concomitant with these studies, evidence was accumulating in favor of the possibility that at least part of the mechanism for the antihypertensive action of clonidine and other related drugs was caused by their ability to inhibit the biosynthesis and release of acetylcholine in certain brain regions (see Buccafusco, 1982). In one such study, both intravenous administration of α-methyldopa and i.c.v. injection of HC-3 were demonstrated to evoke an antihypertensive response in SHR (Buccafusco, 1984c). When subthreshold doses of each drug were administered simultaneously, a fall in blood pressure was recorded that was greater than the sum of the two individual responses. This synergistic action between the centrally acting α_2-adrenergic receptor agonist and the acetylcholine depleting agent suggested that the two drugs were acting through a common mechanism to evoke their antihypertensive responses—inhibition of central cholinergic function.

The most comprehensive study of the cardiovascular actions of central administration of HC-3 was reported by Giuliano and Brezenoff (1987). They compared the ability of i.c.v. injection of HC-3 to lower blood pressure in the SHR and three nongenetic models: DOCA-salt hypertensive rats, Grollman renal hypertensive rats, and aortic coarctation-induced hypertensive rats. In SHR, the ability of HC-3 to lower blood pressure was dependent upon the age of the animal, rather than the hypertensive state. In fact, in SHR whose blood pressure was lowered essentially to normotensive levels through infusion of a peripheral vasodilator, i.c.v. injection of HC-3 was still able to evoke a significant fall in blood pressure. HC-3 was most effective after hypertension was initiated, and did not appear to lower blood pressure in prehypertensive SHR. In concert with the earlier studies, HC-3 produced a much weaker hypotensive action in WKY controls. In DOCA-salt rats, HC-3 not only produced a stable fall in blood pressure at all stages during the development of hypertension, but was also effective in lowering blood pressure in prehypertensive animals. HC-3 lowered blood pressure in the aortic coarctation model, but only after an initial period of at least 1 week of sustained hypertension. The initial phase of hypertension in this model is primarily renin-dependent, but after the return to normal renin levels, hypertension is maintained by other factors, including central sympathetic outflow. The greater antihypertensive effectiveness of HC-3 treatment in aortic coarcted rats as renin levels normalized is consistent with the role of the sympathetic nervous system in mediating the pressor responses to central cholinergic receptor stimulation. In support of this contention was the observation that i.c.v. injection of HC-3 also elicited a hypotensive response in the one-kidney Grollman hypertensive rat, another hypertensive model that is not renin-dependent. The authors concluded, therefore, that although the mechanisms involved in the initiation of hypertension were different in the four models, disruption of cholinergic function interfered with a neural process crucial for the expression of the hypertensive state in these animals. They also demonstrated quite clearly that it is not the hypertensive state that confers antihypertensive potential to HC-3, but that a central cholinergic mechanism is integral to maintaining hypertension in SHR. Once activated, cholinergic mechanisms facilitate the hypertensive process, independent of the perturbations invoked to achieve elevated blood pressure.

In the case of nongenetic models of hypertension, one possible mechanism through which central cholinergic neurons might help to sustain the hypertensive state is the baroreceptor reflex. For example, it is well accepted for both animal and human forms of the disease, that in established hypertension, the baroreceptor reflex is reset about the elevated pressure. This resetting of baroreceptor threshold pressure and gain occurs to a large extent within the central component of the reflex arc (see Korner, 1976). The process of central resetting of baroreceptor reflex sensitivity may involve cholinergic neurons. In fact, central administration of cholinergic drugs have been demonstrated to modify baroreceptor reflex function. Brezenoff and coworkers (1982) were the first to demonstrate that i.c.v. injection of neostigmine or physostigmine enhanced the carotid occlusion pressor reflex in normotensive rats. Subsequently, these results were confirmed and extended to include the head-up tilt reflex (Park and Long, 1991). Administration of physostigmine by the i.c.v. route attenuated the tilt-induced decrease in blood pressure. Both studies demonstrated that i.c.v. pretreatment with atropine or HC-3 blocked

the modulatory effects of the cholinesterase inhibitors on the respective reflexes, but did not interfere with the expression of the reflex itself. In each case, however, brain cholinergic activation appeared to modify the baroreceptor reflex arc in favor of elevated blood pressure. With regard to genetic hypertension, Kawashima and coworkers (1987) observed that a tachycardic response that accompanied the pressor response to intravenous injection of physostigmine in SHR did not occur in WKY. They suggested that the evoked tachycardia in SHR was caused by impairment of baroreceptor reflex function related to cholinergic stimulation. These results were essentially reproduced in a subsequent study in which acetylcholine was directly microinjected into the NTS (Talman and Lewis, 1991). That is, microinjection of acetylcholine into the NTS produced a dose-related fall in both blood pressure and heart rate in WKY. Although the hypotensive response to acetylcholine was similar for both strains, there was virtually no change in heart rate observed in SHR. Taken together, these findings suggest that central cholinergic neurons participate in the resetting of the baroreceptor reflex in hypertension. This possibility is supported by the fact that clonidine's antihypertensive action is achieved in concert with enhancement of the central component of the baroreceptor reflex arc (see Korner, 1976). Because clonidine exerts a significant central 'anticholinergic' action (see Buccafusco, 1982), it is possible that clonidine-induced enhancement of the baroreceptor reflex is mediated, at least in part, through interference with cholinergic function in relevant brain regions.

Although HC-3 injected by the i.c.v. route theoretically has access to all levels of the brain and spinal cord, there is some evidence that one potential site of its antihypertensive action is the hypothalamus, possibly within the posterior aspect of the region. Vargas and Brezenoff (1988) demonstrated that chronic i.c.v. infusion of HC-3 to prehypertensive SHR over a period of 3 weeks essentially forestalled the development of hypertension. After the termination of the infusion, blood pressure returned to hypertensive levels during the next 2 weeks. Hypothalamic levels of acetylcholine appeared to be more affected by the depleting actions of HC-3 than did striatal or brainstem levels. In this respect, the results parallel those from an earlier study in which a single injection of HC-3 was used (Buccafusco and Spector, 1980). Later, Brezenoff and Xiao (1989) demonstrated that bilateral injection of HC-3 into the posterior hypothalamic nucleus lowered blood pressure to the same extent as i.c.v. injection. The antihypertensive action produced by intrahypothalamic injection of HC-3 was blocked when choline was added to the injection solution. Also, bilateral microinjection of choline into the posterior hypothalamic nucleus inhibited the antihypertensive response to i.c.v. injection of HC-3 by up to 60%.

Consistent with the antihypertensive efficacy of brain acetylcholine depletion in SHR are the results of experiments in which central muscarinic receptor blockade was attempted in the hypertensive strain. In fact, i.c.v. injection of the muscarinic receptor antagonist 4-diphenylacetoxy-N-methylpiperidine (4-DAMP) produced a dose-related fall in blood pressure in conscious SHR (Brezenoff et al., 1988). The reduction in blood pressure at the highest dose of 4-DAMP (25 μg) lasted over 2 hours and did not return to baseline hypertensive levels until 24 hours after injection. The drug was not particularly effective in lowering blood pressure in WKY. Because 4-DAMP has some selectivity for non-M_1 receptors (possibly M_2 and M_3), and because the partially selective M_1 muscarinic receptor antagonist pirenzepine failed to decrease blood pressure in SHR, the authors concluded that non-M_1 receptors are important for the cholinergic neuronal processes that maintain hypertension in this model. In support of this contention are the results of their subsequent study in which a mustard analog of 4-DAMP was used to produce a permanent inactivation of predominantly M_2/M_3 muscarinic receptors (Brezenoff et al., 1990). Injection of 4-DAMP mustard by the i.c.v. route resulted in a rapid fall in blood pressure of 51 mmHg in SHR that gradually returned to hypertensive levels over a 10-day period. Bilateral microinjection of the drug into the posterior hypothalamic nucleus produced essentially the same response as that for i.c.v. injection. Some evidence was also provided to indicate the site of action of 4-DAMP mustard. For example, the pressor response to i.c.v. injection of neostigmine was inhibited to a much greater extent than was the pressor response to intravenous administration of physostigmine in SHR pretreated with the mustard. Also, muscarinic receptor binding sites in mustard-treated rats were reduced in number in the hypothalamus, but not in the brainstem. Thus, it appears that cholinergic pathways within the hypothalamus, and particularly within the posterior hypothalamic nucleus, must contribute to the processes that maintain blood pressure in genetically induced experimental hypertension. Table 1 summarizes the literature findings regarding the ability of central administration of cholinergic antagonists to elicit an antihypertensive response in SHR.

C. Neurochemical Considerations

1. Estimates of cholinergic neuronal activity. The ability of cholinomimetic drugs to evoke exaggerated hypertensive responses in SHR is suggestive of an alteration in the function of cholinergic neurons or their receptive sites. An alternate possibility is that the defect producing the altered physiological response in SHR is downstream from the cholinergic site being affected by the drugs. Although the alterations in the acetylcholine synthetic and degredative enzymes purported to exist in hypertensive compared with normotensive rats as cited above suggest some derangement in cholinergic neurobiology, these markers are not predictive of cholinomimetic drug action. Neurochemical estimates of cholin-

ergic neuronal activity and receptor function are more relevant to the action of drugs in the CNS. Beyond the enzyme measurements cited above, however, there is a paucity of information regarding the status of pre- and postsynaptic function in SHR. Perhaps part of the reluctance to pursue this line of research stems from the knowledge that high blood pressure is not the only phenotypical difference between SHR and normotensive rats. Thus, correlation of neurochemical changes with the expression of hypertension could be problematic. This situation is compounded by the apparent controversy regarding which normotensive strain is most suitable as a control for the SHR (Kurtz and Morris, 1987; Henry et al., 1990). Indeed, for a particular neurochemical alteration to be considered as a contributing factor to the development or maintenance in genetic hypertension, several experimental approaches should be considered: (*a*) The neurochemical alteration should predict the response to pharmacological agents directed into the specific site of action. (*b*) Both the development of the neurochemical alterations and the pharmacological response predicted by the alteration should precede or at least occur concomitantly with the development of hypertension. (*c*) The neurochemical and pharmacological responses observed in SHR should be compared with age-matched controls, which should include inbred WKY and at least one other normotensive strain. (*d*) SHR should be back-crossed with the normotensive strain to determine whether the neurochemical or pharmacological responses observed in SHR cosegregate with hypertension. Although these stringent criteria have rarely been met with regard to the cholinergic system, some relevant information has been obtained to suggest that specific alterations in central cholinergic function do exist in SHR, and that they may be contributing factors to the development or maintenance of hypertension.

Table 4 lists a number of studies in which cholinergic neuronal markers were compared in hypertensive and normotensive strains of rats. With few exceptions, the activities of cholinergic enzymes acetylcholinesterase

TABLE 4

Cholinergic neurochemical alterations measured in hypertensive and normotensive rat strains

Reference	Hypertensive Strain	Normotensive Strain	Cholinergic Marker	Brain Region	Marker Ratio: Hypertensive/Normotensive
Yamori, 1976	SHR	WKY/Wistar	AChE	lower brainstem	↑
			ChAT	whole brainstem	↑
Bagjar et al., 1979	SHR	WKY	AChE	whole brain	↑
			ChAT	whole brain	↑
Helke et al., 1980	SHR	WKY	ChAT	PVN, PH, TAV	↓
			ChAT	LC, RG	↓
Cantor et al., 1981	SHR	WKY	mAChR	5 regions	–
Hershkovitz et al., 1983	SHR	WKY	mAChR	PH	↑
			mAChR	pons/medulla	–
Buccafusco, 1986	SHR	WKY	ACh TOR	AH, PH	↑
Kawashima et al., 1986	SHR	WKY	ACh	AH, medulla	↓
Trimarchi and Buccafusco, 1987	SHR	WKY/Wistar	HAChU	hypothalamus	↑
				medulla	↑
Yamada et al., 1987	SHR_{SP}	WKY	mAChR	PH	↑
			mAChR	pons/medulla	–
Yamada et al., 1987	SHR	WKY	nAChR	most brain regions	↓
Khan et al., 1994c	SHR	WKY	nAChR	most brain regions	↓
Gattu et al., 1995a	SHR	WKY	nAChR	most brain regions	↓
Gattu et al., 1995b	SHR	WKY	M_1	PH, CP, CA3	↑
			M_1	cingulate cortex	↑
			M_1	TMN, SN	↑
			M_2	nV, nVII	↑
			M_2	RVL, RG	↑
			M_2	amygdala, nAc	↑
			M_2	olfactory tubercle	↑
			M_2	parietal cortex	↓
			M_2	TAV, VLT	↓
Kubo et al., 1995	SHR	WKY	ACh release	RVL	↑
			ACh release	RD, CV, CD	–
			ChAT	RVL	↑

ACh, acetylcholine; AChE, acetylcholinesterase; ACh TOR, acetylcholine turnover rate; ChAT, choline acetyltransferase; HAChU, high affinity choline uptake; mAChR, muscarinic acetylcholine receptors; nAChR, nicotinic acetylcholine receptors; SHR_{SP}, SHR stroke prone; AH, anterior hypothalamus; CA3, CA3 region of the hippocampus; CD, caudo-dorsal medulla; CP, caudate/putamen; CV, caudo-ventral medulla; LC, locus ceruleus; nAc, nucleus accumbans; PH, posterior hypothalamic nucleus; PVN, paraventricular nucleus; RD, rostro-dorsal medulla; RG, nucleus reticularis gigantocellularis; TAV, anterior ventral thalamic nucleus; TMN, tuberomammillary nucleus; VLT, ventrolateral thalamus.

↑, increase; ↓, decrease; –, no change.

and choline acetyltransferase were reported to be elevated in the hypertensive strain. Estimation of more dynamic aspects of cholinergic function resulted in more consistent neurochemical alterations in SHR. For example, when i.c.v. injection of [^{3}H]choline was used to follow the synthesis of [^{3}H]acetylcholine in age-matched hypertensive SHR and normotensive WKY (Buccafusco, 1986), the biosynthesis of [^{3}H]acetylcholine was increased by up to 56% in both rostral and caudal regions of the hypothalamus of SHR. There were no differences observed between strains for the cerebral cortex or hippocampus. The observation that the enhanced biosynthesis of acetylcholine in medullary tissue derived from SHR apparently is not maintained in vivo (Arneric et al., 1990) suggests that the neuronal factors driving the release of acetylcholine within the RVL in vivo, were eliminated in the in vitro release experiment. One such factor might include the enhanced number or sensitivity of postsynaptic muscarinic receptors.

Unlike other in vitro markers used to assess cholinergic function, the high affinity uptake of choline into freshly prepared crude brain synaptosomal fractions has been used as a relative indicator of cholinergic neuronal activity. That is, in vivo treatments that interfere with cholinergic neuronal impulse flow also reduce the capacity of the synaptosomal high affinity choline carrier as measured in vitro. The converse is also true (Kuhar and Murrin, 1978; Jope, 1979). The capacity of the synaptosomal high affinity choline uptake system measured in vitro not only provides a marker for the presence of cholinergic nerve terminals, but also provides information related to the activity of cholinergic neurons before death. The high affinity uptake of choline into synaptosomal fractions derived from hypothalamus and medulla was measured in both young prehypertensive and adult hypertensive SHR and two age-matched normotensive control groups, the inbred Wistar Lewis strain and WKY (Trimarchi and Buccafusco, 1987). Although there were no differences among the strains regarding the affinity [K_m (substrate concentration at half-maximal velocity)] for the choline carrier, there were marked differences observed for the capacity [maximal velocity (V_{max})] of the carrier between SHR and the two normotensive strains. In fact, for both brain regions, the rank order of values for V_{max} were SHR > WKY > Wistar Lewis. In 5-, 12- and 22-week-old SHR, there was an age-dependent increase in both blood pressure and the V_{max} for the high affinity choline carrier for the medulla-pons, with the oldest rats exhibiting a 78% increase in V_{max} compared with WKY means. There also was a significant correlation between the level of systolic blood pressure and the V_{max} for the high affinity choline uptake system for the medulla-pons region. Although not as robust as for the medulla-pons, there was also a significant correlation between blood pressure and the V_{max} for hypothalamus. Data taken from all three strains were used in the correlation. A strong correlation also existed between age and V_{max} values derived from the medulla-pons. Finally, using systemic administration of pressor agents, blood pressure in normotensive WKY was elevated to hypertensive levels for 3 hours. This elevation in blood pressure did not affect V_{max} values relative to untreated controls. Thus, the neurochemical studies described above using direct (turnover) and indirect (choline uptake) measures of cholinergic neuronal activity are consistent with enhanced activity of cholinergic neurons in two large brain regions derived from SHR. Whether such changes constitute the result of some genetics-based alteration in cholinergic function in the hypertensive strain is not known. However, the concept of enhanced cholinergic function as a causative or sustaining factor in spontaneous hypertension is consistent with the pharmacological data described above for cholinergic agonists and antagonists used in SHR.

Using anesthetized and ventilated SHR and WKY, Kubo and colleagues (1995) demonstrated that direct microinjection of physostigmine into the RVL evoked an exaggerated pressor response in adult hypertensive SHR compared with that in WKY. This heightened pressor response was not reproduced after microinjection of either acetylcholine or carbachol into the nucleus. The exaggerated response to physostigmine in the RVL was suggested to be related to enhanced acetylcholine release. This possibility was confirmed by experiments in which the release of endogenous acetylcholine was measured through the use of microdialysis probes implanted within the RVL. Not only was the basal release of acetylcholine elevated more than two-fold in SHR, but the evoked release of acetylcholine produced after systemic administration of physostigmine was also significantly greater in SHR compared with WKY. Of particular interest was their observation that the pressor response evoked by microinjection of glutamate in the RVL of WKY was not different from that in SHR. This observation had been reported previously (Smith and Barron, 1990a, b; Tseng et al., 1994). Thus, although cholinergic neurons within the RVL function to enhance the output of the local glutamatergic bulbospinal pressor neurons, the derangement within the nucleus associated with spontaneous hypertension appears to be selective for the cholinergic system.

2. Muscarinic receptors. The possibility that central cholinergic neuronal activity and perhaps the release of endogenous brain acetylcholine is elevated in SHR has ramifications with regard to the regulation of postsynaptic cholinergic receptors. In most instances in which muscarinic receptors are exposed to prolonged or elevated levels of transmitter or exogenously administered agonists, the physiological response is usually postsynaptic receptor down-regulation. In the muscarinic system, this occurs acutely through receptor internalization, and over long time-courses through reduced levels of transcription for the genes encoding the receptor protein (Aronstam et al. 1987; McDonald et al., 1988; Wall

et al., 1992). Receptor down-regulation serves as one mechanism to limit the effects of excessive cholinergic stimulation. Should receptors not undergo down-regulation, or rather, should postsynaptic receptors actually up-regulate in the face of enhanced release of acetylcholine, the result would undoubtedly be marked amplification of cholinergic neuronal transmission.

The first attempt to compare muscarinic receptor density in brain regions derived from SHR and WKY was reported by Cantor and coworkers (1981). Using a single nonsaturating concentration of [^{3}H]quinuclidin-3-yl benzilate ([^{3}H]QNB), an irreversible muscarinic receptor antagonist, they reported no interstrain differences in specific [^{3}H]QNB binding in five brain regions, including the hypothalamus. In contrast, using the same labeled ligand, but using a full range of concentrations in a series of saturation-binding experiments, Hershkowitz and colleagues (1983) reported that the apparent density of [^{3}H]QNB binding sites in the posterior hypothalamus was significantly greater in SHR compared with age-matched WKY. Using rats from 1 to 50 weeks old, the density of [^{3}H]QNB binding sites was shown to increase from the first week through the eleventh week of age for both groups. However, levels of hypothalamic muscarinic receptors were always greater for SHR compared with WKY, and this difference included the 1-week-old animals. Alternatively, there was no interstrain difference in the number of [^{3}H]QNB binding sites for the pons-medulla. The authors did not rule out the possibility, however, that their inability to detect such differences in the pons-medulla could be related to the large size of the tissue diluting out differences in interstrain muscarinic receptor binding localized to specific nuclei. These results were largely confirmed in a subsequent study in which the agonist [^{3}H]*cis*-methyldioxolane was used to label hypothalamic and medullary muscarinic receptors (Yamada et al., 1987). In this case, the offshoot strain, the stroke-prone SHR, was compared with aged-matched normotensive WKY. The stroke-prone strain shares many similar characteristics with SHR except that their resting arterial pressures tend to be even higher, and the disease process appears to be fulminant, in that most of the animals succumb to cerebrovascular emboli. Of the eight brain regions studied (including the medulla oblongata), the density of [^{3}H]*cis*-methyldioxolane binding sites was significantly greater in the stroke-prone SHR. It was already alluded to in Section V.B. that different subtypes of muscarinic receptors may play different roles in the cholinergic defect related to spontaneous hypertension. Because muscarinic receptors can regulate both presynaptic and postsynaptic function within the synapse, measured changes in receptor numbers may not be predictive of the specific effect on synaptic neurotransmission. To begin to make some headway regarding the role of muscarinic receptor subtypes and specific brain sites in spontaneous hypertension, one preliminary study (Gattu et al., 1995b) used the partially selective ligands for the M_1 and M_2 muscarinic receptors, [^{3}H]pirenzepine and [^{3}H]AF-DX 384, respectively, (Brann et al., 1993; McKinney, 1993) in an autoradiographic brain mapping study. SHR (12-week-old) with established hypertension exhibited significantly increased numbers of apparent M_1 binding sites in the posterior hypothalamic nucleus compared with age-matched WKY. Other areas exhibiting enhanced M_1 binding in SHR included, the cingulate cortex, caudate putamen, CA3 region of the hippocampus, tuberomammillary nucleus, and the substantia nigra. There were no decreases in the M_1 subtype measured in SHR relative to WKY controls, and none of the 17 other brain regions examined exhibited interstrain differences. SHR exhibited significantly increased numbers of apparent M_2 binding sites in several medullary sites including the RVL, facial nucleus, trigeminal nucleus, and nucleus paragigantocellularis lateralis. The nucleus accumbens and basolateral amygdala also exhibited significant increases in M_2 binding sites in SHR. Several sites also exhibited decreases in M_2 binding in SHR relative to WKY. These included the parietal cortex, anteroventral thalamic nucleus and ventrolateral thalamic nucleus. None of the other 15 brain regions examined exhibited interstrain differences in M_2 binding sites.

3. Nicotinic receptors. The preponderance of pharmacological studies that have been designed to help characterize the role of central cholinergic neurons in experimental hypertension have used receptor nonselective compounds. Although in most of these studies, the muscarinic nature of the evoked pharmacological responses to these mixed agonists has been ascertained, there exists a significant body of evidence to indicate that central nicotinic receptor stimulation evokes a hypertensive response (see Brezenoff and Giuliano, 1982). In a series of more recent studies, it was demonstrated that nicotine or the nicotinic receptor agonist cytisine evoked pressor responses upon either direct injection into the RVL of urethane anesthetized rats (Sundaram and Sapru, 1988; Tseng et al., 1994) or after i.t. injection in conscious rats (Khan et al., 1994b, c). Moreover, the pressor response to nicotinic receptor stimulation in the RVL, and after i.t. injection (Khan et al., 1994a), was much greater in magnitude in SHR compared with WKY. At no time did any of the variety of nicotinic receptor antagonists used in these studies result in a fall in blood pressure, even in SHR, suggesting that nicotinic receptors serve only to modulate pressor pathways in these brain regions. Unlike the pressor response to i.c.v. injection of nicotine (Buccafusco and Yang, 1993), the response to stimulation of nicotinic receptors in the RVL was not blocked with atropine (Tseng et al., 1994). However, the interpretation of the results of the latter study should be considered tentative, because it is not likely that the dose of atropine used intravenously (2.9 μg) could produce blockade of muscarinic receptors in the RVL of

sufficient magnitude to inhibit the effects of the locally applied nicotine.

Although the relative distribution and levels of the subtypes of nicotinic receptors in the spinal cord have not been characterized, Khan and his colleagues (1994b, c) have demonstrated that the pharmacological response to i.t. injection of nicotine and cytisine differ somewhat in normotensive rats. They demonstrated that nicotine and cytisine, which generally exhibit similar binding profiles, exhibit different profiles of sensitivity to the blocking actions of a series of nicotinic receptor antagonists. Also, cytisine evoked a biphasic response, an initial pressor phase that desensitized rapidly, and a more prolonged secondary pressor phase. Nicotine evoked only a single pressor response. The early phase of the cytisine-evoked pressor response, as well as the pressor response to nicotine, were shown to be caused by direct stimulation of the spinal sympathetic outflow. The secondary pressor phase to cytisine appeared to be mediated over a spinobulbar cardiovascular pathway possibly linked to the drug's antinociceptive action. In the SHR, the authors (Khan, 1994a) reported that there was a diminished ability of the initial pressor phase to cytisine to desensitize, as well as a diminished sensitivity of the pressor response to blocking agents. The authors suggested that the enhanced pressor responsiveness to spinal nicotinic receptor stimulation in SHR was related to a reduced number of spinal nicotinic receptors and a reduced ability to desensitize to both the pressor and behavioral effects of cytisine.

In the rat (WKY), the binding of [^{3}H](−)-nicotine to brain membranes varied three-fold, with the lowest binding observed in the cerebellum, and the greatest in the thalamus. The medulla exhibited an intermediate level of [^{3}H](−)-nicotine binding (Yamada et al., 1987). Nicotinic receptors within the rat (WKY) spinal cord estimated by using [^{3}H]cytisine binding revealed a substantially lower density of sites than for the medulla (Khan et al., 1994c). The results of both studies were consistent in that SHR exhibited lower densities of nicotinic receptor sites than WKY. In the medulla and spinal cord, the interstrain differences in the density of nicotinic binding sites amounted to approximately 35% and 20%, respectively. In each study, the authors attributed the altered cardiovascular and behavioral responses to nicotinic receptor stimulation in SHR to the lower density of nicotinic receptors in that strain. The implication was that alterations in the expression of nicotinic receptors may play some role in the expression of hypertension. One alternative explanation consistent with the findings is that the lower expression of nicotinic receptors in SHR is a phenotype of the strain, not associated with hypertension; perhaps not even associated with the enhanced pressor responsiveness to nicotinic receptor stimulation. In support of this alternative possibility is the lack of regional brain and spinal selectivity of the lowered levels of binding sites in SHR. For example, of the nine major regions tested in the two studies, six regions exhibited significantly lower nicotinic receptor densities in SHR. In fact, in all regions tested, except for the hypothalamus, on average, there were fewer numbers of binding sites in SHR. This finding was confirmed for one preliminary study (Gattu et al., 1995a). Using autoradiographic techniques, 20 of 27 brain regions exhibited a significantly lower number of [^{3}H]cytisine binding sites in hypertensive SHR compared with WKY. Again, the average number of binding sites was lower in SHR for almost every region from cerebral cortex to lower brainstem, and no brain region exhibited an elevated level of binding sites in the hypertensive strain. As with the [^{3}H]cytisine binding, sites labeled with ^{125}I-α-bungarotoxin were lower in SHR; however, the interstrain differences were much more localized. Brain regions from SHR that included reduced numbers of both [^{3}H]cytisine and ^{125}I-α-bungarotoxin binding sites included the frontal cortex, olfactory nucleus, and ventrolateral geniculate nucleus. Thus, whereas the SHR may provide an outstanding genetic model for the reduced expression of [^{3}H]nicotine/[^{3}H]cytisine binding sites, the physiological consequences of such a neurochemical alteration remains to be elucidated. However, the localization of nicotinic receptors, particularly of the $\alpha4\beta2$ subtype, to areas of the brain involved in sensory gating such as the thalamus, suggests that they play a role in the heightened responsiveness of SHR to alerting stimuli (see Yamori, 1977; Casto and Printz, 1990; Svensson et al., 1991) and perhaps to the enhanced behaviorally evoked cardiovascular responses to nicotine (Khan et al., 1994c). In support of this contention is the well known ability of nicotine to enhance short-term memory in animals and humans (Elrod et al., 1988; Buccafusco and Jackson, 1991; Levin, 1992; Terry et al., 1993; Arneric et al., 1995a; 1995b).

VI. Molecular Aspects of Muscarinic Function in Spontaneously Hypertensive Rats

A. Polymerase Chain Reaction Studies

With five subtypes of muscarinic receptors to be considered for potential roles in central cardiovascular regulation, and with ligands partially selective for only two or three, the use of standard ligand binding techniques becomes quite limited. Two additional methods are available to estimate the role of all five subtypes, and both are associated with limitations. The most direct method is the receptor antibody precipitation approach in which antisera, usually polyclonal, are directed against epitopes on each receptor subtype. The level of each subtype in a tissue sample is then estimated from the difference between the binding of a nonselective ligand before and after precipitation of the subtype by the antisera (Yasuda et al., 1993). One limitation of the method is the complexity inherent in developing bacterial cells lines that express each receptor protein. Also,

the specificity and titre of the antisera can vary from batch to batch, and the amount of antisera that can be produced can be limiting for in situ studies. Nevertheless, the method has been used with great success to estimate the quantity of muscarinic subtype-specific receptor protein in brain. Unfortunately, the receptor antibody precipitation method has not been applied to the issue of brain muscarinic receptor subtypes in experimental hypertension. An alternate approach estimates the level of mRNA encoding each specific subtype and provides information regarding the regulation of the receptor subtype at the level of transcription. Although changes in the levels of mRNA do not always reflect changes in the synthesis of the encoded protein, as indicated above (see Section I.C.), functional alterations in the level muscarinic receptor protein are generally accompanied by the respective alteration in mRNA level.

The polymerase chain reaction-reverse transcriptase (RT-PCR) methodology has been used to detect low abundance mRNA for the five muscarinic receptor subtypes. PCR products can be quantitated using a weak anion exchange high performance liquid chromatographic technique. In this laboratory, we have begun to test the hypothesis that alterations of muscarinic receptor function occur in critical brain regions in SHR and that these changes reflect alterations in the expression of mRNA coding for specific subtypes (Wei et al., 1995). Total RNA was extracted from hypothalamus and medulla of both prehypertensive (4-week-old) and hypertensive (12-week-old) SHR and age-matched WKY and used for the RT-PCR methodology. PCR-amplified products (cDNA oligos) representing all five subtypes of muscarinic receptors were quantitated by high performance liquid chromatography (Wei et al., 1994). The mRNA of the housekeeping gene, glyceraldehype-3-phosphate dehydrogenase (G3PDH), was used as the internal standard that controlled for any differences in extraction or amplification efficiencies among samples. There were no significant differences between the hypothalamic mRNA encoding the internal standard gene between SHR and WKY rats. In the prehypertensive SHR, the levels of mRNA encoding the m1 and m4 subtypes were altered. The level of m1 mRNA was significantly increased (by 53%), and the level of m4 mRNA was significantly decreased (by 15%) in the SHR. Similar differences were observed in samples derived from the 12-week-old animals; however, the decrease in m4 mRNA levels observed in SHR was greater in magnitude (54%) in the older hypertensive animals. There were no differences between the strains for the other subtypes derived from the hypothalamus; however, in a separate experiment using cerebral cortex from 12-week-old rats, no differences were found among the five subtypes between strains. Thus, at the transcriptional level, there exists a difference in message that suggests overexpression of a subtype (m1) that is linked to excitatory synaptic transmission and an underexpression of a subtype (m4) that is linked to inhibitory transmission. Obviously, it will require additional experimentation to determine whether either or both of these subtypes are important for the expression of elevated blood pressure in SHR. Hypothalamic m4 mRNA levels decreased with age, paralleling the development of hypertension. Because both m1 and m4 mRNAs were altered in SHR, potentially both receptors may interact and play a role in enhanced sympathetic outflow in this strain. The selectivity of alterations in receptor regulation in the SHR was underscored by the observations that (*a*) the level of the control gene G3PDH was not different between strains of either age group; (*b*) the levels of the other subtypes, m2, m3 and m5, were similar between strains; and (*c*) the density of muscarinic receptors in the cerebral cortex was similar between the strains. It is easy to speculate that if mRNA levels reflect functional receptor levels, then hypothalamic cholinergic function is enhanced in SHR; and cholinergic systems have already been considered to play a role in mediating enhanced sympathetic activity during activation of the defense reaction.

A different profile exists in the medulla of SHR (Wei and Buccafusco, 1994). Once again, the m1 mRNA was expressed to a much greater degree in SHR; however, in this case, the inhibitory receptor m2 mRNA is also elevated. This scenario fits well with the known cholinergic neuronal interactions that exist in the medulla (see Figure 2). In the RVL, for example, the predominant M_2 subtype provides inhibitory tone that disinhibits (via a GABA interneuron) bulbospinal glutamatergic excitatory sympathetic activity. Enhanced expression of inhibitory M_2 receptors in this brain region could account for the enhanced sympathetic activity in SHR. The fact that these receptor changes, both at the mRNA and protein level, occurred before the onset of significant hypertension suggests that, if these factors are involved, they play an initiation role as well as a maintenance role in hypertension.

B. Genetic Approaches

In recent years, several groups have attempted to determine the nature of hereditary transfer of genes responsible for hypertension in the SHR by back-crossing SHR with normotensive controls. Estimates have ranged from a single gene with simple additive allelic effects to as many as six major genes that determine high blood pressure (see Kurtz et al., 1990). Application of biometric genetic analysis to this problem has suggested that for SHR reared under normal laboratory conditions, that the level of blood pressure fits an additive-dominance model of inheritance in which alleles decreasing blood pressure are partially dominant (Kurtz et al., 1990). This finding is particularly interesting and relevant because it often has been considered that hypertension would be related to factors increasing blood pressure that are overexpressed. As suggested in the latter study, however, altered expression of factors de-

creasing blood pressure may prove to be the underlying mechanism. For example, the cardiovascular response to startle in SHR is different from that of WKY rats. A characteristic transient bradycardia (inhibitory response) before tachycardia that was observed in WKY rats to startle does not occur in SHR. This transient bradycardia is vagally mediated and represents a phenotype that cosegregates with hypertension in crosses between SHR and WKY rats (Casto and Printz, 1988, 1990). The involvement of parasympathetic tone to the heart is particularly interesting because the target receptors are almost exclusively of the M_2 (inhibitory) subtype. In the mRNA studies described above, hypothalamic m4 mRNA levels decreased with age, paralleling the development of hypertension. Because the M_4 subtype is linked to inhibitory synaptic responses, it is possible that this gene alteration associated with hypertension may reflect, in part, the reduced expression of factors decreasing blood pressure as mentioned above. However, a comprehensive test of this hypothesis will require experiments subject to the four criteria described above (section V.C).

VII. Novel Cholinergic Drugs as Antihypertensive or Sympatholytic Agents

One of the first points of discussion in the article was the lack of ability of atropine and related antimuscarinic compounds to lower blood pressure in animals or humans. To the extent that this issue has now been addressed, it should noted that several approaches have been considered to target central cholinergic neurons directly for the purpose of eliciting antihypertensive activity. Clearly, central administration of a variety of cholinergic antagonists have exhibited antihypertensive action. Indeed, direct administration of atropine, scopolamine, or HC-3 into the RVL lowered blood pressure, even in normotensive rats. Perhaps one of the earliest more novel approaches taken to inhibit the function of central cholinergic pressor neurons was the use of precursors to false cholinergic transmitters. The concept originally was derived from, and was first successful with, the use of methyldopa as a precursor to the false adrenergic transmitter, methylnorepinephrine, a potent α-adrenergic agonist. For the cholinergic system, deanol was used in the late 1970s (see, Goldberg, 1977) as a precursor to choline and hence acetylcholine synthesis; although the promise realized with methyldopa was not attained with deanol (dimethylaminoethanol). Nevertheless, the door was opened for the development of choline analogs that were shown to replace choline for uptake, acetylation, and release from cholinergic neurons, both in vitro and in vivo (see Collier et al., 1979). For the most part, acetylated choline analogs had less intrinsic activity than acetylcholine and were expected to cause inhibition of central cholinergic function. Two studies demonstrated the feasibility of this approach for central cardiovascular systems (Aronstam et al., 1988; Buccafusco and Aronstam, 1988). The ability of i.c.v. infusion of mono-, di- and triethylcholine to inhibit cholinergic cardiovascular neurons was demonstrated by their ability to inhibit the pressor response to intravenous injection of physostigmine. This ability to inhibit the physostigmine response was inversely correlated with the ability of each respective acetylated derivative to act as a muscarinic receptor agonist. The utility of this approach to experimental hypertension was never demonstrated, perhaps because it required i.c.v. infusion of the analogs to produce significant precursor effects in the rats.

A more direct approach to the problem was taken by Coram and Brezenoff (1983). They examined the ability N-(4-diethylamino-2-butynyl)-succinimide (DKJ-21) to lower blood pressure in SHR and WKY. DKJ-21 was one of a series of compounds previously synthesized (Dahlbom et al., 1966) to produce selective blockade of central, rather than peripheral muscarinic receptors. The drug produced a marked antihypertensive response in SHR (the average maximal decrease in blood pressor achieved was 43 mmHg) without lowering blood pressure in WKY, and was effective after either intravenous or i.c.v. injection. The duration of the antihypertensive response after a single dose of DKJ-21 was greater than 24 hours. DKJ-21 did not interfere with peripheral sympathetic ganglionic transmission, nor did it exhibit any sympatholytic or adrenoceptor blocking activity. As such, it was able to inhibit the expression of the pressor response to intravenous injection of physostigmine without significantly altering the fall in blood pressure to intravenous injection acetylcholine. This compound was revisited by the same research group some years later (H. E. Brezenoff, personal communication); the group found that intravenous administration of DKJ-21 was also capable of blocking the prolonged pressor response to soman, an organophosphorus, irreversible cholinesterase inhibitor. The antagonist was also effective in preventing the expression of some of the behavioral toxicity to soman. The significant degree of central selectivity of DKJ-21 appeared to be caused by its favorable distribution into the CNS related to its chemical properties of low basicity and high lipophilicity.

Using an alternative chemical approach, Vargas and Ringdahl (1990) examined the central selectivity of a series of oxotremorine derivatives. As with DKJ-21, these compounds were effective centrally acting muscarinic receptor antagonists that inhibited the pressor response to intravenous injection of physostigmine but failed to produce significant peripheral muscarinic receptor blockade. The derivatives also appeared to retain some of oxotremorine's partial selectivity for the M_2 subtype. The drugs had virtually no effect on resting blood pressure in urethane-anesthetized normotensive rats; unfortunately, however, hypertensive animals were not used in the study. Therefore, the antihyperten-

sive potential of these oxotremorine derivatives remains untested.

VIII. Conclusions and Future Directions

The role of central cholinergic neurons in cardiovascular regulation and in the development and maintenance of experimental hypertension has been under investigation for most of this century. Because of the efforts of numerous investigators throughout the world, a significant body of evidence exists that is consistent with this role. It is clear, for example, that normotensive animals and humans can be made hypertensive upon exposure to cholinomimetic drugs that act within the CNS. Generally, this hypertensive state is reversible after a single injection. However, prolonged infusion of carbachol into the CSF in conscious normotensive rats elicited a continuous hypertensive state over 7 days (Wu and Wei, 1982). This protracted hypertensive state could not be produced by the central infusion of other pressor agents, including histamine, prostaglandin E_2, and thyrotropin-releasing hormone. This interesting observation with carbachol has not been further studied. Although stimulation of central muscarinic receptors generally elicits hypertension, it is clear that inhibitory cardiovascular cholinergic neurons also exist. The relationship between excitatory and inhibitory cholinergic pathways is not yet apparent, and less is known regarding the function of the inhibitory system. Some evidence is available to suggest that the inhibitory cardiovascular cholinergic system may be linked to modulation and integration of cardiovascular changes associated with nociceptive input.

Both nicotinic and muscarinic receptors mediate hypertensive responses to pharmacological activation of cholinergic neurons. Again, much less is known about the nicotinic system, although in some situations, nicotinic receptor-mediated pressor responses may be evoked through the enhanced release of acetylcholine that in turn stimulates muscarinic receptors. Nicotinic and muscarinic receptor subtypes play varying roles in different brain regions regarding cardiovascular regulation. The role of each subtype may depend upon the nature of the neuronal interactions for a specific brain region integrating cardiovascular information. Thus, subtypes that mediate inhibitory or hyperpolarizing events in the postsynaptic cell, upon stimulation, may elicit an excitatory cardiovascular response if the receptors are located on the cell body of a neuron that releases an inhibitory transmitter such as GABA. Such a relationship between cholinergic and GABAergic neurons appears to exist within the RVL. In fact, the RVL may serve as one of the primary sources of efferent sympathetic activity to spinal preganglionic neurons. Cholinergic neurons synapsing within the RVL play an important role in the expression of sympathetic tone from the RVL. In this respect, RVL cholinergic neurons may play a role in the integration of cardiovascular information associated with a variety of behavioral and physiological events integrated at all levels of the neuraxis. Accordingly, the centrally mediated vasoconstrictor effects of a wide variety of neurotransmitters, modulators, hormones and their synthetic counterparts introduced into the CNS may rely on the release of acetylcholine in the mediation of their actions. On the other hand, cholinergic neurons clearly do not carry bulbospinal sympathetic information from the RVL, but rely on interactions with amino acid neurotransmitters and perhaps with the gaseous transmitter NO.

Interference with the function of central cholinergic neurons has been demonstrated to both avert and reverse the development of experimental hypertension in a wide variety of rat models. The effectiveness of 'anticholinergic' approaches in nongenetic, induced hypertension is consistent with the possibility that central cholinergic neurons play a role in sustaining the hypertensive state. Maintenance of hypertension could be attained through the participation of central cholinergic neurons in the resetting of the baroreceptor reflex about the elevated blood pressure. Although similar adaptive events may participate in maintaining genetically induced hypertension, in the SHR and perhaps other related strains, alterations in the regulation and function of acetylcholine release and receptive mechanisms may have their origins in the genetic makeup of the hypertensive strain. The alterations in the mRNA-encoding muscarinic receptor proteins in SHR have not been substantial. In fact, most neurochemical changes that have been measured in this strain (as compared with normotensive WKY and other normotensive rats) are usually considerably less than 100% of control values. Therefore, the question must posed as to the relevance of, for example, a 30% increase in the mRNA encoding a muscarinic receptor, or a 20% increase in the binding of a muscarinic receptor ligand to tissues derived from brain regions in SHR. However, neurochemical, molecular, and pharmacological evidence exists to indicate that, in many brain regions of SHR, both release mechanisms and receptor numbers are enhanced. Pharmacologically, this situation is rarely encountered. That is, pharmacological enhancement of cholinergic function usually induces pre- and/or postsynaptic down-regulation, manifested by reduced turnover and release of acetylcholine and reduced numbers of functional postsynaptic receptors. In the SHR, the consequence of both pre- and postsynaptic up-regulation, even though each event alone is relatively small, would be substantial amplification of cholinergic neurotransmission. The hypothesis that site-specific dysregulation of cholinergic neuronal regulation may be encoded into the genetic makeup of SHR and related strains should be testable with modern molecular, genetic, and neurochemical techniques. Potentially interacting with cholinergic receptor regulation at the gene level are the mediators and neurotropins released from cholinergic or nearby neurons. A case in

point is the ability of endogenous prostaglandins to modulate the feedback mechanisms that function to maintain the level of cholinergic neurotransmission within certain limits.

Although a substantial body of evidence has been presented in support of the possibility that central cholinergic neurons play an important role in experimental hypertension, the extent of this evidence clearly does not always outweigh other possibilities that have been discussed over the years. Although the surgical or pharmacological elimination of any one central neurotransmitter may alter the rate of development of hypertension in genetically induced models, with time, blood pressure invariably reaches hypertensive levels (see Buccafusco and Brezenoff, 1986). Whether this is a reflection of the brain's use of redundant circuitry or of the underlying pathogenesis of peripheral cardiovascular organs is not known. For the SHR (which is only one imperfect model of human essential hypertension), however, the findings in support of the cholinergic hypothesis that have been replicated in several laboratories include the following: (*a*) The pressor response to central cholinergic stimulation is exaggerated compared with normotensive strains. (*b*) Cholinergic antagonists are antihypertensive in SHR. (*c*) Pre- and postsynaptic biochemical markers of cholinergic function are altered in SHR, usually with the changes favoring facilitation of neural responses that would elevate blood pressure. (*d*) Each of the above three findings has been demonstrated to occur both before and concomitant with the development of significant hypertension.

Although some evidence was presented above to suggest the feasibility of designing novel anticholinergic drugs for the treatment of hypertension, it is not clear whether such compounds would have any advantage over the current wide selection of antihypertensive medications. The pervasive involvement of cholinergic neurons in virtually every brain function may relegate even the most subtype-specific antagonist to attend with significant side effects. Nevertheless, if enhanced cholinergic function underlies the common forms of human hypertension, selectively acting anticholinergic agents may exhibit some specificity for reducing the effects of such heightened cholinergic systems. If, as suggested in the first paragraphs of this review, the current crop of antihypertensive drugs is not able to completely prevent the morbidity and mortality associated with chronic hypertension, selectively acting anticholinergic medication might be expected to inhibit the expression of exaggerated sympathetic tone to the vasculature almost at its origin. As such, there exists a great need for new clinical studies designed to test the hypothesis of central cholinergic involvement in essential hypertension.

At present, perhaps more than at any other time in history, humans are receiving centrally acting cholinergic agonists for therapy. The cognitive and memory impairments associated with Alzheimer's disease and related dementias is related in part to a loss of cholinergic neurons of the frontal cortex, hippocampus, and other brain regions. Currently, and for several years to come, one of the mainstays of treatment will be centrally acting cholinesterase inhibitors and other cholinergic agonists, both muscarinic and nicotinic. Because Alzheimer's disease affects millions of Americans—and is the fourth leading cause of death and the first leading cause of institutionalization in the country—the use of cholinergic therapy will be enormous. Many pharmaceutical companies currently involved in development of Alzheimer's therapeutics are evaluating some type of cholinergic therapy, and many new compounds will soon be marketed. Undoubtedly, many of these drugs will have cardiovascular consequences through peripheral and/or central mechanisms. As both Alzheimer's disease and essential hypertension occur with greater frequency with age, the potential for patients developing both diseases is quite high. The cardiovascular consequences of delivering cholinomimetic drugs to patients with hypertensive disease may prove to be problematic for the treatment of CNS diseases. Also, it is not apparent how agents that are therapeutic for Alzheimer's disease will interact with patients' antihypertensive medications. Therefore, even if the potential for antihypertensive medication based upon the antagonism of central cholinergic function is not realized, continued research in the area of central cholinergic mechanisms in hypertensive disease clearly is warranted.

Acknowledgements. The author would like to acknowledge the fine secretarial assistance provided by Ms. Patricia Ryan in the preparation of this manuscript. Support for some of the author's work described in this manuscript was provided by the Office of Research and Development, Medical Research Service of the Department of Veterans Affairs.

REFERENCES

ADAMS, M. L., KALICKI, J. M., MEYER, E. R., AND CICERO, T. J.: Inhibition of the morphine withdrawal syndrome by a nitric oxide inhibitor, N^G-nitro-L-arginine methyl ester. Life Sci. **52:** PL245–PL249, 1993.

ALLY, A., HARA, Y., AND MURAYAMA, S.: Cardiovascular effects of central administration of cholinomimetics in anesthetized cats. Neuropharmacology **32:** 185–193, 1993.

ALLY, A., WILSON, S. B., NOBREGA, C. L., AND MITCHELL, J. J.: Cardiovascular effects elicited by central administration of physostigimine via M_2 muscarinic receptors in conscious cats. Brain Res. **677:** 268–276, 1995.

AQUILONIUS, S.-M., AND SJOSTROM, R.: Cholinergic and dopaminergic mechanisms in Huntington's chorea. Life Sci. **10:** 405–414, 1971.

ARNERIC, S. P., ANDERSON, E. J., BANNON, A. W., BRIGGS, C. A., BUCCAFUSCO, J. J., BRIONI, J., CANNON, J. B., DECKER, M. W., DONNELLY-ROBERTS, D., GOPALAKRISHNAN, M., HOLLADAY, M. W., KYNCL, J., MARSH, K. C., PAULY, J., RADEK, R. J., RODRIGUES, A. D., AND SULLIVAN, J. P.: Preclinical pharmacology of ABT-418: a prototypical cholinergic channel activator for the potential treatment of Alzheimer' s disease. CNS Drug Rev. **1:** 1–26, 1995a.

ARNERIC, S. P., GIULIANO, R., ERNSBERGER, P., UNDERWOOD, M. D., AND REIS, D. J.: Synthesis, release and receptor binding of acetylcholine in the C1 area of the rostral ventrolateral medulla: contributions in regulating arterial pressure. Brain Res. **511:** 98–112, 1990.

ARNERIC, S. P., SULLIVAN, J. P., AND WILLIAMS, M.: Neuronal nicotinic acetylcholine receptors. Novel targets for central nervous system therapeutics. *In* Psychopharmacology, the Fourth Generation of Progress, ed. by F. E. Bloom and D. J. Kupfer, pp. 95–110, Raven Press, New York, 1995b.

ARONSTAM, R. S., SMITH, M. D., AND BUCCAFUSCO, J. J.: Clonidine prevents the short-term down regulation of muscarinic receptors in the mouse brain induced by the acetylcholinesterase inhibitor soman. Neurosci. Lett. **78:** 107–112, 1987.

ARONSTAM, R. S., MARSHALL, D. C., AND BUCCAFUSCO, J. J.: Cholinergic false

transmitters: physiological and biochemical actions in central and peripheral systems. Neuropharmacology **27:** 217–225, 1988.

ASANUMA, M., OGAWA, N., HABA, K., HIRATA, H., AND MORI, A.: Effects of chronic catecholamine depletions on muscarinic M1-receptor and its mRNA in rat brain. J. Neurol. Sci. **110:** 205–214, 1992.

BAGETTA, G., CORASANITI, M. T., MELINO, G., PAOLETTI, A. M., FINAZZI-AGRÓ, A., AND NISTICÓ, G.: Lithium and tacrine increase the expression of nitric oxide synthase mRNA in the hippocampus of rat. Biochem. Biophys. Res. Comm. **197:** 1132–1139, 1993a.

BAGETTA, G., MASSOUD, R., RODINÓ, FEDERICI, G., AND NISTICÓ, G.: Systemic administration of lithium chloride and tacrine increases nitric oxide synthase activity in the hippocampus. Eur. J. Pharmacol. **237:** 61–64, 1993b.

BAGJAR, J., HRDINA, V., AND GOLDA, V.: Development of cholinergic enzymes in the brain of normal and hypertensive rats. Prog. Brain Res. **49:** 471b, 1979.

BANNON, A. W., DECKER, M. W., WILLIAMS, M., AND ARNERIC, S. P.: Psycholtherapeutic potential of selective neuronal nicotinic acetylcholine receptor ligands. *In* Brain Imaging of Nicotine and Tobacco Smoking, ed. by E. F. Domino, pp. 311–334, NPP Books, Ann Arbor, MI, 1995.

BARBER, R. P., PHELPS, P. E., HOUSER, C. R., CRAWFORD, G. D. SALVATERRA, P. M., AND VAUGHN, J. E.: The morphology and distribution of neurons containing choline acetyltransferase in the adult rat spinal cord: an immunocytochemical study. J. Comp. Neurol. **229:** 329–346, 1984.

BARNES, J. C., AND ROBERTS, F. F.: Central effects of muscarinic agonists and antagonists on hippocampal theta rhythm and blood pressure in the anaesthetised rat. Eur. J. Pharmacol. **195:** 233–240, 1991.

BENTLY, G. A., AND LI, D. M. F.: Studies of the new hypotensive drug ST 155. Eur. J. Pharmacol. **4:** 124–134, 1968.

BHARGAVA, K. P., PANT, K. K., TANGRI, K. K.: Cholinergic influences on the spinal cardiovascular neurones. J. Auton. Pharmac. **2:** 225–230, 1982.

BICKFORD, M. E., GUNLUCK, A. E., GUIDO, W., AND SHERMAN, S. M.: Evidence that cholinergic axons from the parabrachial region of the brainstem are the exclusive source of nitric oxide in the lateral geniculate nucleus of the cat. J. Comp. Neurol. **334:** 410–430, 1993.

BIRKENHAGER, W. H., AND REID J. L.: Experimental and genetic models of hypertension. *In* Handbook of Hypertension, ed. by W. De Jong, Elsevier Science Publishers B. V., New York, 1984.

BLESSING, W. W., AND REIS, D. J.: Inhibitory cardiovascular function of neurons in the caudal ventrolateral medulla of rabbits: relationship to the area containing A_1 noradrenergic cells. Brain Res. **253:** 161–171, 1982.

BONNER, T. L., BUCKLEY, N. J., YOUNG, A. C., AND BRANN, M. R.: Identification of a family of muscarinic acetylcholine receptor genes. Science (Wash. DC) **237:** 527–532, 1987.

BONNER, T. L., YOUNG, A. C., BRANN, M. R., AND BUCKLEY, N. J.: Cloning and expression of the human and rat m5 muscarinic acetylcholine receptor genes. Neuron **1:** 403–410, 1988.

BORGES, L. F., AND IVERSEN, S. D.: Topography of choline acetyltransferase immunoreactive neurons and fibers in the rat spinal cord. Brain Res. **362:** 140–148, 1986.

BOUSQUET, P., FELDMAN, J., BLOCH, R., AND SCHWARTZ, J.: Central cardiovascular effects of alpha-adrenergic drugs: differences between catecholamines and imidazolines. J. Pharmacol. Exp. Ther. **230:** 232–236, 1984.

BRANN, M. R., JØRGENSEN, J. E. H., HILL-EUBANKS, D., AND JONES, S. V. P.: Muscarinic acetylcholine receptor subtypes: localization and structure/function. Prog. Brain Res. **98:** 121–127, 1993.

BREDT, D. S., AND SNYDER, S. H.: Nitric oxide, a novel neuroal messenger. Neuron **8:** 3–11, 1992.

BREZENOFF, H. E.: Cardiovascular regulation by brain acetylcholine. Fed. Proc. **43:** 17–20, 1984.

BREZENOFF, H. E., CARNEY, K., AND BUCCAFUSCO, J. J.: Potentiation of the carotid artery occlusion reflex by a cholinergic system in the posterior hypothalamic nucleus. Life Sci. **30:** 391–400, 1982.

BREZENOFF, H. E., AND CAPUTI, A. P.: Intracerebroventricular injection of hemicholinium-3 lowers blood pressure in conscious spontaneously hypertensive rats but not in normotensive rats. Life Sci. **26:** 1037–1048, 1980.

BREZENOFF, H. E., EL-FAKAHANY, E. S., AND XIAO, Y-F.: The effectof 4-DAMP mustard on blood pressure and muscarinic binding in spontaneously hypertensive rats. Ann. Neurosci. **1:** 24–33, 1990.

BREZENOFF, H. E., AND GIULIANO, R.: Cardiovascular control by cholinergic mechanisms in the central nervous system. Ann. Rev. Pharmacol. Toxicol. **22:** 341–381, 1982.

BREZENOFF, H. E., AND JENDEN, D. J.: Modification of arterial blood pressure in rats following microinjection of drugs into the posterior hypothalamus. Int. J. Neuropharmacol. **8:** 593–600, 1969.

BREZENOFF, H. E., AND JENDEN, D. J.: Changes in arterial blood pressure after microinjections of carbachol into the medulla and IVth ventricle of the rat brain. Neuropharmacology **9:** 341–348, 1970.

BREZENOFF, H. E., LI, J., AND TKACS, N.: Cholinergic stimulation of the brain increases blood pressure and evokes C-FOS expression in specific brain nuclei. Am. J. Hypertension **7:** 137A, 1994.

BREZENOFF, H. E., VARGAS, H., AND XIAO Y.-F.: Blockade of brain M_2 muscarinic receptors lowers blood pressure in spontaneously hypertensive rats. Pharmacology **36:** 101–105, 1988.

BREZENOFF, H. E., AND XIAO, Y-F.: Acetylcholine in the posterior hypothalamic nucleus is involved in the elevated blood pressure in the spontaneously hypertensive rat. Life Sci. **45:** 1163–1170, 1989.

BRISTOW, M. R., HERSHBERGER, R. E., PORT, J. D., GILBERT, E. M., SANDOVAL, A., RASMUSSEN, R., CATES, A. E., AND FELDMAN, A. M.: β-Adrenergic pathways in nonfailing and failing human ventricular myocardium. Circulation **82** (suppl. 1): I-12–I-25, 1990.

BRODY, M. J., FINK, G. D., BUGGY, J., HAYWOOD, J. R., GORDON, F. J., AND JOHNSON, A. K.: The role of the anteroventral third ventricle (AV3V) region in experimental hypertension. Circ. Res. **43** (suppl. 1): I-2–I-13, 1978.

BUCCAFUSCO, J. J.: Mechanism of the clonidine-induced protection against acetylcholinesterase inhibitor toxicity. J. Pharmacol. Exp. Ther. **222:** 595–599, 1982.

BUCCAFUSCO, J. J.: Inhibition of regional brain acetylcholine biosynthesis by clonidine in spontaneously hypertensive rats. Drug Dev. Res. **4:** 627–633, 1984a.

BUCCAFUSCO, J. J.: Inhibition of brain acetylcholine biosynthesis by methyldopa in spontaneously hypertensive rats. Brain Res. Bull. **13:** 257–262, 1984b.

BUCCAFUSCO, J. J.: Effect of methyldopa on brain cholinergic neurons involved in cardiovascular regulation: a study in conscious spontaneously hypertensive rats. Hypertension **6:** 614–621, 1984c.

BUCCAFUSCO, J. J.: Inhibition of brain acetylcholine biosynthesis by clonidine and methyldopa: Relevance to hypertensive disease. *In* Advances in Experimental Medicine, ed. by I. Hanin, pp. 1109–1111, Plenum Press, New York, 1986.

BUCCAFUSCO, J. J.: Neuropharmacologic and behavioral actions of clonidine: interactions with central neurotransmitters. Int. Rev. Neurobiol. **33:** 55–107, 1992.

BUCCAFUSCO, J. J., AND ARONSTAM, R. S.: Precursors of cholinergic false transmitters: central effects on blood pressure and direct interactions with cholinergic receptors. Neuropharmacology **27:** 227–233, 1988.

BUCCAFUSCO, J. J., AND BREZENOFF, H. E.: Mechanisms involved in the cardiovascular response to intracerebroventricular injection of noradrenaline and phentolamine. Neuropharmacology **16:** 775–780, 1977.

BUCCAFUSCO, J. J., AND BREZENOFF, H. E.: Pharmacological study of a central cholinergic mechanism within the posterior hypothalamic nucleus which mediates a hypertensive response. Brain Res. **165:** 295–310, 1979.

BUCCAFUSCO, J. J., AND BREZENOFF, H. E.: Opposing influences on behavior mediated by muscarinic and nicotinic receptors in the rat posterior hypothalamic nucleus. Psychopharmacology **67:** 249–254, 1980.

BUCCAFUSCO, J. J., AND BREZENOFF, H. E.: Brain neurotransmitters and the development and maintenance of hypertension. Prog. Drug Res. **30:** 127–150, 1986.

BUCCAFUSCO, J. J., AND JACKSON, W. J.: Beneficial effects of nicotine administered prior to a delayed matching-to-sample task in young and aged monkeys. Neurobiol. Aging **12:** 233–238, 1991.

BUCCAFUSCO, J. J., LAPP, C. A., ARONSTAM, R. S., HAYS, A. C., AND SHUSTER, L. C.: Role of prostanoids in the regulation of central cholinergic receptor sensitivity. J. Pharmacol. Exp. Ther. **266:** 314–322, 1993.

BUCCAFUSCO, J. J., LAPP, C. A., WESTBROOKS, K. L., AND ERNSBERGER, P.: Role of medullary I_1- imidazoline and α_2-adrenergic receptors in the antihypertensive responses evoked by central administration of clonidine analogs in conscious spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. **273:** 1162–1171, 1995a.

BUCCAFUSCO, J. J., AND MAGRI, V.: The pressor response to spinal cholinergic stimulation in spontaneously hypertensive rats. Brain Res. Bull. **25:** 69–74, 1990.

BUCCAFUSCO, J. J., MAKARI, N. F., AND HAYS, A. C.: Pharmacological analysis of enhanced pressor response to central cholinergic stimulation in spontaneously hypertensive rats. Jpn. J. Pharmacol. **54:** 105–112, 1990.

BUCCAFUSCO, J. J., AND SERRA, M.: Role of cholinergic neurons in the cardiovascular responses evoked by central injection of bradykinin or angiotensin II in conscious rats. Eur. J. Pharmacol. **113:** 43–51, 1985.

BUCCAFUSCO, J. J., AND SPECTOR, S.: Role of central cholinergic neurons in experimental hypertension. J. Cardiovascular Pharmacol. **2:** 347–355, 1980a.

BUCCAFUSCO, J. J., AND SPECTOR, S.: Influence of clonidine on experimental hypertension induced by cholinergic stimulation. Experientia **36:** 671–672, 1980b.

BUCCAFUSCO, J. J., TERRY, JR., A. V., AND SHUSTER, L.: Spinal NMDA receptor: nitric oxide mediation of the expression of morphine withdrawal symptoms in the rat. Brain Res. **679:** 189–199, 1995b.

BUCCAFUSCO, J. J., AND YANG, X-H: Mechanism of the hypertensive response to central injection of nicotine in conscious rats. Brain Res. Bull. **32:** 35–41, 1993.

BUCKLEY, N. J., BONNER, T. I., AND BRANN, M. R.: Localization of a family of muscarinic receptor mRNAs in rat brain. J. Neurosci. **8:** 4646–4652, 1988.

BYLUND, D. B., EIKENBERG, D. C., HIEBLE, J. P., LANGER, S. Z., LEFKOWITZ, R. J., MINNEMAN, K. P., MOLINOFF, P. B., RUFFOLO, R. R., AND TRENDELENBURG, U.: International union of pharmacology nomenclature of adrenoceptors. Pharmacol. Rev. **46:** 121–136, 1994.

CANTOR, E. H., ABRAHAM, S., AND SPECTOR, S.: Central neurotransmitter receptors in hypertensive rats. Life Sci. **28:** 519–526, 1981.

CAPPENDIJK, S. L. T., DE VRIES, R., AND DZOLJIC, M. R.: Inhibitory effect of nitric oxide (NO) synthase inhibitors on naloxone-precipitated withdrawal syndrome in morphine-dependent mice. Neurosci. Lett. **162:** 97–100, 1993.

CASTO, R., AND PRINTZ, M. P.: Genetic transmission of hyperresponsivity in

crosses between spontaneously hypertensive and Wistar-Kyoto rats. J. Hypertension **6** (suppl. 4): S52–S54, 1988.

CASTO, R., AND PRINTZ, M. P.: Exaggerated response to alerting stimuli in spontaneously hypertensive rats. Hypertension **16:** 290–300, 1990.

CASTOLDI, A. F., MANZO, L., AND COSTA, L. G.: Cyclic GMP formation induced by muscarinic receptors is mediated by nitric oxide synthesis in rat cortical primary cultures. Brain Res. **610:** 57–61, 1993.

CHENG, J. B., AND SHIBATA, S.: Pressor response to 5-hydroxytryptamine, norepinephrine and KCl in the perfused hindquarter preparation form the spontaneously hypertensive rat. J. Pharmacol. Exp. Ther. **214:** 488–495, 1980.

COCHRANE, K. L., BUCHHOLZ, R. A., HUBBARD, J. W., KEETON, T. K., AND NATHAN, M. A.: Hypotensive effects of lesions of the rostral ventrolateral medulla in rats are anesthetic-dependent. J. Autonom. Nerv. Sys. **22:** 181–187, 1988.

COHN, J. N.: Abnormalities of peripheral sympathetic nervous system control in congestive heart failure. Circulation **82** (suppl. 1): I-59–I-67, 1990.

COLLIER, B., BOKSA, P., AND LOVAT, S.: Cholinergic false transmitters. Prog. Brain Res. **49:** 107–121, 1979.

COLLINS, R., PETO, R., MACMAHON, S., HEBERT, P., FIEBACH, N. H., EBERLEIN, K. A., GODWIN, J., QIZILBASH, N., TAYLOR, J. O., AND HENNEKENS, C. H.: Blood pressure, stroke, and coronary heart disease. Lancet **335:** 827–838, 1990.

CORAM, W. M., AND BREZENOFF, H. E.: The antihypertensive effect of a selective central muscarinic cholinergic antagonist: N-(4-diethylamino-2-butynyl)-succinimide. Drug Dev. Res. **3:** 503–516, 1983.

CRISCIONE, L., REIS, D. J., AND TALMAN, W. T.: Cholinergic mechanisms in the nucleus tractus solitarii and cardiovascular regulation in the rat. Eur. J. Pharmacol. **88:** 47–55, 1983.

CRUICKSHANK, J. M., THORP, J. M., AND ZACHARIAS, F. J.: Benefits and potential harm of lowering high blood pressure. Lancet **8533:** 581–583, 1989.

CUTLER, J. A., MACMAHON, S. W., AND FURBERG, C. D.: Controlled clinical trials of drug treatment for hypertension. Hypertension **13** (suppl. 1): I-36–I-44, 1989.

DAGE, R. C.: A centrally mediated prolonged hypotension produced by oxotremorine or pilocarpine. Br. J. Pharmac. **65:** 15–21, 1979.

DAHLBOM, R., KARLEN, B., GEORGE, R., AND JENDEN, D. J.: Synthesis and pharmacology of N-(4- dietylamino-2-butynyl)-succinimide (DKJ-21), a new central anticholinergic agent. Life Sci. **5:** 1625–1631, 1966.

DALY, P. A., AND SOLE, M. J.: Myocardial catecholamines and the pathophysiology of heart failure. Circulation **82** (suppl. 1): I-35–I-43, 1990.

DAMPNEY, R. A. L.: Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. **74:** 323–364, 1994.

DECK, R., OBERDORF, A., AND KRONEBERG, G.: Die wirkung von 2-(2, 6-dichlorophenylamino-2- imidazolin-hydrochlorid (Clonidin) auf die kontrakton und die acetylcholin-freisetzung am isolierten koaxiel eletrisch gereizten meerschweincheileum. Arzneim-Forsch **21:** 1580–1584, 1971.

DIETZ, R., SCHÖMIG, A., HAEBARA, H., MANN, J. F. E., RASCHER, W., LÜTH, J. B., GRÜNHERZ, N., AND GROSS, F.: Studies on the pathogenesis of spontaneous hypertension of rats. Circ. Res. **43** (suppl. 1): I98–I106, 1978.

DREW, G. M.: Pharmacological charaterization of the presynaptic α-adrenoceptors regulating cholinergic activity in the guinea-pig brain. Br. J. Pharmacol. **64:** 293–300, 1978.

ECKERSDORF, B., GOLBIEWSKI, H., AND LEWINSKA, M. K.: Kainic acid lesions of the cat's anteromedial hypothalamus and emotional-defensive response evoked by carbachol injection to the same loci. Behav. Brain Res. **24:** 161–166, 1987.

EDERY, H., GEYER, M. A., TAYLOR, P., AND BERMAN, H. A.: Target sites for anticholinesterase on the ventral surface of the medulla oblongata: hypotension elicited by organophosphorous agents. J. Auton. Pharmacol. **6:** 195–205, 1986.

ELROD, K., BUCCAFUSCO, J. J., AND JACKSON, W. J.: Nicotine enhances delayed matching-to-sample performance by primates. Life Sci. **43:** 277–287, 1988.

ERNSBERGER, P., MEELEY, M. P., MANN, J. J., AND REIS, D. J.: Clonidine binds to imidazole binding sites as well as α_2-adrenoceptors in the ventrolateal medulla. Eur. J. Pharmacol. **134:** 1–13, 1987.

ERNSBERGER, P., MEELEY, M. P., AND REIS, D. J.: An endogenous substance with clonidine-like properties: selective binding to imidazole sites in the ventrolateral medulla. Brain Res. **441:** 309–318, 1988.

EVA, C., FUSCO, M., BONO, C., TRIA, M. A., GAMALERO, S. R., LEON, A., AND GENAZZANI E.: Nerve growth factor modulates the expression of muscarinic cholinergic receptor messenger RNA in telencephalic neuronal cultures from newborn rat brain. Molecular Br. Res. **14:** 344–351, 1992.

FELDBERG, W., AND GUERTZENSTEIN, P. G.: Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. J. Physiol. **258:** 337–355, 1976.

FELDMAN, D. S., AND BUCCAFUSCO, J. J.: Role of NMDA receptors and nitric acid synthase in the pressor response to spinal cholinergic stimulation. Pharmacol. Abs. **34:** 166, 1992.

FELDMAN, D. S., AND BUCCAFUSCO, J. J.: Localization of cholinergic neurons involved in the cardiovascular response to intrathecal injection of carbachol. Eur. J. Pharmacol. **250:** 483–487, 1993a.

FELDMAN, D. S., AND BUCCAFUSCO, J. J.: Role of glutamate and nitric oxide on the pressor response to spinal cholinergic stimulation in freely moving rats. Soc. Neurosci. Abs. **19:** 313, 1993b.

FOLKOW, B., AND RUBINSTEIN, E. H.: Cardiovascular effects of acute and chronic stimulation of the defense area in the rat. Acta Physiol. Scand. **68:** 48–57, 1966.

FREEMAN, H., AND CARMICHAEL, H. P.: A pharmacodynamic study of the autonomic nervous system in normal men. The effects of intravenous injection of epinephrine, atropine, ergotomine and physostigmine upon the blood pressure and pulse rate. J. Pharmacol. Exp. Ther. **58:** 409–416, 1936.

GATTU, M., URBANAWIZ, S. E., PAULY, J. R., AND BUCCAFUSCO, J. J.: Estimation of nicotinic receptor binding sites in the CNS of spontaneously hypertensive (SHR) and normotensive rats by autoradiography. Soc. Neurosci. Abs. **21:** 1403, 1995a.

GATTU, M., WEI, J., URBANAWIZ, S. E., PAULY, J. R., AND BUCCAFUSCO, J. J.: Autoradiographic comparison of muscarinic M_1 and M_2 binding sites in the CNS of spontaneously hypertensive (SHR) and normotensive rats. FASEB J. **9:** A46, 1995b.

GIBSON, I. C., AND LOGAN, S. D.: Effects of muscarinic receptor stimulation of sympathetic preganglionic neurones of neonatal rat spinal cord in vitro. Neuropharmacology **34:** 309–318, 1995.

GILLIS, R. A., WALTON, D. P., QUEST, J. A., NAMATH, I. J., HAMOSH, P., AND DRETCHEN, K. L.: Cardiorespiratory effects produced by activation of cholinergic muscarinic receptors on the ventral surface of the medulla. J. Pharmacol. Exp. Ther. **247:** 765–773, 1988.

GIULIANO, R., AND BREZENOFF, H. E.: Increased central cholinergic activity in rat models of hypertension. J. Cardiovasc. Pharmacol. **10:** 113–122, 1987.

GIULIANO, R., RUGGIERO, D. A., MORRISON, S., ERNSBERGER, P., AND REIS, D. J.: Cholinergic regulation of arterial pressure by the C1 area of the rostral ventrolateral medulla. J. Neurosci. **9:** 923–942, 1989.

GOLDBERG, A. M.: Is deanol a precursor of acetylcholine? Practical J. Psych./ Neurol. **38:** 16–20, 1977.

GORDON, F. J., BRODY, M. J., FINK, G. D., BUGGY, J., AND JOHNSON, A. K.: Role of central catecholamines in the control of blood pressure and drinking behavior. Brain Res. **178:** 161–173, 1979.

GORDON, F. J., BRODY, M. J., AND JOHNSON, A. K.: Regional depletion of central nervous system catecholamines: effects on blood pressure and drinking behavior. Brain Res. **345:** 285–297, 1985.

GORDON, F. J.: Spinal GABA receptors and central cardiovascular control. Brain Res. **328:** 165–169, 1985.

GORDON, F. J.: Aortic baroreceptor reflexes are mediated by NMDA receptors in caudal and ventrolateral medulla. Am. J. Physiol. **252:** R628–R633, 1987.

GRANATA, A. R., RUGGIERO, D. A., PARK., D. H., JOH, T. H., AND REIS, D. J.: Brain stem area with C1 epinephrine neurons mediates baroreflex vasodepressor responses. Am. J. Physiol. **248:** H547–H567, 1985.

GUYENET, P. G., FILTZ, T. M., AND DONALDSON, S. R.: Role of excitatory amino acids in rat vagal and sympathetic baroreflexes. Brain Res. **407:** 272–284, 1987.

HALLBÄCK, M.: Interaction between central neurogenic mechanisms and changes in cardiovascular design in primary hypertension. Experimental studies in spontaneously hypertensive rats. Acta Physiol. Scand. **424** (suppl.): 3–59, 1975.

HARA, Y., ALLY, A., AND MURAYAMA, S.: Central cardiovascular effects of physostigmine in anesthetized cats. Neuropharmacology **31:** 923–928, 1992.

HARTLE, D. K., AND BRODY, M. J.: The angiotensin II pressor system of the rat forebrain. Circ. Res. **54:** 355–366, 1984.

HAYS, A. C., SULLIVAN, M. H., HALLOWES, W. C., AND BUCCAFUSCO, J. J.: Acceleration of central muscarinic receptor tolerance by indomethacin. Soc. Neurosci. Abs. **15:** 1218, 1989.

HELKE, C. J., MUTH, E. A., AND JACOBOWITZ, D. M.: Changes in central cholinergic neurons in the spontaneously hypertensive rat. Brain Res. **188:** 425–436, 1980.

HENRY, R., CASTO, R., AND PRINTZ, M. P.: Diurnal cardiovascular patterns in spontaneously hypertensive and Wistar-Kyoto rats. Hypertension **16:** 422–428, 1990.

HERSHKOWITZ, M., ELIASH, S., AND COHEN, S.: The muscarinic cholinergic receptors in the posterior hypothalamus of hypertensive and normotensive rats. Eur. J. Pharmacol. **86:** 229–236, 1983.

HOFFMAN, W. E., AND PHILLIPS, M. I.: A pressor response to intraventricular injections of carbachol. Brain Res. **105:** 157–162, 1976.

HOFFMAN, W. E., SCHMID, P. G., AND PHILLIPS, M. I.: Central cholinergic and noradrenergic stimulation in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. **206:** 644–651, 1978.

HOLLAND, L. N., SHUSTER, L. C., AND BUCCAFUSCO, J. J.: Role of spinal and supraspinal muscarinic receptors in the expression of morphine withdrawal symptoms in the rat. Neuropharmacology **32:** 1387–1395, 1993.

JANOWSKY, D. S., DAVIS, J. M., EL-YOUSEF AND SEKERKE, H. J.: Acetylcholine and depression. Psychosom. Med. **34:** 459, 1973.

JANOWSKY, D. S., RISCH, S. C., HEUY, L. Y., KENNEDY, B., AND ZIEGLER, M.: Effects of physostigmine on pulse, blood pressure, and serum epinephrine levels. Am. J. Psych. **142:** 738–740, 1985.

JANOWSKY, D. S., RISCH, S. C., KENNEDY, B., ZIEGLER, M., AND HUEY, L.: Central muscarinic effects of physostigmine on mood, cardiovascular function, pituitary and adrenal neuroendocrine release. Psychopharmacology **89:** 150–154, 1986.

JOPE, R. S.: High affinity choline transport and acetylCoA production in brain and their roles in the regulation of acetylcholine synthesis. Brain Res. Rev. **1:** 313–344, 1979.

JUDY, W. V., WATANABE, A. M., HENRY, D. P., BESCH, H. R., MURPHY, W. R., AND HOCKEL, G. M.: Sympathetic nerve activity: Role in regulation of blood pressure in the spontaneously hypertensive rat. Circ. Res. **38:** 1121–1129, 1976.

JUDY, W. V., WATANABE, A. M., MURPHY, W. R., APRISON, B. S., AND YU, P. L.: Sympathetic nerve activity and blood pressure in normotensive backcross rats genetically related to the spontaneously hypertensive rat. Hypertension **1:** 598–604, 1979.

KARMOS-VÁRSZEGI, M., AND KARMOS, G.: A comparative study of autonomic, somatic and bioelectric correlates of emotional reactions elicited by cholinergic stimulation of the hypothalamus and the ventral tegmentum. Act. Nerv. Super. (Praha) **19:** 123–124, 1977.

KAWASHIMA, K., FUJIMOTO, K., MIWA, Y., AND MAEMURA, S.: A relationship of central cholinergic functions with enhanced cardiovascular responses to physostigmine in the spontaneously hypertensive rat. J. Hypertens. **4** (suppl. 3): S167–S169, 1986.

KAWASHIMA, K., MIWA, Y., AND FUJIMOTO, K.: Possible involvement of an impaired baroreflex mechanism but not the renin-angiotensin system and vasopressin in the enhanced pressor responsiveness to physostigmine in spontaneously hypertensive rats. Gen. Pharmac. **18:** 327–330, 1987.

KENNEDY, B., JANOWSKY, D. S., RISCH, S. C., AND ZIEGLER, M.: Central cholinergic stimulation causes adrenal epinephrine release. J. Clin. Invest. **74:** 972–975, 1984.

KHAN, I. M., PRINTZ, M. P., YAKASH, T. L., AND TAYLOR, P.: Augmented responses to intrathecal nicotinic agonists in spontaneous hypertension. Hypertension **24:** 611–619, 1994a.

KHAN, I. M., TAYLOR, P., AND YAKASH, T. L.: Stimulatory pathways and sites of action of intrathecally administered nicotinic agents. J. Pharmacol. Exp. Ther. **271:** 1550–1557, 1994b.

KHAN, I. M., TAYLOR, P., AND YAKASH, T. L.: Cardiovascular and behavioral responses to nicotinic agents administered intrathecally. J. Pharmacol. Exp. Ther. **270:** 150–158, 1994c.

KOBINGER, W.: Central α-adrenergic systems as targets for hypotensive drugs. Rev. Physiol. Biochem. Pharmacol. **81:** 39–100, 1978.

KORNER, P. I.: Central control of blood pressure: implications in the pathophysiology of hypertension. *In* Regulation of blood pressure by the central nervous system, ed. by G. Onesti, M. Fernandes and K. E. Kim, pp. 3–20, Grune & Stratton, New York, 1976.

KRUKOFF, T. L.: Decreased hexokinase activity in paraventricular nucleus of adult SHR and renal hypertensive rats. Am. J. Physiol. **254:** R508–R512, 1988.

KRSTIĆ, M. K.: A further study of the cardiovascular responses to central administration of acetylcholine in rats. Neuropharmacology **21:** 1151–1162, 1982.

KRSTIĆ, M. K., AND DJURKOVIĆ, D.: Cardiovascular response to intracerebroventricular administration of acetylcholine in rats. Neuropharmacology **17:** 341–347, 1978.

KRSTIĆ, M. K., AND DJURKOVIĆ, D.: Modification by physostigmine of the cardiovascular responses to intracerebroventricular administration of 5-hydroxytryptamine in rats. Arch. Int. Physiol. Biochem. **95:** 153–158, 1987.

KUBO T., FUKUDA K., MIKAMI A., TAKAHASHI H., MISHINA M., HAGA T., HAGA K., ICHIYAMA A., KANGAWA K., KOJIMA M., MATSUO H., HIROSE T., AND NUMA S.: Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. Nature (Lond.) **323:** 560–561, 1986.

KUBO, T., ISHIZUKA, T., FUKUMORI, R., ASARI, T., AND HAGIWARA, Y.: Enhanced release of acetylcholine in the rostral ventrolateral medulla of spontaneously hypertensive rats. Brain Res. **686:** 1–9, 1995.

KUBO, T., AND TATSUMI, M.: Increased pressor responses to physostigmine in spontaneously hypertensive rats. Naunyn-Schmiedeberg's Arch. Pharmacol. **306:** 81–83, 1979.

KUHAR, M. J., AND MURRIN, L. C.: Sodium-dependent high affinity choline uptake. J. Neurochem. **30:** 15–21, 1978.

KURTZ T. W., CASTO R., SIMONET L., AND PRINTZ M. P.: Biometric genetic analysis of blood pressure in the spontaneously hypertensive rat. Hypertension **16:** 718–724, 1990.

KURTZ, T., AND MORRIS, R.: Biological variability in Wistar-Kyoto rats: implications for research with the spontaneously hypertensive rat. Hypertension **10:** 127–131, 1987.

LAUBIE, M.: Recent Advances in Hypertension, ed. by P. Milliez and M. Safar, Societe Aliena, Reims,1975.

LEE, N. H., AND FRASER, C. M.: Post-transcriptional regulation of the m1 muscarinic acetylcholine receptor (abstract). Life Sci. **52:** 562, 1993.

LEIŠIĆ, R., AND VARAGIĆ, V.: Factors influencing the hypertensive effect of eserine in the rat. Br. J. Pharmacol. **16:** 99–107, 1961.

LEVEY, A. I., KITT, C. A., SIMONDS, W. F., PRICE, D. L., AND BRANN, M. R.: Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J. Neurosci. **11:** 3218–3226, 1991.

LEVIN, E. D.: Nicotinic systems and cognitive function. Psychopharmacology **108:** 417–431, 1992.

LIN, Q., AND LI, P.: Rostral medullary cholinergic mechanisms and chronic stress-induced hypertension. J. Auton. Nerv. Syst. **31:** 211–218, 1990.

LIN, R. F., LIN, T-S., TILTON, R. G., AND CROSS, A. H.: Nitric oxide localized to spinal cords of mice with experimental allergic encephalomyelitis: an electron paramagnetic resonance study. J. Exp. Med. **178:** 643–648, 1993.

LIUZZI, F. J., WU, W., SCOVILLE, S. A., AND SCHINCO, F. P.: Development of nitric oxide synthase expression in the superficial dorsal horn of the rat spinal cord. Exp. Neurol. **121:** 275–278, 1993.

LONGONE, P., MOCCHETI, I., RIVA, M. A., AND WOJCIK, W. J.: Characterization of a decrease in muscarinic m_2 mRNA in cerebellar granule cells by carbachol. J. Pharmacol. Exp. Ther. **265:** 441–446, 1993.

LORENZ, R. G., SAPER, C. B., WONG, D. L., CIARANELO, R. D., AND LOEWY, A. D.: Co-localization of substance P- and phenylethanolamine-N-methyltransferase-like immunoreactivity in neurons of ventrolateral medulla that project to the spinal cord: potential role in control of vasomotor tone. Neurosci. Lett. **55:** 255–260, 1985.

MAGRI, V., AND BUCCAFUSCO, J. J.: Hypertension following intrathecal injection of cholinergic agonists in conscious rats: role of endogenous acetylcholine. J. Auton. Nerv. Syst. **25:** 69–77, 1988.

MAKARI, N. F., TRIMARCHI, G. R., AND BUCCAFUSCO, J. J.: Contribution of pre-and post-synaptic components to heightened central cholinergic activity in spontaneously hypertensive rats. Neuropharmacology **28:** 379–386, 1989.

MALHOTRA, V., KACHROO, A., AND SAPRU, H. N.: Role of α1-adrenergic receptors in the intermediolateral column in mediating the pressor responses elicited by the stimulation of ventrolateral medullary pressor area. Brain Res. **626:** 278–286, 1993.

MARSHALL, D. C., AND BUCCAFUSCO, J. J.: Supraspinal and spinal mediation of naloxone induced morphine withdrawal in rats. Brain Res. **329:** 131–142, 1985.

MARSHALL, D. C., AND BUCCAFUSCO, J. J.: Spinal cholinergic neurons and the expression of morphine withdrawal symptoms in the rat. J. Neurosci. **7:** 621–628, 1987.

MCCALL, R. B., AND CLEMENT, M. E.: Role of serotonin$_{1A}$ and serotonin$_2$ receptors in the central regulation of the cardiovascular system. Pharmacol. Rev. **46:** 231–243, 1994.

MCCARTY, R., CHIUEH, C. C., AND KOPIN, I. J.: Spontaneously hypertensive rats: adrenergic hyperresponsivity to anticipation of electric shock. Behav. Biol. **23:** 180–188, 1978.

MCCARTY, R., KIRBY, R. F., CIERPIAL, M. A., AND JENAL, T. J.: Accelerated development of cardiac sympathetic responses in spontaneously hypertensive (SHR) rats. Behav. Neural. Biol. **48:** 321–333, 1987.

MCDONALD, B. E., COSTA, L. G., AND MURPHY, S. D.: Spatial memory impairment and central muscarinic receptor loss following prolonged treatment with organophosphates. Toxicol. Lett. **40:** 47–56, 1988.

MCGRIFF, J. C., AND QUILLEY, C. P.: The rat with spontaneous genetic hypertension is not a suitable model of human essential hypertension. Circ. Res. **48:** 455–463, 1981.

MCKINNEY, M.: Muscarinic receptor subtype-specific coupling to second messengers in neuronal systems. Prog. Brain Res. **98:** 333–340, 1993.

MCKINNEY, M., AND COYLE, J. T.: The potential for muscarinic receptor subtype-specific pharmacotherapy for Alzheimer's disease. Mayo Clin. Proc. **66:** 1225–1237, 1991.

MELLER, S. T., DYKSTRA, C., AND GEBHART, G. R.: Production of endogenous nitric oxide and activation of soluble guanylate cyclase are required for N-methyl-D-aspartate-produced facilitation of the nonciceptive tail-flick reflex. Eur. J. Pharmacol. **214:** 93–96, 1992.

MENANI, J. V., SAAD, W. A., ARRUDA CAMARGO, L. A., RENZI, A., DE LUCA JR., L. A., AND COLOMBARI, E.: The anteroventral third ventricle (AV3V) region is essential for pressor, dipsogenic and natriuretic responses to central carbachol. Neurosci. Lett. **113:** 339–344, 1990.

MILNER, T. A., PICKEL, V. M., GIULIANO, R., AND REIS, D. J.: Ultrastructural localization of choline acetyltransferase in the rat rostral ventrolateral medulla: Evidence for major synaptic relations with non-catecholaminergic neurons. Brain Res. **500(1–2):** 67–89, 1989.

MIYAZAKI, T., COOTE, J. H., AND DUN, N. J.: Excitatory and inhibitory effects of epinephrine on neonatal rat sympathetic preganglionic neurons in vitro. Brain Res. **497:** 108–116, 1989.

MONTASTRUC, J. L., DANG TRAN, L., HUCHET-BRISAC, A. M., CHELLY, J., ROSTIN, M., AND MONTASTRUC, P.: Involvement of cholinergic mechanisms in the central cardiovascular actions of nicardipine in dogs. Arch. Int. Pharmacodyn. **289:** 212–216, 1987.

MOORE, A. F., AND DREXLER A. P.: A cholinergic link in the centrally mediated actions of angiotensin II. Drug Dev. Res. **2:** 241–250, 1982.

MORRISON, S. F., MILNER, T. A., AND REIS, D. J.: Reticulospinal vasomotor neurons of the rat rostral ventrolateral medulla: relationship to sympathetic nerve activity and the C1 adrenergic cell group. J. Neurosci. **8:** 1286–1301, 1988.

MURUGAIAN, J., SUNDARAM, K., AND SAPRU, H.: Cholinergic mechanisms in the ventrolateral depressor area. Brain Res. **501:** 355–363, 1989.

MYERS, R. D.: Emotional and autonomic responses following hypothalamic chemical stimulation. Can. J. Pharmacol. **18:** 6–14, 1964.

NATTEL, S., BAYNE, L., AND RUEDY, J.: Physostigmine in coma due to drug overdose. Clin. Pharmacol. Ther. **25:** 96–102, 1979.

NILSSON, E., MERETOJA, O. A., AND NEUVONEN, P.: Hemodynamic responses to physostigmine in patients with a drug overdose. Aesth. Analg. **62:** 885–888, 1983.

OGAWA, N., ASANUMA, M., MIZUKAWA, K., HIRATA, H., CHOU, H., AND MORI, A.: Post-ischemic administration of bifemelane hydrochloride prohibits ischemia-induced depletion of the muscarinic M_1-receptor and its mRNA in the gerbil hippocampus. Brain Res. **591:** 171–175, 1992.

OHTSU, E., AND MATSUZAWA, T.: Metabolic alterations in the endogenous for-

mation of 6-keto- prostaglandin $F_{1\alpha}$, thromboxane B_2 and prostaglandin E_2 in the brain-stem of stroke- resistant spontaneously hypertensive rats. J. Nutr. Sci. Vitaminol. **26:** 343–347, 1980.

OISHI, S., YURENEV, A., PARFYONOVA, E., LEE, D. D., DUBOV, P., NIKULIN, I., KOSENKO, A., KONYAEVA, E., BALYAKINA, E., ATAKHANOV, S., AND DEQUATTRO, V.: Altered sympathetic tone in hypertensives with angina and lowered threshold for pacing ischemia. Amer. J. Hypertens. **5:** 154S–163S, 1991.

OKAMOTO, K., AND AOKI, K.: Development of a strain of spontaneously hypertensive rats. Jpn. Circ. J. **27:** 282–293, 1963.

OKUDA, C., MIZOBE, T., AND MIYAZAKI, M.: The involvement of central cholinergic mechanisms in cardiovascular responses to intracerebroventricular and intravenous administration of thyrotropin-releasing hormone. Life Sci. **40:** 1293–1299, 1987.

OPPENHEIMER, E., AKAVIA, E., SHAVIT, S., AND KORCZYN, A. D.: Sympathomimetic amines and cardiac arrhythmias. Cardiovasc. Res. **24:** 754–757, 1990.

PAGANI, M., MAZZUERO, G., FERRARI, A., LIBERATI, D., CERUTTI, S., VAITL, D., TAVAZZI, L., AND MALLIANI A.: Sympathovagal interaction during mental stress. Circulation **83** (suppl. 2): II-43–II-51, 1991.

PARK, K. H., AND LONG, J. P.: Modulation by physostigmine of head-up tilt- and bilateral carotid occlusion-induced baroreflexes in rats. J. Pharmacol. Exp. Ther. **257** (suppl. 1): 50–55, 1991.

PAULETTO, P., SCANNAPIECO, G., AND PESSINA, A. C.: Sympathetic drive and vascular damage in hypertension and atherosclerosis. Hypertension **17** (suppl. 3): III-75–III-81, 1991.

PAZO, J. H., AND MEDINA, J. H.: Cholinergic mechanisms within the caudate nucleus mediate changes in blood pressure. Neuropharmacology **22:** 717–720, 1983.

PAZOS, A., WIEDERHOLD, K. H., PALACIOS, J. M.: Central pressor effects induced by muscarinic receptor agonists: Evidence for a predominant role of the M_2 receptor subtype. Eur. J. Pharmacol. **125:** 63–70, 1986.

PERALTA E. G., ASHKENAZI A., WINSLOW J. W., SMITH D. H., RAMACHANDRAN J., AND CAPON D. J.: Distinct primary structures, ligand-binding properties and tissue-specific expression of four human muscarinic acetylcholine receptors. EMBO J **6:** 3923–3929, 1987.

PHILIPPU, A.: Involvement of cholinergic systems of the brain in the central regulation of cardiovascular functions. J. Auton. Pharmacol. **1:** 321–330, 1981.

PODRID, P. J., FUCHS, T., AND CANDINAS, R.: Role of the sympathetic nervous system in the genesis of ventricular arrhythmia. Circulation **82** (suppl. 1): I-103–I-113, 1990.

POLINSKY, R. J., BROWN, R. T., CURRAS, M. T., BASER, S. M., BAUCOM, C. E., HOOPER, D. R., AND MARINI, A. M.: Central and peripheral effects of arecoline in patients with autonomic failure. J. Neurol. Neurosurg. Psychiatry **54:** 807–812, 1991.

PRICE, R. H. JR., MAYER, B., AND BEITZ, A. J.: Nitric oxide synthase neurons in rat brain express more NMDA receptor mRNA than non-NOS neurons. Neuroreport **4:** 807–810, 1993.

PUNNEN, S., WILLETTE, R. N., KRIEGER, A. J., AND SAPRU, H. H.: Medullary pressor area: Site of action of intravenous physostigmine. Brain Res. **382:** 178–184, 1986.

REIS, D. J., DOBA, N., AND NATHAN, M. A.: Neurogenic arterial hypertension produced by brainstem lesions. *In* Regulation of Blood Pressure by the Central Nervous System, ed. by G. Onesti, M. Fernandes and K. E. Kim, pp. 35–51, Grune & Stratton, New York, 1976.

REIS, D. J., ROSS, C. A., GRANATA, A. R., AND RUGGIERO, D. A.: Role of the C1 area of rostroventrolateral medulla in cardiovascular control. *In* Brain Peptides and Catecholamines in Cardiovascular Regulation, ed. by J. P. Buckley and C. M. Ferrario, pp. 1–14, Raven Press, New York, 1987.

RETTORI, V., GIMENO, M., LYSON, K., AND MCCANN, S. M.: Nitric oxide mediates norepinephrine- induced prostaglandin E_2 release from the hypothalamus. Proc. Natl. Acad. Sci. USA **89:** 1543–11546, 1992.

RIBEIRO-DA-SILVA, A., AND CUELLO, A. C.: Choline acetyltransferase-immunoreactive profiles are presynaptic to primary sensory fibers in the rat superficial dorsal horn. J. Comp. Neurol. **295:** 370–384, 1990.

RISCH, S. C., KALIN, N. H., AND JANOWSKY, D. S.: Cholinergic challenges in affective illness, behavioral and neuroendocrine correlates. J. Clin. Psychopharmacol. **1:** 186–192, 1981.

ROBINSON, S. E.: Interaction of the median raphe nucleus and hypothalamic serotonin with cholinergic agents and pressor responses in the rat. J. Pharmacol. Exp. Ther. **223:** 662–668, 1982.

RUGGIERO, D. A., GIULIANO, R., ANWAR, M., STORNETTA, R., AND REIS, D. J.: Anatomical substrates of cholinergic-autonomic regulation in the rat. J. Comp. Neurol. **292:** 1–53, 1990.

RUGGIERO, D. A., STRONETTA, R. L., MORRISON, S. F., AND REIS, D. J.: Spinal projections to brainstem autonomic centers. Soc. Neurosci. Abstr. **12:** 1156, 1986.

SAITO, R., KAMIYA, H., AND ONO, N.: Role of the central muscarinic receptor of prostaglandin I_2 in cardiovascular function in rat. Brain Res. **330:** 167–169, 1985.

SAKAGUCHI, A., LEDOUX, J. E., AND REIS, D. J.: Sympathetic nerves and adrenal medulla: Contributions to cardiovascular-conditioned emotional responses in spontaneously hypertensive rats. Hypertension **5:** 728–738, 1983.

SAPRU, H. N.: Cholinergic mechanisms subserving cardiovascular function in the medulla and spinal cord. Prog. Brain Res. **81:** 171–179, 1989.

SCHMITT, H.: The pharmacology of clonidine and related products. *In* Handbook of Experimental Pharmacology, Antihypertensive Agents, ed. by F. Gross, vol. 39, pp. 299–396, Springer-Verlag, New York, 1977.

SCHUMAN, E. M., AND MADISON, D. V.: The intracellular messenger nitric oxide is required in long-term potentiation. Science (Wash. DC) **254:** 1503–1506, 1991.

SESSA, W. C., HARRISON, J. K., LUTHIN, D. R., POLLOCK, J. S., AND LYNCH, K. R.: Genomic analysis and expression patterns reveal distinct genes for endothelial and brain nitric oxide synthase. Hypertension **21:** 934–938, 1993.

SHIBUKI, K., AND OKADA, D.: Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. Nature (Lond.) **349:** 326–328, 1991.

SMITH, J. K., AND BARRON, K. W.: Cardiovascular effects of L-glutamate and tetrodotoxin microinjected into the rostral and caudal ventrolateral medulla in normotensive and spontaneously hypertensive rats. Brain Res. **506:** 1–8, 1990a.

SMITH, J. K., AND BARRON, K. W.: The rostral and caudal ventrolateral medulla in young spontaneously hypertensive rats. Brain Res. **506:** 153–158, 1990b.

SMITH, M. D., YANG, X., NHA, J.-Y., AND BUCCAFUSCO, J. J.: Antinociceptive effect of spinal cholinergic stimulation: interaction with substance P. Life Sci. **45:** 1255–1261, 1989.

STAMENOVIC, B. A., AND VARAGIC, V. M.: The effect of reserpine on the efferent neuronal activity in the cervical sympathetic of the rat. Neuropharmacology **9:** 561–566, 1970.

STEWART, R. D.: Tricyclic antidepressant poisoning. Am. Family Physician **19:** 136–144, 1979.

SUH, T. H., WANG, C. H., AND LIM, R. K. S.: The effect of intracisternal applications of acetylcholine and the localization of the pressor centre and tract. Chin. J. Physiol. **10:** 61–79, 1936.

SUNDARAM, K., KRIEGER, A. J., AND SAPRU, H.: M_2 muscarinic receptors mediate pressor responses to cholinergic agonists in the ventrolateral medullary pressor area. Brain Res. **449:** 141–149, 1988.

SUNDARAM, K., MURUGAIAN, J., KRIEGER, A., AND SAPRU, H.: Microinjections of cholinergic agonists into the intermediolateral cell column of the spinal cord at T_1-T_3 increase heart rate and contractility. Brain Res. **503:** 22–31, 1989a.

SUNDARAM, K., MURUGAIAN, J., WATSON, M., AND SAPRU, H.: M2 muscarinic receptor agonists produce hypotension and bradycardia when injected into the nucleus tractus solitarii. Brain Res. **477:** 358–362, 1989b.

SUNDARAM, K., AND SAPRU, H.: Cholinergic nerve terminals in the ventrolateral medullary pressor area: pharmacological evidence. J. Auton. Nerv. Syst. **22:** 221–228, 1988.

SURPRENANT, A., HORSTMAN, D. A., AKBARALI, H., AND LIMBIRD, S. E.: A point mutation of the α_2-adrenoceptor that blocks coupling to potassium but not calcium currents. Science (Wash. DC) **257:** 977–980, 1992.

SVENSSON, L., HARTHON, C., AND LINDER, B.: Evidence for a dissociation between cardiovascular and behavioral reactivity in the spontaneously hypertensive rat. Physiol. Behav. **49:** 661–665, 1991.

TAIRA, C. A., AND ENERO, M. A.: Central α_1- and α_2-adrenoceptors and brain cholinergic stimulation in sinoarotic denervated rats. Eur. J. Pharmacol. **271:** 9–16, 1994.

TAKAHASHI, H., AND BUCCAFUSCO, J. J.: The sympathoexcitatory response following selective activation of a spinal cholinergic system in anesthetized rats. J. Auto. Nerv. Sys. **34:** 59–68, 1991a.

TAKAHASHI, H., AND BUCCAFUSCO, J. J.: Spinal cholinergic modulation of cardiovascular tone and a somatosympathetic reflex response. Brain Res. Bull. **27:** 47–51, 1991b.

TAKAHASHI, H., AND BUCCAFUSCO, J. J.: Excitatory modulation by a spinal cholinergic system of a descending sympathoexcitatory pathway in rats. Neuropharmacology **31:** 259–269, 1992.

TAKAHASHI, H., INOUE, A., TAKEDA, K., OKAJIMA, H., SASAKI, S., YOSHIMURA, M., NAKAGAWA, M., AND IJICHI, H.: Augmented central cholinergic mechanisms in spontaneously hypertensive rats: Involvement of deranged noradrenergic mechanisms in the brain. Jpn. Heart J. **25:** 397–410, 1984.

TALMAN, W. T., COLLING, J-M. E. K., AND ROBERTSON, S. C.: Glycine microinjected into nucleus tractus solitarii of rat acts through cholinergic mechanisms. Am. J. Physiol. **260:** H1326–H1331, 1991.

TALMAN, W. T., AND LEWIS, S. J.: Altered cardiovascular responses to glutamate and acetylcholine microinjected into the nucleus tractus solitarii of the SHR. Clin. Exp. Hypertens. **13** (suppl. 5): 661–668, 1991.

TERENGHI, G., RIVEROS-MORENO, V., HUDSON, L. D., IBRAHIM, N. B. N., AND POLAK, J. M.: Immunohistochemistry of nitric oxide synthase demonstrates immunoreactive neurons in spinal cord and dorsal root ganglia of man and rat. J. Neurolog. Sci. **118:** 34–37, 1993.

TERRY, A. V., JR., BUCCAFUSCO, J. J., AND JACKSON, W. J.: Scopolamine reversal of nicotine enhanced delayed matching-to-sample performance by monkeys. Pharmacol. Biochem. Behav. **45:** 925–929, 1993.

TRIMARCHI, G. R., AND BUCCAFUSCO, J. J.: Changes in regional brain synaptosomal high affinity choline uptake during the development of hypertension in spontaneously hypertensive rats. Neurochem. Res. **12:** 247–254, 1987.

TRIMARCHI, G. R., GLISSON, W. C., THOMPSON, W. M., VANLINGEN, J., AND BUCCAFUSCO, J. J: Cholinergic neurons and the cardiovascular response produced by central injection of substance P in the normotensive rat. Life Sci. **39:** 1579–1588, 1986.

TRIPPODO, N. C., AND FROHLICH, E. D.: Similarities of genetic (Spontaneous) hypertension, man and rat. Circ. Res. **48:** 309–319, 1981.

TSENG, C.-J., GER, L.-P., LIN, H.-C., AND TUNG, C.-S.: The pressor effect of nicotine in the rostral ventrolateral medulla of rats. Chin. J. Physiol. **37:** 83–87, 1994.

VARAGIĆ, V. M.: The action of eserine on the blood pressure of the rat. Br. J. Pharmacol. **10:** 349–353, 1955.

VARAGIĆ, V. M., AND KRSTIC, M.: Adrenergic activation by anticholinesterases. Pharmacol. Rev. **18:** 799–800, 1966.

VARAGIĆ, V. M., MRSULJA, B., STOSIC, N., PASIC, M., AND TERZIC, M.: Glycogenolytic and hypertensive effects of physostigmine in the anti-nerve-growth-factor-serum treated rats. Eur. J. Pharmacol. **12:** 194–202, 1970.

VARAGIĆ, V. M., PROSTRAN, M. S., STEPANOVIC, S., SAVIC, J., AND VUJNOV, S.: Transmitter interactions in the central cholinergic control of blood pressure regulation. Drug Metab. Drug Interact. **9:** 49–76, 1991.

VARGAS, H. M., AND BREZENOFF, H. E.: Suppression of hypertension during chronic reduction of brain acetylcholine in spontaneously hypertensive rats. J. Hypertens. **6:** 739–745, 1988.

VARGAS, H. M., AND RINGDAHL, B.: Centrally active antimuscarinic analogs of oxotremorine selectively block physostigmine-induced hypertension, but not peripheral muscarinic vasodepression. J. Pharmacol. Exp. Ther. **253:** 165–170, 1990.

VERRIER, R. L., AND DICKERSON, L. W.: Autonomic nervous system and coronary blood flow changes related to emotional activation and sleep. Circulation **83** (suppl. 2): II-81–II-89, 1991.

WALKER, J. L., AND WEETMAN, D. F.: Oxotremorine-induced hypertension in the anaesthetized rat. Br. J. Pharmac. **39:** 490–500, 1970.

WALL, S. J., YASUDA, R. P., LI, M., CIESLA, W., AND WOLFE, B. B.: Differential regulation of subtypes m1-m5 of muscarinic receptors in forebrain by chronic atropine administration. J. Pharmacol. Exp. Ther. **262:** 584–588, 1992.

WANG, S-Z., SHENG, S-Z., JOSEPH, J. A., AND EL-FAKAHANY, E. E.: Comparison of the level of mRNA encoding m1 and m2 muscarinic receptors in brains of young and aged rats. Neurosci Lett. **145:** 149–152, 1992a.

WANG, S-Z., ZHU, S-Z., MASH, D. C., AND EL-FAKAHANY, E. E.: Comparison of the concentration of messenger RNA encoding four muscarinic receptor subtypes in control and Alzheimer brains. Molecular Br. Res. **16:** 64–70, 1992b.

WEI, J., AND BUCCAFUSCO, J. J.: Transcriptional changes in the expression of central muscarinic receptors in the spontaneously hypertensive rat (SHR). Soc. Neurosci. Abstr. **20:** 108, 1994.

WEI, J., MILICI, A., AND BUCCAFUSCO, J. J.: Alterations in the expression of the genes encoding specific muscarinic receptor subtypes in the hypothalamus of spontaneously hypertensive rats. Circ. Res. **76:** 142–146, 1995.

WEI, J., WALTON, E. A., MILICI, A., AND BUCCAFUSCO, J. J.: m1-m5 Muscarinic receptor distribution in rat CNS by RT-PCR and HPLC. J. Neurochem. **63:** 815–882, 1994.

WEINER, H.: Stressful experience and cardiorespiratory disorders. Circulation **83** (suppl. 1): II-2–II-8, 1991.

WERNER, U., STARKE, K., AND SCHUMANN, H. J.: Actions of clonidine and 2-(2-methyl-6-ethylcyclohexylamine)-2-oxazoline on postganglionic autonomic nerves, Arch. Int. Pharmacodyn. Ther. **195:** 282–290, 1972.

WILLETTE, R. N., DOORLEY, B. M., AND SAPRU, H. N.: Activation of cholinergic mechanisms in the medulla oblongata reverse intravenous opioid-induced respiratory depression. J. Pharmacol. Exp. Ther. **240:** 352–358, 1987.

WILLETTE, R. N., KRIEGER, A. J., BARCAS, P. P., AND SAPRU, H. N.: Medullary GABA receptors and the regulation of blood pressure in the rat. J. Pharmacol. Exp. Ther. **226:** 893–899, 1983.

WILLETTE, R. N., PUNNEN, S., KRIEGER, A. J., AND SAPRU, H. N.: Cardiovascular control by cholinergic mechanisms in the rostral ventrolateral medulla. J. Pharmacol. Exp. Ther. **231:** 457–463, 1984.

WU, Y. Y., AND WEI, E. T.: Infusions of chemicals into the brain and the development of sustained elevations of blood pressure in the rat. Life Sci. **30:** 1537–1545, 1982.

XIAO, Y-F., AND BREZENOFF, H. E.: The role of m2 muscarinic receptors in the posterior hypothalamus in the pressor response to intracerebroventricularly-injected neostigmine. Neuropharmacology **24:** 1061–1065, 1988.

YAKSH, T. L.; DIRKSEN, R.; HARTY, G. J.: Antinociceptive effects of intrathecally injected cholinomimetic drugs in the rat and cat. Eur. J. Pharmacol. **117:** 81–88, 1985.

YAMADA, S., KAGAWA, Y., USHIJIMA, H., TAKAYANAGI, N., TOMITA, T., AND HAYASHI, E.: Brain nicotinic cholinoceptor binding in spontaneous hypertension. Brain Res. **410:** 212–218, 1987.

YAMAMURA, H. I., WAMSLEY, J. K., DESHMUKH, P., AND ROESKE, W. R.: Differential light microscopic autoradiographic localization of muscarinic cholinergic receptors in the brainstem and spinal cord of the rat using (^{3}H)pirenzepine. Eur. J. Pharmacol. **91:** 147–149, 1983.

YAMORI, Y.: Neurogenic mechanisms of spontaneous hypertension. *In* Regulation of Blood Pressure by the Central Nervous System, ed. by G. Onesti, M. Fernandes and K. E. Kim, pp. 65–76, Grune & Stratton, New York, 1976.

YAMORI, Y.: Pathogenesis of spontaneous hypertension as a model for essential hypertension. Jap. Circ. J. **41:** 259–266, 1977.

YAMORI, Y.: Development of the spontaneously hypertensive rat (SHR) and of various spontaneous rat models, and their implications. *In* Handbook of Hypertension: Experimental and Genetic Models of Hypertension, ed. by W. de Jong, vol. 4, pp. 224–239, Elsevier Science Publishers, New York, 1984.

YASUDA, R. P., CIESLA, W., FLORES, L. R., WALL, S. J., LI, M., SATKUS, S. A., WEISSTEIN, J. S., SPAGNOLA, B. V., AND WOLFE, B. B.: Development of antisera selective for m4 and m5 muscarinic cholinergic receptors: distribution of m4 and m5 receptors in rat brain. Mol. Pharmacol. **43:** 149–157, 1993.

ZHUO, M., MELLER, S. T., AND GEBHART, G. F.: Endogenous nitric oxide is required for tonic cholinergic inhibition of spinal mechanical transmission. Pain **54:** 71–78, 1993.