Ann. Rev. Pharmacol. Toxicol. 1982. 22:341–81

CARDIOVASCULAR CONTROL BY CHOLINERGIC MECHANISMS IN THE CENTRAL NERVOUS SYSTEM

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INTRODUCTION

Despite an extensive research effort over the last few decades, understanding of how the central nervous system (CNS)[1] controls the circulation is at best still incomplete. Many symposia and reviews have detailed the function of catecholaminergic (CCA) pathways in the central connections of the autonomic nervous system (1–6). It is widely recognized that several methodological advances [e.g. chemical ablation by 6-hydroxydopamine (6-OHDA), sensitive and selective methods for detection and quantitation of CCA, use of specific substances which can alter formation, metabolism or release of catecholamines], paralleled by the development of drugs which lower blood pressure (BP) through stimulation of central adrenergic receptor sites (e.g. alpha-methyldopa, clonidine), have fostered precocious advances involving the biogenic amines. Until recently, however, far less

[1]*Abbreviations used in this paper:* ACh, acetylcholine; AChE, acetylcholinesterase; ADH, antidiuretic hormone; BP, blood pressure; CAOR, carotid arterial occlusion reflex; CCA, catecholamine(rgic); ChAT, choline acetyltransferase; CNS, central nervous system; CV, cardiovascular; DFP, diisopropylfluorophosphate; DMPP, dimethylphenylpiperazinium; HR, heart rate; HC-3, hemicholinium-3; 6-OHDA, 6-hydroxydopamine; ia, intraarterial(ly); ic, intracisternal(ly); ica, intracarotid arterial(ly); icv; intracerebroventricular(ly); iv, intravenous(ly); iva, intravertebral arterial(ly); LC, locus coeruleus; LV, lateral ventricle; NE, norepinephrine; NTS, nucleus tractus solitarius; PHN, posterior hypothalamic nucleus; sc, subcutaneous(ly); SH rat, spontaneously hypertensive rat; TEPP, tetraethylpyrophosphate; and TMA, tetramethylammonium.

0362-1642/82/0415-0341$02.00

progress had been made in identifying and mapping cholinergic pathways, in quantitating regional brain cholinergic enzyme and neurotransmitter levels, or in modifying acetylcholine (ACh) synthesis, metabolism, or release by pharmacological interventions. With the potential availability of a recently described cholinergic neurotoxin (7, 8), new areas of investigation undoubtedly will open.

Using a variety of pharmacological and neurophysiological techniques, numerous investigators have contributed substantial data which we feel now warrant consolidation and perspective. A comprehensive review of the literature regarding central muscarinic mechanisms which modulate BP and heart rate (HR) has been conspicuously lacking. Accordingly, this paper will (*a*) critically summarize recent experimental evidence for central cholinergic, particularly muscarinic, influence on cardiovascular (CV) function; (*b*) discuss mechanisms by which such muscarinic involvement may be expressed; and (*c*) probe the functional implications of these muscarinic regulatory systems. The literature published from about 1950–1981 will be examined primarily. However, earlier articles, where particularly pertinent to an issue, may be cited. For a more comprehensive overview of the literature appearing prior to this period, the reader is referred to reports (9, 10) which survey the early findings.

EFFECTS OF CENTRAL CHOLINERGIC STIMULATION ON CARDIOVASCULAR FUNCTION

Cholinergic stimulation of the CNS both by direct receptor agonists and acetylcholinesterase (AChE) inhibitors elicits a pressor response in several species. Although most investigations have been conducted in the rat, there is substantial evidence that the cholinergically-mediated pressor response occurs also in the cat, dog, and in man. This hypertensive effect can be observed following either systemic or central routes of administration, and appears to involve activation of central muscarinic receptor sites. The following two subsections will survey experimental findings that substantiate these generalizations.

Cardiovascular Effects of Acetylcholine and Other Direct Receptor Agonists

Cumulative data from several different laboratories have implicated central cholinergic neurons and receptors in CV regulation. Much of the published literature concerning the central CV effects of cholinomimetics appears contradictory, pressor and/or depressor effects variously having been reported. However, closer analysis of these studies suggests that, while unresolved issues still remain, such discrepant findings are largely attributable

to species differences and variation in dose, site, and mode of administration, and to the use of anesthetized preparations in some studies and conscious subjects in others. Early investigators (9, 10) also recognized that such factors could account for the lack of uniformity in the nature of reported responses to anticholinesterase agents. Our review of the literature reinforces their contention, and furthermore, supports the thesis that, when such considerations are taken into account, a substantial and continually growing body of data emerges indicating that brain ACh mediates a rise in BP via activation of muscarinic receptor sites.

While the focus of this review will be such muscarinic participation in central circulatory regulation, a possible contributory role of nicotinic pathways in the brain cannot be discounted. Relevant experiments concerning central nicotinic stimulation will be cited here. However, this review in no way purports to comprehensively evaluate the literature on the CNS effects of nicotine in CV function. Rather, as we hope to amply document, the preponderance of experimental evidence indicates that physiological central pathways which modulate the CV effects of endogenous ACh show specific muscarinic properties.

CAT AND DOG Acetylcholine has been reported to have excitatory or biphasic excitatory/inhibitory actions in central mechanisms involved in CV regulation. These discordant observations are more apparent than real, however, arising mainly from differences in details of administration, use of anesthetics, and possibly also to some extent, to yet undefined species variations.

As early as 1935, Suh, Wang, and Lim (11) described a rise in BP in the anesthetized dog after intracisternal (ic) injection or application of ACh to the floor of the fourth ventricle. According to several subsequent investigators, intracerebroventricular (icv) injection of ACh in anesthetized (12, 13) or unanesthetized (14) dogs and in conscious cats (15) produces a small, transient pressor response of almost immediate onset, and a fall in HR. These effects of ACh are abolished by icv atropine (13, 14).

As little as 10 μg ACh injected icv elicited CV responses in the unanesthetized dog (14), whereas a dose more than 10-fold greater (150 μg) was required to obtain comparable effects in the unanesthetized cat (15). Such difference in sensitivity to ACh-induced CV changes could be species-related, attributable perhaps to the more rapid rate of drug clearance from the cerebral ventricles in the cat than in the dog (12). Alternatively, it may be due to differences in the method of icv drug administration. In the dog, Lang & Rush (14) administered ACh as a bolus injection into the lateral ventricle (LV) (total injection volume 100–200 μl with 100 μl NaCl wash); this could produce rapid distribution through the ventricular system, thus

achieving effective concentrations in lower brainstem regions. In contrast, Day & Roach (15) infused a smaller volume (100 μl) of ACh into the LV of the cat over a period of 4 min. Compared with bolus injection, drug diffusion by this mode of administration could depend more on the physiological flow of cerebrospinal fluid; the effective drug concentrations reaching CV-sensitive areas of caudal brain regions would be lower, and the cat would appear to be more resistant to the CV stimulant effects of ACh. Similar considerations might be applied in other instances of conflicting reports in the literature.

Using anesthetized animals, other investigators have reported depressor effects in cats (16), or both pressor and depressor effects in cats (12) and dogs (17), following icv administration of ACh or superfusion of various cat brain regions (18) with ACh. Since purely pressor responses are obtained in the cat and dog without anesthesia, the use of anesthetics may have contributed to these discrepant observations. Cardiovascular responses to pharmacological agents are profoundly altered by various anesthetics (19–25). Pentobarbital, for example, reduces high-affinity choline uptake in the cortex and hippocampus (26, 27), decreases ACh turnover (28), reduces cholinergic neuronal activity (26–28), and can inhibit pressor responses to electrical stimulation of various brain regions (29). There is also evidence that general anesthetics block excitatory muscarinic actions of cholinergic agonists on cortical neurons (30, 31). Such blunting of CV responsiveness is suggested in the studies at issue by the observation that higher doses of ACh were required to produce pressor effects in anesthetized cats and dogs (approximately 300–500 μg) than in conscious animals (10–150 μg). The possible role of anesthesia in explaining variable responses to other cholinergic agonists will become apparent in subsequent paragraphs.

When various direct-acting cholinomimetics are centrally administered in the cat or dog, pressor responses, usually accompanied by tachycardia, also are observed. In the anesthetized dog, icv carbachol (13, 17) or ic administered oxotremorine (32) evokes more marked and more persistent CV stimulant effects than does ACh. Similar responses are elicited in the conscious dog by icv injection of methacholine (14) and in the conscious cat by icv carbachol (15). Intracisternal administration of oxotremorine also elevates BP in the conscious dog (32). These effects are consistently antagonized by atropine administered by the same route (13, 15, 17, 32), indicating the involvement of muscarinic receptors in a central pressor mechanism.

In the anesthetized cat, however, carbachol lowers arterial pressure (16, 33). In view of the depressant effect of anesthetics discussed above, and of the observations that without anesthesia, purely pressor responses are obtained in the conscious cat (15), it is possible that the anesthetic agent blocks the muscarinic excitatory action of cholinergic stimulation, thereby un-

masking a nicotinic depressor component. Such selective inhibition by anesthetic agents could apply also to the variable pressor responses observed after central administration of nicotine. Whereas icv injection of nicotine or of the nicotinic agonist tetramethylammonium chloride (TMA) produces pressor (14, 15) or biphasic (15, 34) responses in the unanesthetized cat or dog, icv nicotine is depressor in the anesthetized cat (16, 33). Additional evidence corroborating this view was provided by Armitage & Hall (16, 33), who observed that a pressor response to icv nicotine in the conscious cat could be converted to a fall after induction of anesthesia with chloralose. Schaeppi (34) also reported that variable pressor responses to intravertebral injection of nicotine in the conscious dog were converted to entirely depressor ones after thiopentone.

Although experiments with specific muscarinic (e.g. atropine, scopolamine) and nicotinic (e.g. mecamylamine, hexamethonium) blockers indicate that significant CV stimulant effects of cholinergic activation are mediated via central muscarinic receptor sites, the existence of nicotinic pressor pathways cannot be dismissed. Based on the specific blocking action of muscarinic and nicotinic receptor antagonists in cats, Armitage & Hall (16, 33) and Day & Roach (15) have indicated both nicotinic and muscarinic receptors are involved in central cholinergic pathways of BP regulation. Cardiovascular excitatory mechanisms involving nicotinic, but not muscarinic, excitatory CV mechanisms (18, 35–37), as well as inhibitory nicotinic (38) or inhibitory muscarinic (18, 35–37) CV mechanisms, also have been described.

RAT ACh (1.5–6 μg) administered ic (39) or icv (40) in the anesthetized or conscious rat produces an immediate rise in arterial pressure which can be abolished by spinal cord transection or atropine administered by the same route (40). Thus, in the rat, as in the cat and dog, ACh can mediate a pressor response by activation of central muscarinic receptor sites.

In agreement with the previously cited studies in other species, intraventricular injection of ACh (1.5–6 μg) causes variable HR changes (40, 41), but notably in the anesthetized rat the pressor response to icv ACh is not consistently associated with bradycardia (40). Furthermore, following spinal cord transection, this bradycardia can occur in the absence of a rise in BP. These results suggest, therefore, that, at least in the majority of rats, the observed bradycardia is not of reflex origin. That icv ACh might produce central stimulation capable of overriding reflex bradycardia is suggested by the finding that icv injection of ACh often produced tachycardia which was abolished by cutting the vagosympathetic trunks.

Other cholinergic agonists also have been shown to alter CV parameters when injected into the cerebral ventricular system or directly into various

brain regions of the rat. Brezenoff's (42–44) initial studies of intrahypothalamically injected muscarinic agonists (e.g. ACh, muscarine, methacholine, oxotremorine) suggested that muscarinic receptors in various hypothalamic regions might mediate a hypotensive response. After more localized injections, BP responses to intrahypothalamic application of carbachol and various AChE inhibitors were found to depend greatly on the specific region of the hypothalamus involved (45).

Microinjection of carbachol (1–3 μg) into the posterior or ventromedial hypothalamic nuclei of urethane-anesthetized rats increases both BP and HR, while injection of similar doses into the dorsomedial or premammillary hypothalamic areas decreases these parameters (45). In the posterior hypothalamic nucleus (PHN) of *unanesthetized* freely-moving rats, as well as in the supramammillary nucleus and pars medialis of the medial mammillary nucleus, carbachol (0.1–100 μmol, equivalent to 18.2 ng–18.2 μg) also evokes a dose-dependent pressor response. However, changes in HR are variable, correlating well ($r = 0.9$) with the degree of locomotor activity evoked by carbachol (41, 46). Both the increase in BP and the bradycardia are significantly reduced by prior intrahypothalamic injection of atropine, but are unaffected by mecamylamine, indicating that muscarinic receptors mediate these responses (41). In contrast, the increase in locomotor activity evoked by intrahypothalamic carbachol is specifically blocked by prior intrahypothalamic injection of mecamylamine but not atropine (47). Thus, the carbachol-induced increase in locomotor activity at nicotinic receptor sites may mask the bradycardia produced by muscarinic stimulation in this brain region.

Similar observations suggesting a central cholinergic excitatory CV mechanism have been described following ic administration of carbachol (1 μg) in the chloralose-urethane anesthetized rat (39) or injection of carbachol (0.25 ng–250 ng) into the lateral (48–51) or third (52) ventricle of conscious rats. Since prior icv treatment with atropine, but not hexamethonium, blocked the carbachol-induced rise in BP and bradycardia, Hoffman (52) concluded that central muscarinic mechanisms are involved. Ozawa & Uematsu (39) observed that the pressor response to ic carbachol could be prevented almost completely by either ic atropine or hexamethonium, and concluded that nicotinic receptor sites may be located along the pathway of a primary muscarinic receptor-activated mechanism. The dose of hexamethonium used in this study was quite high (0.5 mg), however, making such a conclusion tenuous. In agreement with Buccafusco & Brezenoff (41) that a central endogenous mechanism may directly mediate the bradycardic response to carbachol, Ozawa & Uematsu (39) found that considerable bradycardia persisted even after bilateral sectioning of the cervical vagal nerves; however, ic phentolamine significantly attenuated the fall in HR.

Additional observations suggesting central muscarinic participation in an excitatory CV neuronal system is given by reports that in anesthetized rats ic administration of the muscarinic agonist bethanechol (39) or iv injection of arecoline in rats pretreated with methylatropine (53) can increase arterial BP. Pressor responses have been observed as well following oxotremorine administered iv (54, 55), sc (25), or ic (39) to anesthetized rats, or sc (25) and intrahypothalamically (41) to unanesthetized rats. These pressor responses appear to involve activation of muscarinic receptor sites since specific blockade can be demonstrated with atropine or scopolamine (25, 54, 55).

Variable CV changes have been described following nicotinic stimulation (ic, icv, intrahypothalamic) with DMPP (dimethylphenylpiperazinium) (39) or nicotine itself (39, 40, 42, 52, 56), suggesting that nicotinic receptors may play a role in central pathways which regulate CV functions.

MAN The role of direct cholinergic agonists in CNS cardiovascular regulation in man is not well documented in the literature. For the most part, relevant observations are in the form of individual case reports or small group evaluations.

Several examples of pressor and tachycardic responses in man have appeared in association with certain clinical applications of AChE inhibitors, particularly physostigmine (see Effects of Central Cholinergic Stimulation on Cardiovascular Function: Cardiovascular Effects of Acetylcholinesterase Inhibitors; Man). Earlier indications that a direct receptor cholinergic agonist (ACh) might act in the human CNS to elevate BP (57) are supported by recent evidence that activation of central muscarinic receptors increases BP and HR in man. In a study reported by Nutt et al (58), six patients pretreated with methylscopolamine (to prevent peripheral cholinergic symptoms) each received a single sc injection of arecoline in an effort to ameliorate striatal cholinergic hypofunction associated with Huntington's disease. At doses ranging from 5 to 20 mg, arecoline produced significant dose-related increases in systolic BP and HR. These findings thus concur with abundant experimental evidence in animals in which similar CV changes appear to be centrally-mediated muscarinic phenomena.

Cardiovascular Effects of Acetylcholinesterase Inhibitors

Acetylcholinesterase inhibitors, by blocking the rapid inactivation of ACh, prolong and enhance the action of the neurotransmitter released from cholinergic terminals. Pharmacological manipulations which increase effective endogenous brain ACh at central neuronal synapses evoke a pressor response which is specifically antagonized by atropine or by depletion of brain ACh with hemicholinium-3 (HC-3) [HC-3 rapidly blocks high-affinity uptake of choline into cholinergic nerve terminals, thereby inhibiting ACh

synthesis and depleting ACh stores (59–62)]. It is precisely this antagonism which convincingly implicates muscarinic receptor sites in the central physiological circuits that mediate CV information. Experimental evidence that central cholinesterase and endogenous brain ACh are involved in the hypertensive response to AChE inhibitors will be examined in Central Mechanisms Mediating Cardiovascular Effects of Muscarinic Activation: Brain AChE and ACh. The following paragraphs will document reports of the phenomenon in various species, citing particularly data which suggest that central muscarinic receptors are involved.

CAT, DOG, AND RAT It has been known for many years that AChE inhibitors elevate BP in the cat and dog. According to Hornykiewicz & Kobinger (9), one of the first published reports (1867) not only described a marked pressor response to physostigmine in dogs, but attributed the effect to a central action of the drug [see (9, 10) for other literature published from 1867 to about 1950]. It has since been shown by several different laboratories that AChE inhibitors can evoke a rise in BP in the cat, dog, and rat.

Both carbamate (e.g. physostigmine, neostigmine), as well as organophosphorus (e.g. sarin, DFP, paraoxon) AChE inhibitors increase arterial pressure in the cat, (10), dog (12, 14, 32, 63–67), and rat (9, 20, 23, 41, 42, 45–47, 53, 54, 63, 68–76, 78–91). Those AChE inhibitors which enter the CNS (e.g. physostigmine, sarin) consistently increase BP after iv injection (9, 10, 20, 23, 53, 54, 63, 64, 66, 68–73, 75–78, 80–90, 92), as well as administration into the cerebral ventricles (12, 14, 20, 63, 68, 74), the carotid artery (10, 63, 79) or vertebral artery (63), cisternum magnum (9, 32, 63), or into specific brain regions (41, 42, 45–47, 69, 70, 91). In contrast, quarternary ammonium AChE inhibitors which do not readily penetrate the CNS (e.g. neostigmine, edrophonium, echothiophate) either elicit a very small rise in BP (10, 77, 80, 81, 89) or have no pressor effect at all (9, 80, 81, 93) when injected iv. However, when administered directly into the brain (whether ic, icv, intrahypothalamically, or by injection into the carotid artery) (9, 10, 41, 45, 93), quarternary AChE inhibitors consistently produce an increase in BP.

Generally the doses required to elicit a pressor response by central routes of administration are considerably lower than those required by iv administration. Hornykiewicz & Kobinger (9) noted, for example, that ic injection of 2.7 μg physostigmine in the rat produced pressor responses comparable to that evoked by six-fold higher doses administered iv. In the cat also, intraarterial (ia) doses of physostigmine, parathion, or neostigmine only 1/10 as great as those injected iv produced significant increases in BP (10).

The pressor response to iv or centrally administered AChE inhibitors

occurs in both conscious and anesthetized preparations. In rats, anesthesia does not appear to influence the consistency with which a pressor response can be produced. This may be a function of the particular anesthetic agent —urethane—usually used in these studies. The selective inhibition of cholinergically stimulated pressor effects, which may occur with other general anesthetics in the cat and dog (see Effects of Central Cholinergic Stimulation on Cardiovascular Function), may not occur as readily with urethane in rats. Calaresu (94) found that whereas alpha-chloralose blocked the BP increase produced by electrical stimulation of the lateral septum in rats, urethane left the pressor response unchanged. Nevertheless, cardiac effects of central cholinergic stimulation in the rat appear to be modified profoundly by anesthetic agents, including urethane. In the unanesthetized rat, iv or icv administration of physostigmine evokes an immediate bradycardia, even before the pressor response develops (20, 74). However, in the urethane-anesthetized rat, tachycardia occurs concomitantly with the rise in BP (20). Similarly, whereas neostigmine injected into the PHN of the conscious rat results in a dose-dependent bradycardia coincident with an increase in BP (41), in the urethane-anesthetized animal it consistently produces tachycardia (45).

With respect to the pressor response itself, several features are noteworthy. In general, the pressor response to AChE inhibitors is dose-dependent and, in the case of the carbamates, non-tachyphylactic (10, 23, 73, 75, 76, 83, 86). However, tachyphylaxis has been reported to organophosphorus AChE inhibitors (10, 40, 83, 95). The onset of the pressor response to centrally administered AChE inhibitors usually is considerably longer (on the order of 1–5 min) than that observed following ACh, carbachol, and other direct receptor agonists [immediate to less than 1 min; (9, 10, 39, 41, 44, 52, 80–83, 96]. This observation suggests that the AChE inhibitors act through an indirect mechanism and that the longer latency could reflect the time required for adequate enzyme inhibition and accumulation of effective ACh levels. A longer duration of pressor and cardiac changes following injection of AChE inhibitors (up to about 90 min) also is consonant with inhibition of the enzyme that normally rapidly terminates the action of ACh released from nerve terminals.

Finally, it has been a consistent finding in these studies that atropine antagonizes the pressor and cardiac effects of AChE inhibitors. When injected prior to administration of the AChE inhibitor, atropine prevents the CV responses to the anticholinesterase; when given after injection of the AChE inhibitor, it aborts them.

While early investigators reported that iv atropine could abolish the pressor responses to various AChE inhibitors administered by the same route (63, 80) or by icv injection (63), their doses of antagonist were heroic

(up to 10 mg/kg). Subsequent work has demonstrated complete or nearly complete blockade of anticholinesterase-induced pressor responses with far smaller doses of atropine [0.15–1.0 mg/kg (9, 32, 76, 85, 89)], suggesting the possibility that such antagonism could be due to central anticholinergic effects. At a dose (1.5 mg/kg) which did not at all influence the ganglionic stimulation produced by high doses of ACh, Hornykiewicz & Kobinger (9) showed that atropine abolished the pressor effect of iv physostigmine. We have found that systemic administration of 1 mg/kg of atropine blocks the pressor effect of physostigmine injected either iv or icv in urethane-anesthetized rats (68). Icv pretreatment with 0.3 μg atropine blocks the pressor and bradycardic effects of icv physostigmine (5 μg) in conscious rats (74, 99).

Further evidence that atropine abolishes the CV effects of AChE inhibitors via a central site of action is given by the finding that iv administration of methylatropine, a quarternary ammonium derivative of atropine which does not as readily penetrate the blood brain barrier (97, 98), fails to inhibit the CV response to iv or icv injection of the AChE inhibitors (14, 54, 68).

MAN Isolated reports that physostigmine (100) and propoxur (101) elevate BP in normal human volunteers are finding confirmation in recent literature on the clinical uses of physostigmine.

Currently, physostigmine, already an established antidote in the treatment of intoxication with atropine-like agents (e.g. tricyclic antidepressants, benztropine), is now also used in the management of the central toxic effects of overdosage with certain non-anticholinergic drugs (e.g. benzodiazepines, barbiturates) (102–107). Nattel et al (106) have reported that in patients poisoned with non-anticholinergic agents, physostigmine (2 mg administered iv as a slow bolus injection) within 10 min evoked a rise in BP and an increase in HR. On the other hand, treatment with an AChE inhibitor which does not penetrate the blood brain barrier (edrophonium, up to 20 mg) produced a fall in both CV parameters. On the basis of these observations and of the unaccountable occurrence of mydriasis following physostigmine injection, these investigators suggested that physostigmine might be acting centrally to increase sympathetic outflow. In cases of anticholinergic overdose, physostigmine reduced BP and HR, prompting the speculation that the anticholinergic drugs might have antagonized the centrally-mediated cholinergic effects of physostigmine. Aquilonius & Sjöström (108) used physostigmine in an effort to restore the diminished brain cholinergic function in patients with Huntington's chorea. After pretreatment with methylscopolamine, administration of physostigmine (40 μg/kg, iv) elicited tachycardia and an elevation of systolic and diastolic pressure. Physostigmine also has been employed to investigate the possible role of central cholinergic mechanisms in various behavioral and cognitive func-

tions (109). Here too, this inhibitor of AChE has been observed to elevate BP and HR in patients pretreated with methylscopolamine (K. Davis, personal communication).

CENTRAL ORIGIN OF CHOLINERGIC CARDIOVASCULAR STIMULATION

Localization

Even before more conclusive evidence of CNS involvement was provided by direct administration of cholinergic agonists into the brain, or by specific pharmacological interventions with muscarinic antagonists or HC-3, other experimental approaches demonstrated the central origin of cholinergically induced CV responses. Several investigators have reported, for example, that transection of the spinal cord rostral to the spinal sympathetic outflow either abolishes or severely reduces CV responses to icv injection of ACh (40), oxotremorine (39) and carbachol (39) or systemic injection of oxotremorine (55), physostigmine (75, 81), and sarin (15).

A possible action of cholinergic agonists on sensory fibers carried in the vagi is eliminated by reports that bilateral vagotomy does not influence the pressor response to ACh (12, 40), carbachol (39), oxotremorine (55), sarin (63), or physostigmine (20, 89). While these studies do not exclude a possible effect on other afferent pathways to the brainstem, they reinforce observations that the rise in BP results from a direct action on the CNS, and not from a reflex increase in CV activity.

Other corroborative evidence of a central site of action of the anticholinesterase-induced pressor response derives from cross-circulation studies. Polet & DeSchaepdryver (67) reported that when sarin was injected into the carotid artery perfusing the vascularly isolated but neurally intact head of a dog (connected to trunk only by spinal cord), marked dose-dependent pressure increases could be demonstrated. Intracarotid administration of atropine caused the pressor effects to disappear. Since the use of this preparation precludes the possibility of peripheral drug effects in the recipient dog, these results indicate that the pressor response to sarin arises from stimulation of atropine-sensitive sites within the brain.

While spinal transection and cross-circulation studies demonstrate that cholinergically-mediated pressor responses are initiated at a level not lower than the medulla, they do not specify the brain regions involved. A variety of other techniques have further defined the site(s) of these neuronal events and have indicated, in addition, that different loci may mediate the CV responses to cholinomimetics administered by iv versus direct injection into the brain.

MIDBRAIN/MEDULLA/PONS When physostigmine is administrered iv in the decerebrate rat [(81); H. Sapru and R. Willette, personal communication] or ia in the decerebrate cat (10, 110), the pressor response usually seen in the intact animal is unaffected. Similarly, decerebration does not affect the rise in BP evoked in the cat by intravertebral arterial (iva) injection of neostigmine or parathion (10). These results, indicative of anticholinesterase action in the pons/medulla, are in accord with results obtained by brain transection. Brezenoff & Rusin (20) reported that in urethane-anesthetized rats, serial transections of the brain did not attenuate the pressor response to prior iv injection of physostigmine until a cut was made near the rostral regions of the pons. Since transections immediately posterior to hypothalamic mammillary bodies, as well electrolytic lesions of the mid- and caudal hypothalamus did not modify the pressor response, these findings suggest that regions in or caudal to the midbrain mediate the hypertensive effect of iv physostigmine.

Studies comparing the hypertensive response to ia versus iv administration of anticholinesterases support brainstem regions as a likely site of the physostigmine pressor response in the rat. Krstić & Varagić (79) observed that BP elevations produced by physostigmine injected into the jugular vein were considerably greater than those obtained by injection into either the left or right carotid artery. However, if the drug was directed toward the heart by retrograde injection into the ligated right common carotid artery, the response was nearly as great as that following intrajugular administration of the same dose. On the other hand, if the injection was made into the left common carotid artery, also directed toward the heart, the pressor effect was smaller than that following intrajugular injection. Citing anatomical differences in the arborization of the carotid arteries, these investigators concluded that injections into the jugular vein or retrogradely into the right common carotid artery result in a relatively high concentration of the drug reaching the vertebral arteries.[2] In contrast, the amount of physostigmine reaching the left vertebral artery after injection into the left carotid artery toward the heart is probably smaller, since most of the drug enters the aorta and is diluted or inactivated in the general circulation. On the basis of these observations, Krstić & Varagić (79) suggested that in the rat the brain regions supplied by the vertebral arteries (e.g. medulla and pons) (111) are of greater functional importance for the physostigmine pressor response than regions supplied by the common carotid arteries (e.g. mesencephalon, diencephalon, cerebral cortex).

[2]The right common carotid artery in the rat arises from the innominate artery close to the origin of the right subclavian artery, whereas the left carotid artery and left subclavian artery each arises directly from the aorta. The vertebral arteries arise from the subclavian arteries.

It is apparent from the work of Eickstedt et al (10) that the brainstem is also an important region for anticholinesterase-induced pressor responses in the cat. When physostigmine, neostigmine, or parathion was injected retrogradely through the right common carotid artery into the right vertebral artery of the decerebrate cat, increases in BP were obtained at doses (50–100 μg/kg) which were completely ineffective when administered iv.

The participation of higher brain regions cannot be excluded, however, because comparably low doses of the same three AChE inhibitors also evoked a rise in BP when injected into the internal carotid artery of the intact anesthetized cat. The internal carotid artery supplies primarily higher brain regions of the cat (111). (Unfortunately, it is not possible to compare the pressor responses to iva and intracarotid arterial (ica) injections since data are not shown). AChE inhibitors also have been injected directly into the vertebral artery of the dog (12, 32, 63). However, similar conclusions regarding the importance of the pontomedullary region in the pressor responses to these drugs are not justified on the basis of these observations. In this species the vertebral arteries supply a much more diffuse area (thalamus, hypothalamus, midbrain, as well as pons and medulla) than in the rat or cat (primarily medulla/pons) (111, 112).

Although the precise brainstem location of cholinergically-mediated pressor responses is not yet known, two possible loci are the nucleus tractus solitarius (medulla) and the locus coeruleus (pons). Microinjections of physostigmine into the nucleus tractus solitarius (NTS) of the rat have been reported to produce a rise in BP (91). Intravenous administration of physostigmine, or microiontophoretic application of ACh, oxotremorine, muscarine, or methacholine increased the firing rate of the locus coeruleus (LC) (30, 113). These observations will be considered in greater detail (see Central Mechanisms Mediating Cardiovascular Effects of Muscarinic Activation).

HYPOTHALAMUS Not unexpectedly, diverse lines of evidence indicate that the hypothalamus also plays an important role in the CV responses to cholinergic agonists. The hypothalamus long has been known to be a region highly involved in CV regulation (114–117). The implication of most brain transection studies cited above that hypothalamic areas are not required for the pressor response to systemically administered cholinergic agonists or AChE inhibitors (10, 20, 81, 110) does not necessarily mean that cholinergic mechanisms in this brain region are not involved in CV function. It is possible, for example, that brainstem regions are more sensitive to iv administered cholinergic agonists, that iv injected cholinomimetics do not as readily reach hypothalamic sites, or that drug clearance from circumventricular areas is rapid. Varagić et al (87) reported that acute electrolytic

lesions in the ventrolateral parts of either the posterior or anterior hypothalamus significantly depressed or completely abolished the iv physostigmine pressor response. However, they do not relate these findings to the prior conclusion that brainstem regions are more important in the pressor response (79).

Certainly there is sufficient evidence that when cholinergic agonists are administered close to or directly into hypothalamic areas, CV changes ensue. Thus, an indirect indication that cholinergic agonists might be activating brain structures close to the walls of the ventricles, including possibly hypothalamic regions, is the observation that onset of pressor responses to icv administered cholinergic agonists carbachol (52), choline (118), or physostigmine (74) in conscious rats is almost immediate (10" – 90"). In addition, Hoffman and co-workers (48–50, 52) have reported findings suggesting that periventricular muscarinic activation releases antidiuretic hormone (ADH), which is involved in the CV and antidiuretic responses to icv carbachol in the conscious rat. The work of this laboratory justifies the conclusion that direct microinjection of various direct- and indirect-acting muscarinic agonists into the PHN and the ventromedial nucleus consistently evokes a rise in BP (41, 45, 46). Additional active sites include the supramammillary nucleus, pars medialis of the medial mammillary nucleus (41), and the premammillary nucleus (45). Finally, a recent report from this laboratory indicates that ACh in the PHN exerts a modulatory influence on baroreceptor reflex activity (69, 70).

Modulation of Baroreceptor Reflexes

Indicative of the possibility that cholinergic agonists act at multiple central sites to influence CV function are several observations that brain ACh exerts a modulatory effect on baroreceptor reflexes. Both medullary and hypothalamic reflex pathways involved in cardiac and pressor function, respectively, appear to contain at least one cholinergic synapse.

BARORECEPTOR HEART RATE REFLEXES Injection into the fourth cerebral ventricle of the muscarinic receptor blocking agents *l*-hyoscyamine or ethylbenztropine in anesthetized dogs (119), or propantheline in anesthetized cats (120), inhibits reflex bradycardia evoked by iv administered vasoconstrictor agents. In contrast, injection of the AChE inhibitors physostigmine into the LV of the conscious rat (74) or neostigmine into the fourth ventricle of the anesthetized cat (120) potentiates this reflex slowing of the heart. Similarly, LV injection of physostigmine in the unanesthetized rat inhibits reflex tachycardia induced by iv administration of the potent vasodilator, sodium nitroprusside (74). Intravenous injection of physostigmine fails to modify either the reflex bradycardia or the reflex tachycardia.

In addition, neither the antimuscarinic agents nor the AChE inhibitors alter the pressor effect of the vasoconstrictor agents (74, 120) or the depressor effect of the vasodilator (74). Collectively, these data indicate that the effects on the baroreceptor HR reflexes of both the antimuscarinic agents and AChE inhibitors are centrally mediated. Furthermore, these effects occur independently of any BP changes produced by the vasoconstrictor or vasodilator agents, suggesting that the observed potentiation/inhibition of HR changes is at the level of the reflex mechanism itself.

In these same studies administration of the antimuscarinic agent, propantheline, into the fourth ventricle counteracted the enhancing effect of neostigmine on reflex bradycardia (120) and central pretreatment with atropine (0.3 μg) blocked physostigmine-induced potentiation of reflex bradycardia or inhibition of reflex tachycardia (74). Also, HC-3, at doses (10–20 μg, LV) known to maximally deplete brain stores of ACh (20, 60, 121, 122) significantly inhibited both norepinephrine-induced reflex bradycardia and nitroprusside-induced tachycardia, and completely abolished the effects of physostigmine on the bradycardic and tachycardic reflex changes. These observations suggest that brain ACh can modulate baroreceptor cardiac reflexes, and that muscarinic receptors mediate transmission at the cholinergic synapse in this reflex pathway.

Possible involvement of a nicotinic mechanism in this pathway cannot be excluded, however. While central administration of the nicotinic blocking agents hexamethonium (120) or mecamylamine (74) had no effect on either reflex bradycardia (74, 120), reflex tachycardia (74), or physostigmine-induced inhibition of reflex tachycardia, doses of mecamylamine as low as 1 μg icv reversed the potentiating effect of physostigmine on reflex bradycardia (74). That is, with mecamylamine pretreatment, physostigmine inhibited reflex bradycardia. Additional studies are needed to further define the role of this nicotinic mechanism in the central regulation of baroreceptor cardiac reflexes.

It is noteworthy that, while low doses of atropine (0.3 μg) injected into the LV effectively antagonized effects of physostigmine on both cardiac reflexes, doses of atropine up to 1 μg icv did not affect the control reflex bradycardia or tachycardia induced by norepinephrine (NE) or sodium nitroprusside, respectively. The inhibition of reflex bradycardia produced by the antimuscarinic agents in the cat (120) and dog (122) experiments cited above may be related to species variation, use of anesthesia, or differences in the intraventricular site of injection. Alternatively, it is possible that two (or more) cholinergic sites modulate baroreceptor cardiac reflex activity. Physostigmine, administered into the LV, may exert its potentiating effect at some more rostral site (e.g. midbrain) which is blocked by atropine administered by the same route. In this case, the concentration of

atropine or physostigmine reaching the brainstem region may be insufficient to have a direct effect on the latter, perhaps more critical, cholinergic site. *l*-Hyoscyamine, ethylbenztropine, or propantheline, injected into the fourth ventricle (120, 122), may have reached this brainstem region in sufficient concentration to inhibit reflex bradycardia.

CAROTID ARTERIAL OCCLUSION REFLEX Occlusion of the common carotid arteries elicits a reflex increase in systemic BP (CAOR, carotid arterial occlusion reflex). In addition to producing the typical anticholinesterase-induced rise in arterial pressure, physostigmine injected either iv (9, 69), ic (9), icv (69, 70), or by microinjection into the PHN (69, 70) potentiates the CAOR in the rat up to threefold. By direct administration into the brain, much smaller doses are required to demonstrate this enhancement of the reflex than via iv injection. The enhanced reflex pressor effect is superimposed on the initial BP increase evoked by the AChE inhibitor, and is most evident at the peak of this usual pressor response. As the pressor effect of the AChE inhibitor declines, the magnitude of the CAOR reverts to control values (9). This reflex also can be potentiated by physostigmine in the dog (123). In addition, administration of TEPP (tetraethylpyrophosphate) iv, or of neostigmine ic or icv, but not iv, potentiates the CAOR in the rat (9, 69, 70). On the basis of the observations obtained by direct administration of these various anticholinesterases into the brain, and in view of the lack of peripheral effects on carotid sinus pressoreceptors (124, 125), the AChE inhibitor-induced potentiation of the CAOR appears to be a central phenomenon.

Both the typical initial pressor response and the potentiation of the CAOR produced by icv injection of physostigmine can be blocked by icv pretreatment with HC-3 (69, 70). These results indicate that both responses are mediated by endogenous ACh. However, while Brezenoff and co-workers (69, 70) have reported that low doses of HC-3 (20 μg) did not affect the magnitude of the control CAOR in rats, Sinha et al (13) observed that in the dog, either icv injection or topical application of much larger doses of HC-3 (1.0 mg) onto the floor of the fourth ventricle completely blocked the pressor response to carotid arterial occlusion. The response could be restored within 10 min by icv administration or topical application of choline.

Injection of atropine iv (9) or icv (69, 70) prevents both the BP elevation and the potentiation of the reflex evoked by iv or icv injection of physostigmine, respectively. However, injection of atropine bilaterally into the PHN inhibits only the potentiation of the carotid arterial occlusion pressor reflex produced by iv injection of physostigmine; the rise in basal arterial pressure following administration of the AChE inhibitor is not antagonized (69, 70).

Thus, the PHN appears to contain the final cholinergic synapse in the pathway modulating the pressor response to carotid arterial occlusion. The initial increase in baseline BP is due to an action of physostigmine at some site other than the posterior hypothalamus, and is not related to the effects of the AChE inhibitor on baroreceptor pressor reflexes. The finding that intrahypothalamic injection of mecamylamine in the PHN does not inhibit the physostigmine-induced potentiation of the CAOR indicates that nicotinic receptors are not involved in this response.

These observations may be relevant to the inhibitory action of clonidine on the CAOR (126--128). Tadepalli & Mills (128) demonstrated that injection of clonidine into the fourth ventricle inhibited the CAOR in dogs with an intact nervous system. The inhibitory effect was blocked, however, by either midcollicular transection or restriction of the drug to supracollicular brain areas. These results suggest that clonidine acts in the brainstem to inhibit the CAOR, but that the pathway travels rostrally to involve supracollicular regions. In view of demonstrated interactions between clonidine and cholinergic mechanisms in other parts of the CNS (32, 53, 72, 129), it is possible that clonidine may express at least part of its antagonistic action on the CAOR via inhibition of an excitatory cholinergic mechanism in the posterior hypothalamus.

PERIPHERAL MECHANISMS MEDIATING CARDIOVASCULAR EFFECTS OF CENTRAL MUSCARINIC ACTIVATION

Increased Sympathetic Outflow

ENHANCED PERIPHERAL ADRENERGIC ACTIVITY The preponderance of experimental evidence indicates that cholinergically-evoked impulses originating in the brain produce CV responses via increased peripheral sympathetic activity.

Once the early investigations so consistently showed that CV responses to diverse cholinergic agonists failed to appear after sectioning of the spinal cord, but not after bilateral vagotomy (see Central Origin of Cholinergic Cardiovascular Stimulation: Localization), it was only reasonable to consider that peripheral sympathetic activation was affected along the obvious emergent pathway from the brain. This, in fact, has been supported by independent findings that in rats and cats destruction of the brain and spinal cord by pithing abolishes the pressor effects of ACh (143), oxotremorine (55), neostigmine (10), parathion (10), and physostigmine (73).

Recordings of neuronal efferent activity have directly demonstrated enhanced sympathetic outflow following central muscarinic stimulation. The

electrical activity in preganglionic sympathetic fibers (splanchnic nerve or cervical sympathetic chain) was found to be amplified by iv administered paraoxon in the rat (130) or physostigmine in the rat (84, 131), cat (84), and dog (132), and by ic injection of oxotremorine in the dog (32). Stamenović & Varagić (131) showed the enhancement of sympathetic discharge following iv physostigmine to be dose-dependent, with good correlation between the duration of the anticholinesterase-induced pressor effect and that of the preganglionic neuronal facilitation. This increased efferent sympathetic activity continued even when the associated pressor response to physostigmine or paraoxon was blocked by phentolamine or reserpine (130, 131). Atropine, but not methylatropine (130, 131) or hexamethonium (131), was found to block the enhanced neuronal activity, suggesting that the increased sympathetic discharge produced by these AChE inhibitors was triggered by central activation of muscarinic mechanisms.

Further indication that central stimulation of muscarinic processes initiates a generalized peripheral sympathetic activation is given by studies demonstrating the lipolytic and glycogenolytic actions of physostigmine. In the anesthetized rat, iv physostigmine has a lipolytic (133) and glycogenolytic action (134–136), whereas peripheral cholinergic stimulation by iv injected neostigmine produces neither of these effects. Both atropine and propranolol antagonize the glycogenolytic response in liver (135) and brain (134). Pretreatment with the centrally-acting antihypertensive agent mebutamate or with reserpine or guanethidine also blocks physostigmine-induced glycogenolysis in liver (135). Significantly, physostigmine has no glycogenolytic effect in vitro, but does in vivo even in the adrenalectomized animal. All these data reinforce the proposition that, like the increase in arterial pressure, the metabolic effects produced by physostigmine represent a centrally-evoked cholinergic activation of sympathetic outflow.

DRUG STUDIES Using a wide variety of direct and indirect-acting muscarinic agonists to produce centrally-mediated CV responses, numerous investigations have evaluated the effects of drugs known to impair the function of the sympathetic nervous system. In general, these studies also support the hypothesis that the pressor effects of muscarinic activation originating in the brain are mediated by enhanced transmission through the sympathetic ganglia. Irrespective of whether this pharmacological interference was effected by total sympathectomy, ganglionic blockade, alpha-adrenoceptor blockade, or by impairment of the synthesis, storage, or release of CCA, the majority of studies have reported significant attenuation or complete abolition of cholinergically-induced pressor responses.

Thus, chemical ablation of the peripheral sympathetic nervous system with the CCA neurotoxin 6-OHDA severely attenuates or completely

abolishes the pressor response to iv physostigmine in the rat (86, 137). Varagić et al (136) reported, however, that immunosympathectomy with anti-nerve growth factor (at birth) only partially reduced the hypertensive effect of physostigmine (tested 5 months later). It is likely that some degree of regeneration of noradrenergic nerve terminals occurred.

When blockade of ganglionic transmission is achieved in the rat with nicotine (23, 75, 81), mecamylamine (76), TMA (23), P286 (*N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-diisopropylurea) (75) or hexamethonium (63, 81), the pressor responses to subsequent administration of physostigmine can be abolished. Similarly, pentolinium prevents the hypertensive effect of oxotremorine (55), and hexamethonium (usually at high doses) that of carbachol (39) and various AChE inhibitors (sarin, dyflos, TEPP, E600 (63).

Often it has been found that hexamethonium, even when given in doses far in excess of that sufficient to block ganglionic nicotinic receptors, fails to antagonize the pressor effects of ACh (40), carbachol (48), or physostigmine (23, 40, 63, 75–77, 138, 139). Furthermore, during the early depolarizing phase of ganglionic blockade by nicotine (75) or TMA (23), the pressor effects of physostigmine (23, 75), as well as those of DMPP and the ganglionic muscarinic receptor stimulants (140–142) McN-A-343 and AHR 602, are completely abolished, while during the later nondepolarizing phase of its blocking action the physostigmine-induced pressor response is unaffected (23).

Gokhale (75, 76) reported that doses of hexamethonium which completely blocked the ganglionic effects of DMPP only partially inhibited the pressor response to physostigmine. However, when a small dose (20 μg/kg) of atropine was administered at this stage, the pressor response to physostigmine was completely abolished. This dose of atropine by itself produced only a small reduction in the CV effect of physostigmine, but did prevent the pressor effects of the muscarinic ganglionic stimulants McN-A-343 and AHR-602. Doses of atropine in the range of 50–200 μg/kg, however, almost completely abolished the pressor effect of physostigmine. Whether the transmission of impulses through this muscarinic pathway regularly contributes to the central sympathetic activation initiated by physostigmine and other cholinergic agonists or is functional only when the established hexamethonium-sensitive pathway is blocked, remains unsettled.

To more precisely define the involvement of sympathetic nerve activity in the pressor response to central muscarinic activation, drugs which interfere with sympathetic function at levels beyond the ganglionic synapse also have been studied. Peripheral alpha-adrenoceptor blockade with phenoxybenzamine, phentolamine, tolazoline, ergotamine, or dibenamine dramatically reduces or completely abolishes the pressor response to ACh (40, 143),

carbachol (39, 41), oxotremorine (55), and various AChE inhibitors: sarin (63), neostigmine (41), physostigmine (68, 80, 81), and paraoxon (130). This indicates that the pressor effect of these cholinergic agonists is due to the vasoconstrictor action of catecholamines released from peripheral stores. In contrast with its inhibitory effect on physostigmine-induced glycogenolysis (135), blockade of beta-receptors with systemically administered propranolol has either no effect or slightly potentiates the pressure increase evoked by physostigmine (78, 135). Similar results occur with oxotremorine (25, 55) or carbachol (39). As might be expected, tachycardia associated with carbachol (39) or oxotremorine (55) injection is inhibited. The results of these studies indicate that the physostigmine-induced pressor response is mediated by alpha-adrenoceptors, and the glycogenolytic effect by beta-adrenoceptors.

Drugs which interfere with the synthesis or storage of NE in the adrenergic terminal also have been found to abolish the hypertensive effects of cholinergic agonists. Following pretreatment with alpha-methyldopa, alpha-methylmetatyrosine, or alpha-methylparatyrosine, Walker & Weetman (55) obtained significant inhibition of the pressor response to iv oxotremorine. In reserpinized rats or cats, subsequent administration of oxotremorine (55), paraoxon (130), and physostigmine (9, 84–86, 96) produces markedly depressed or no CV effects at all. Furthermore, the slow iv infusion of NE, alpha-methyldopa, or 5-hydroxytryptamine usually fails to restore the response in these animals (84, 86, 96). Neither is pretreatment with the monamine oxidase inhibitor (MAOI) isopropylisoniazid able to antagonize the inhibitory action of reserpine on the pressor response to physostigmine (96). [This MAOI abolishes the effect of reserpine on the liberation of epinephrine from the adrenal glands (144)]. It is therefore unlikely that physostigmine acts directly by liberating NE from either the postganglionic sympathetic axon or the adrenal gland.

Additional investigations with several adrenergic neuron blocking agents corroborate this view. When rats or cats are treated with bretylium (40, 73, 76, 84, 89, 96), guanethidine (39, 55, 73, 76, 82), bethanidine (15), or TM-10 (choline-2,6-xylyl ether bromide) (84, 96), agents which impair adrenergic transmission by preventing release of adrenergic neurotransmitter from the nerve ending, the hypertensive response to subsequent administration of physostigmine, carbachol, or oxotremorine is abolished or severely depressed. Thus, CV responsiveness to these diverse cholinomimetics requires activation of the sympathetic neuron, rather than a direct action to release NE from the adrenergic terminal itself.

If re-uptake of NE into the terminal is blocked, for example, by desmethylimipramine or cocaine, the pressor effects of central cholinergic activation can be potentiated (39, 75, 81). Presumably, sympathetic im-

pulses arriving at the blood vessel walls are reinforced by this drug-induced failure to dispose of the neurotransmitter.

ADRENAL CATECHOLAMINE RELEASE Since a general increase in activity of the CNS would be expected to liberate CCA from the adrenal glands, even the earliest investigators in this area suspected that the CV responses to cholinergic agonists might involve these structures. This surmise was particularly reinforced by the well-known study by Stewart & Rogoff (145) in 1921 showing that physostigmine produces a 10 to 15-fold increase in the CCA output of the cat adrenal gland. This effect was thought to be centrally mediated because section of the nerve supply to the adrenals abolished the response. Numerous investigators, therefore, undertook to adrenalectomize rats, cats, and dogs in an effort to determine whether all or part of the pressor response to cholinergic agonists was due to CCA release from the adrenal medulla. In general these studies have shown that adrenalectomy, demedullation, or denervation of the adrenal medulla, while antagonizing the increase in plasma CCA (25, 78, 96, 137), do not affect the rise in BP produced by iv administration of physostigmine (9, 73, 76, 78, 80, 81, 85, 96, 137, 139), sarin (63), or oxotremorine (25, 55). Conversely, dissociation of the pressor and adrenal CCA-liberating effects of cholinergic drugs has been demonstrated also by pharmacological manipulations which severely deplete CCA from most peripheral tissues, but leave adrenomedullary stores intact. Thus, in rats pretreated with 6-OHDA, iv physostigmine increased plasma CCA levels, but significantly blocked the rise in BP (137). Similarly, pretreatment with MAOI isopropylisoniazid [which abolishes the effect of reserpine on CCA liberation from the adrenal gland (144)] failed to antagonize the inhibitory action of reserpine on the pressor response to physostigmine (96). Finally, the many previously cited studies with adrenergic neuron blocking agents reinforce the thesis that the adrenal gland plays little or no role in the pressor effects of cholinergic drugs; these drugs, which do not affect the release of CCA from the adrenal medulla, severely depress or abolish the rise in BP produced by various cholinergic substances (see Peripheral Mechanisms Mediating Cardiovascular Effects of Central Muscarinic Activation).

Not only do adrenalectomy, demedullation, or adrenal denervation fail to inhibit the pressor effect of cholinergic activation, but these interventions may actually potentiate this response (25, 68, 137). Kaul & Grewal (137) have suggested that the vasodilator effect of epinephrine in certain vascular beds is normally masked by the vasoconstrictor effects of physostigmine in other regions. When this counteracting influence of epinephrine is removed by adrenalectomy or propranolol, the pressor effect of sympathetic activation is unopposed; hence a potentiated pressor response is seen.

Contrasting with the findings for AChE inhibitors administered iv, Brezenoff (68) has shown that the pressor response to icv injection of physostigmine in the urethane-anesthetized rat is mediated entirely by liberation of adrenal CCA; adrenalectomy completely prevented any rise in BP following injection into the lateral ventricles. However, adrenalectomy does not abolish the pressor response to carbachol or neostigmine injected into the PHN (41).

Antidiuretic Hormone (ADH) Release

Contradictory observations regarding the ability of iv administered phentolamine to block the pressor effect of centrally administered carbachol have prompted speculation that a factor other than increased sympathetic activity might be involved in this response. Whereas Ozawa & Uematsu (39) and Buccafusco & Brezenoff (41) observed that iv phentolamine (5 mg/kg or 2 mg/kg, respectively) significantly reduced the rise in arterial pressure produced by carbachol injected ic (1 μg) in the anesthetized rat (39) or directly into the PHN (0.5 μg) of the conscious rat (41), Hoffman & Phillips (50) reported that this alpha-adrenoceptor blocking agent (10 mg/kg) did not affect the pressor response to carbachol (25 ng) injected into the LV of the conscious rat. Evidence has been presented by Hoffman & co-workers (48–50, 52) that the pressor effect of carbachol injected into the third or lateral cerebral ventricles of unanesthetized rats is a muscarinic receptor-activated mechanism mediated in part by release of ADH.

In contrast, citing blockade of the hypertensive response to icv carbachol by spinal cord transection or systemic injection of hexamethonium, guanethidine, or phentolamine, as well as significant potentiation by iv desmethylimipramine, Ozawa & Uematsu (39) proposed that the sympathetic trunk is the main pathway conveying the central muscarinic pressor effect of ic carbachol. Similarly, Buccafusco & Brezenoff (41) have demonstrated that enhanced peripheral sympathetic activity is entirely responsible for the rise in pressure evoked by intrahypothalamically administered carbachol or neostigmine. Not only was the pressor response to these cholinergic agonists unaltered by hypophysectomy, but the reduction in the pressor response to injection of carbachol or neostigmine into the PHN following phentolamine was comparable to the maximal inhibition that could be obtained when pressor responses were elicited by electrical stimulation of the PHN or by iv injection of NE.

While it is difficult to make direct comparisons among these and other studies in which carbachol was administered centrally because (*a*) similar doses were not injected into the same brain regions in both conscious and anesthetized animals and (*b*) ADH levels were assayed only in the work by Hoffman's group, there actually may be no fundamental disagreement in

the reported findings. Whether sympathetic activation appears to mediate the pressor response to carbachol *in toto* or in part may be a function of the use of anesthetic, the site of injection, and the dose.

On the basis of the investigations by Hoffman & co-workers, it appears that injection of low doses of carbachol into the third or lateral cerebral ventricles of unanesthetized rats causes the release of ADH which, in turn, contributes to a rise in BP. This is consistent with the well-documented central cholinergic regulation of ADH release [(7, 146); see also (49) and (147) for additional references]. The rise in BP and in plasma levels of ADH produced by centrally administered carbachol is accompanied by an increase in drinking and urine osmolality (48–50, 52). It is likely that in the anesthetized rat the ability of icv injected carbachol to release ADH is suppressed, and the pressor response evoked by higher doses of carbachol in these animals is mediated primarily by an increase in sympathetic activity. This is supported both by the observation that pentobarbital anesthesia abolishes the response to 25 ng carbachol icv (50), and by the vastly different doses required to produce pressor responses of nearly comparable magnitude in unanesthetized and anesthetized rats. Thus, while 25 ng carbachol injected into the LV of the conscious rat evokes a pressure rise of about 34 mm Hg (52), it takes an ic dose approximately 40 times higher (1 μg) to increase BP to only a slightly greater extent (44 mm Hg) in the chloralose-urethane anesthetized rat (39). Perhaps, to clarify the role of ADH in the carbachol pressor response, plasma levels of the hormone should be assayed in anesthetized rats following icv administration of low (25 ng) and high (1 μg) doses of carbachol. It would be of some interest also to evaluate the contribution of ADH in the pressure rise produced by the higher dose (1 μg) injected into the LV of the conscious rat.

Varagić & Milić (147) have reported that injection of physostigmine in the conscious or anesthetized rat can produce antidiuresis and chloruresis, presumably via release of ADH. However, the effect is not regularly reproducible and occurs at much higher doses (0.05–0.3 mg/kg iv) than those which induce a pressor response (0.04–0.08 mg/kg iv). Indicative of the possibility that physostigmine-induced pressor and antidiuretic responses are independent mechanisms, these investigators observed that the antidiuresis, unlike the pressor response, is not abolished by atropine (2.5–3 mg/kg iv). Hoffman (52), in contrast, found that pretreatment with atropine (10 μg) into the third ventricle significantly reduced both BP and antidiuresis.

It appears from the work of Buccafusco & Brezenoff (41) described above that the pressor response to carbachol or neostigmine injected into the PHN is mediated exclusively by increased sympathetic activity. Nevertheless, intrahypothalamic administration of nanogram quantities of carbachol

(18.2 ng) evokes a pressor response (17 mm Hg) which is comparable to that obtained (less than 20 mm Hg) by injection of 25 ng carbachol into the third ventricle (50). Assay of ADH activity following carbachol administration in the PHN would help clarify possible involvement of ADH in the pressor response.

Overall, these results suggest there may be no basic discord between those who demonstrate that ADH is a component of the carbachol-induced pressor response and those who find increased sympathetic activity is the primary mechanism; both mechanisms may play a role under different experimental conditions. Perhaps, a more fundamental issue is whether induction of ADH release by centrally administered carbachol reflects a physiological muscarinic mechanism. It is possible that carbachol may act on non-innervated cholinergic receptors to produce effects not evoked by endogenous ACh or AChE inhibition. It has been shown, for example, that while microinjection of carbachol into the dorsomedial or premammillary areas of the hypothalamus evokes hypotension and bradycardia, administration of an AChE inhibitor into these same areas produces hypertension and tachycardia (45). To determine whether endogenous ACh in fact participates in a physiological mechanism that triggers ADH release and a subsequent pressor effect, it would be worthwhile to evaluate the capacity of physostigmine to release the hormone in pressor amounts.

CENTRAL MECHANISMS MEDIATING CARDIOVASCULAR EFFECTS OF MUSCARINIC ACTIVATION

Brain AChE and ACh

Despite the fact the ACh and other direct receptor agonists were shown by many investigators to produce CV effects via central muscarinic receptor activation, it remained to demonstrate experimentally that the AChE inhibitor acted similarly through endogenous brain ACh. In 1968, Varagić et al (83) reported that systemic doses of physostigmine known to evoke an increase in BP produced dose-dependent inhibition of whole brain cholinesterase activity. In addition, the pressor effect of physostigmine was diminished or completely reversed if the animals were pretreated with the organophosphorus inhibitors DFP or paraoxon, suggesting that a functionally competent cholinesterase was required for the physostigmine pressor effect to occur. Recently, Brezenoff et al (70) demonstrated that brain AChE activity is reduced during the course of the pressor response to physostigmine or neostigmine administered into the LV.

Using a more direct approach to demonstrate the involvement of brain ACh in the pressor response to AChE inhibitors, Brezenoff & Rusin (20)

injected the ACh synthesis inhibitor, HC-3, into the LV of rats subsequently given physostigmine either iv or icv. Two hours after injection of HC-3, dose-related reductions in regional ACh content were associated with a parallel inhibition of the pressor response to iv or icv administered physostigmine. While ACh levels also fell in cortex and brainstem, a maximum reduction of 84% occurred in the midbrain following 4 μg HC-3; this depletion of neurotransmitter was paralleled by nearly total suppression of the pressor response to iv injected physostigmine. The response to icv administration of the drug was maximally reduced about 60%. These results indicate that endogenous brain ACh can mediate the pressor response to AChE inhibition. Subsequent work of this laboratory has substantiated this requirement for brain ACh. When synthesis of the neurotransmitter is blocked by HC-3, the pressor and baroreceptor reflex potentiating actions of physostigmine and other AChE inhibitors (41, 46, 70, 71, 74), but not that of direct-acting cholinergic agonists, such as carbachol (41, 46, 118), is abolished. All these data collectively suggest that the activation of muscarinic receptors by ACh released at the synaptic cleft may be of physiological significance in the central pathways involved in BP regulation.

Cholinergic-Catecholaminergic Interactions

One of the more obscure features of the process(es) by which central cholinergic stimulation initiates a pressor response is the precise sequence of neuronal events that follows activation of the muscarinic receptor sites in the brain. Current data support the view that the enhanced peripheral sympathetic activation observed after systemic administration of cholinomimetic agents is preceded by activation of a central adrenergic mechanism.

Such coupling between cholinergic and the CCA systems could be expected on the basis of the distribution of relevant substances. Thus, the brain regions which are probable targets of muscarinic drug action (e.g. hypothalamus, mesencephalon, brainstem) are areas known to be rich in CCA (148–152), ACh (153–155), and in the enzymes responsible for the synthesis and hydrolysis of the latter neurotransmitter, i.e. choline acetyltransferase (ChAT) (153–155) and AChE (151–154, 156), respectively.

In addition, however, much pharmacological data indicate that the CNS pathway which mediates the pressor response to muscarinic stimulation appears to contain at least one CCA synapse. The increase in BP following iv injection of physostigmine (92) or icv injection of carbachol (51) is inhibited by depletion of brain CCA with 6-OHDA. Furthermore the hypertensive effect elicited by icv carbachol is antagonized by central pretreatment with bethanidine or guanethidine (15) and alpha-adrenergic receptor blocking agents (39, 52), and is potentiated by pretreatment with des-

methylimipramine (39). There also may be beta-adrenoceptors along this pathway of CV excitation, since the pressor (15) and tachycardic (15, 39) effects of icv carbachol are blocked by centrally administered propranolol. Thus, the integrity of central CCA neurons appears to be critical for expression of the cholinergic pressor response.

One disputed issue concerns the question whether central alpha-adrenergic mechanisms mediate both the pressor and the antidiuretic effects of icv carbachol. Hoffman (52) has reported that in the unanesthetized rat the rise in BP, as well as the diuresis produced by icv injection of carbachol, were blocked by pretreatment with icv phentolamine. This finding contrasts with observations by Kühn (146) and Gordon & Brody (51), who showed that in the anesthetized and conscious rat, respectively, central phentolamine pretreatment blocked the pressor, but not the antidiuretic, response to icv carbachol.

Overall, these findings are consistent with abundant evidence in the literature which indicates that there are physiological interactions between central cholinergic and CCA pathways involved in CV and other functions, including glycogenolysis (134), regulation of anterior pituitary hormone release (157, 158) locomotor activity (159, 160), and temperature regulation (161, 162). Such cholinergic regulation of CCA phenomena in the CNS has been demonstrated by biochemical as well as pharmacological and physiological approaches. Both physostigmine and oxotremorine increase the synthesis and release of brain CCA (92, 143, 158, 163–172). This increase in turnover probably is mediated through central muscarinic receptors as evidenced by its inhibition by atropine or scopolamine, but not by the peripherally active methylscopolamine (92, 158, 163–168).

In the conscious rat, Kazić (92) has demonstrated a dose-dependent reduction in NE content of brainstem and hypothalamus concomitantly with an increase in BP following iv administration of physostigmine (100–300 μg/kg). This CCA-depleting action of AChE inhibitor could be abolished completely by atropine or propranolol. Furthermore, concurrent with an increase in arterial pressure in the periphery, iv physostigmine increased synthesis of NE in all brain areas. Physostigmine also has been shown to influence NE release in vivo in man, since metabolite concentrations of 3-methoxy-4-hydroxyphenylglycol were significantly increased following iv administration (173). Taken together, these biochemical investigations make it not unlikely that the centrally mediated pressor response to cholinergic agonists involves muscarinic activation of either postsynaptic CCA membranes or CCA nerve terminals.

Cholinergic-CCA interactions in the central control of BP are further supported by single unit recording techniques and microiontophoretic application of drugs. One such site of interaction is the locus coeruleus (LC),

a pontine nucleus consisting of densely-packed NE-containing cell bodies (148, 152). The axonal distribution of these neurons includes the hypothalamus, motor nucleus of the vagus, and the NTS, all important areas in CV control. Pharmacological and physiological studies have directly linked the LC to CV regulation (148, 174–176).

In addition to NE, the LC contains ACh, ChAT, and AChE (148, 152, 153, 156, 177). Based on the relative amount of ACh and ChAT present in this nucleus, Cheney et al (153) have proposed that ACh is localized in cholinergic nerve terminals. Furthermore, the NE and AChE in the LC are found in the same neuronal cell bodies, suggesting that the AChE may be situated on the noradrenergic neurons to inactivate a cholinergic input (152, 156, 178).

Microiontophoretically applied ACh does in fact increase the firing rate of LC neurons (30, 113). Cholinergic agonists with muscarinic activity, including bethanechol, muscarine, and methacholine (30), excite LC neurons when applied microiontophoretically. This stimulatory effect can be blocked by local application of scopolamine, but not by hexamethonium or gallamine (30, 129, 179). Thus the cholinergic receptors involved in this excitation of noradrenergic neurons appear to be specifically muscarinic.

Nicotine, injected iv, produces a transient activation of LC neurons (30, 129), and this effect is abolished by iv administration of the nicotinic receptor antagonist mecamylamine or by scopolamine. However, since nicotine has no direct effect on LC neurons when iontophoretically applied (30, 129), it may be presumed that this drug acts at some more remote nicotinic site to secondarily stimulate the muscarinic cholinergic input to the LC.

Significantly, iv administration of physostigmine or oxotremorine also increases the firing of LC neurons; this effect can be prevented by either iv injected or microiontophoretically applied scopolamine (30). Thus, the existence of excitatory muscarinic receptors on LC neurons, demonstrated by the microiontophoretic data, becomes a physiologically meaningful observation.

Drug administration into another CNS site significantly involved in CV function also supports a possible cholinergic-CCA interaction. The brainstem area which serves as the first link in the sinoaortic baroreceptor reflex arc, the NTS (91, 180), is reported to contain ACh concentrations and ChAT activity similar to that present in the LC (155). A moderate amount of AChE activity also is found in this region (152). DeJong & Nijkamp (181) reported that the hypotensive effect produced by injection of alpha-methylnorepinephrine into the NTS was potentiated by prior systemic injection of atropine (5 mg/kg ip) in vagotomized rats. In addition, the bradycardic effect of this drug was diminished. These findings suggest that a muscarinic CV excitatory mechanism exists, presumably in the NTS, which counter-

acts (and may normally be masked by) the depressor and bradycardic effects of alpha-adrenoceptor activation in the NTS.

Zandberg & DeJong (91) have described a dose-related increase in BP following local injection of physostigmine into the area of the NTS. Furthermore, their mapping studies revealed that these sites of cholinergic reactivity corresponded closely with those sites most responsive to the hypotensive effects of similarly injected alpha-methylnorepinephrine. However, systemic administration of atropine (15 mg/kg ip) did not abolish the pressor response to physostigmine injected into NTS (91). This observation is inconsistent with the inhibitory effects of atropine previously reported by these investigators (181), and also contradicts the finding of this laboratory that iv injection of atropine in doses of 1 mg/kg regularly antagonizes the pressor effect of iv physostigmine. It would be of considerable interest to determine the influence of prior injection of atropine directly into the NTS on the pressor response to physostigmine administered iv or into this brain region.

There is evidence that the relationship between cholinergic-CCA neuronal systems may not be a one-way interaction. Just as alpha-adrenoceptors located on peripheral cholinergic nerve terminals can inhibit the release of ACh (182–185), similar observations suggest a modulatory effect of CCA agonists on cholinergic terminals in the CNS. Thus, drugs which interfere with noradrenergic function prevent the rise in ACh induced by oxotremorine in rat whole brain and hippocampus (186, 187). It was recently demonstrated that treatment with reserpine may reduce brain ACh storage capacity (121). Dopamine agonists are known to inhibit the release of striatal ACh, while dopamine receptor blocking agents enhance ACh release (188). Furthermore, Buccafusco et al (72) have reported that the alpha-receptor agonist, clonidine, significantly reduced turnover of ACh from certain brain regions and markedly inhibited the pressor response to iv administration of physostigmine. It is also noteworthy that NE or clonidine inhibit the firing of LC neurons (189, 190).

An attractive hypothesis which is consistent with the above-cited indications of central cholinergic-CCA interactions is that substantial cholinergic innervation (from an unknown source) converges on noradrenergic neurons in the LC. ACh released by nerve activity from these terminals interacts with muscarinic receptors on the LC cell bodies to enhance NE synthesis and release. Via extensive interrelationships with other brain regions, this central noradrenergic activation triggers an increase in peripheral sympathetic nerve activity and a subsequent increase in BP. Interaction of either endogenous CCA or exogenous alpha-adrenoceptor agonists (e.g. clonidine) with presynaptic alpha-receptors on the cholinergic nerve terminals reduces release of ACh, thus diminishing the firing of the noradrenergic LC neurons

with a resultant fall in BP. It has been postulated that clonidine, which acts centrally to lower arterial pressure, may produce its antihypertensive effect via inhibition of central cholinergic neurons (53, 72).

FUNCTIONAL IMPLICATIONS OF MUSCARINIC REGULATION OF CV FUNCTION

In spite of the demonstrated presence of muscarinic hypertensive mechanisms in the hypothalamus, midbrain, and/or brainstem, depletion of endogenous brain ACh with HC-3 injected icv or intrahypothalamically (13, 20, 41, 45, 46, 71, 74, 99, 191), or blockade of central muscarinic receptors by iv, icv, or intrahypothalamic atropine (14, 39, 41, 44, 74, 77, 99, 192, 193), does not depress basal BP in the normotensive animal. If the model based on cholinergic-catecholaminergic interactions is valid, pharmacological impairment of cholinergic function would be expected to lower BP.

The general lack of significant antihypertensive action of atropine may be related to effects of antimuscarinic agents at several CNS sites which exert opposing influences on CV regulation. Alternatively, it is possible that under normal basal conditions the cholinergic excitatory CV mechanism is quiescent, but, under circumstances of increased neuronal activity, hence of increased ACh release, might become activated (41, 46, 99, 191). An indication that such activation might occur under physiological conditions is given by the observations that centrally administered HC-3 reduced (and choline restored) the hypertensive response to electrical stimulation of the medulla, and abolished the rise in BP elicited by bilateral carotid arterial occlusion (13). In addition, AChE inhibitors, HC-3, and antimuscarinic drugs were shown to significantly influence the pressor and cardiac responses involved in baroreceptor-mediated reflexes (9, 69, 70, 74, 119, 120), suggesting a modulatory role for endogenous brain ACh.

Highly corroborative evidence that a tonically inactive cholinergic system could become activated to produce CV changes is given by recent observations that (*a*) brain ACh may play a role in the development or maintenance of specific forms of hypertension, and (*b*) HC-3 or atropine lower BP in certain animal models of the disease. The spontaneously hypertensive (SH) rat is widely used as a model for human essential hypertension. Although the genesis of the elevated pressure is not known, this model does appear to exhibit enhanced sympathetic activity [(2, 114, 194); see also (71) and (155) for additional references]. Several studies suggest that this activity is related to altered central cholinergic function. Yamori et al (195) first described increased activities of AChE and ChAT in the brainstem of SH rats. Bagjar et al (196) also found increased activities of these enzymes in the SH rat compared to controls. More recently, Helke et al (155), assaying

punctate regions of SH rat brain for ACh and ChAT during development of hypertension, found age-specific biochemical alterations. At 12 weeks of age, the ACh levels and ChAT activity in the LC of the SH rat were significantly elevated in comparison with younger SH rats or age-matched normotensive controls. Although the factor(s) triggering this cholinergic activation are not known, this finding is of great interest because of the well-known importance of LC noradrenergic neurons in the regulation of BP (see Central Mechanisms Mediating CV Effects of Muscarinic Activation: Cholinergic-CCA Interactions). Furthermore, ACh, various muscarinic agonists, and physostigmine have been shown to activate neurons in this brain region (30, 113, 129, 179, 197, 198). While the receptors that mediate this enhanced neuronal firing can be blocked by iv or microiontophoretic application of antimuscarinic agents (30, 179, 198), it is noteworthy that neither atropine nor scopolamine could inhibit basal LC neuronal activity (30, 179). These findings suggest that the excitatory muscarinic input to the LC probably plays a minor role in maintaining the tonic activity of the noradrenergic neurons at this site.

The decrease in ChAT activity found in the posterior and dorsomedial hypothalamic nuclei of SH rats (155) at different ages also may be of significance since these loci have been implicated in CV function (41, 45, 69, 70). Helke et al (199) also have presented evidence that in the DOCA-salt hypertensive rat ChAT activity of the intermediate portion of the NTS is elevated, and that of the dorsomedial nucleus of the hypothalamus is decreased.

These neurochemical findings are consistent with data regarding CV responses of hypertensive rats to central cholinergic manipulation. Intravenous administration of physostigmine in both young (71) and mature (54) unanesthetized SH rats evokes a pressor response which is greatly increased in magnitude compared to that produced in normotensive Wistar-Kyoto controls. Stimulation of autonomic ganglia with DMPP elicits nearly comparable pressure increases in SH and control animals, suggesting that the site of increased responsiveness is the CNS (71). Kubo & Tatsumi (54) further localized the enhanced responsiveness to a presynaptic site, reporting no difference in the hypertensive response to oxotremorine of SH and normotensive rats pretreated with methylatropine. Thus, the potentiated pressor response to muscarinic stimulation in the SH rat appears to involve increased spontaneous release of ACh at some unspecified central site(s).

In contrast to the latter observation, Hoffman et al (48) have reported that the pressor effect of carbachol injected into the third ventricle is enhanced in SH rats. Carbachol appears to elevate BP centrally through a postsynaptic mechanism, at least in the PHN, since this effect is not abolished by HC-3 (41). The discrepant observations may be related to different

routes of administration, which could activate different mechanisms for the pressor response. On the other hand, carbachol is reported to possess presynaptic activity (200, 201), and in this regard the results may be consistent with the presynaptic potentiation described by Kubo & Tatsumi (54). In either instance, central cholinergic responsiveness related to CV function appears to be enhanced in the SH rat.

As correlative findings to these observations, icv injection of HC-3 (10–20 μg) decreases BP in unanesthetized SHR (71, 99, 191), DOCA-salt, and Grollman renal hypertensive (R. Giuliano, unpublished observations) rats. No reduction in arterial pressure occurs following similar injections of HC-3 in their respective controls. The hypotensive response begins after a delay of about 10 min, and in SH rats has been correlated with the rate of decline of brain ACh concentration (71). However, the duration of the fall is only 2–4 hours, while ACh levels are only partially recovered by this time. This return of BP to pretreatment hypertensive levels could be the result of recovery of cholinergic function in some brain region or the institution of a noncholinergic compensatory mechanism.

In addition to the hypotensive effect of HC-3, iv administration of atropine sulfate lowers BP in the SH rat, but does not affect (193) or only minimally reduces (202) BP in the normotensive rat. The doses required to produce this effect are relatively high (up to 20 mg/kg), although Caputi et al (193) reported that older rats were more responsive than younger animals. While 1 mg/kg of atropine was without significant effect in SH rats 11 and 15 weeks old, this dose reduced BP by 45 mm Hg in 20-week-old animals. Apart from the age-related neurochemical changes reported in specific brain nuclei of SH rats (155), age-related differences have been cited in the sensitivity of the rat to cholinergic agonists and antagonists (203) and to brain receptor binding (204).

The site of the hypotensive action of atropine is controversial. Caputi et al (193) reported that since methylatropine fails to produce any fall in BP in SH rats, the site of atropine's hypotensive action is the brain. Paradoxically, however, icv injection of as much as 0.2 mg atropine does not affect arterial pressure. These investigators postulated that atropine does not diffuse to the active site following this route of administration. This possibility is supported by the observation that while icv atropine blocks the pressor response to icv physostigmine, it does not antagonize the hypertensive effect of physostigmine injected iv (H. Brezenoff, unpublished data).

Abraham et al (205) have reported that atropine blocks peripheral alpha-adrenergic receptors in the normotensive rat. However, Caputi et al (193) reported that in the SH rat even high doses of atropine (10–20 mg/kg) do not reduce the pressor response to iv injected NE or angiotensin.

Taken together, all these observations support the thesis that the

proposed muscarinic CV excitatory mechanism in the CNS may be a highly physiologically relevant system. Although under usual conditions of CV functioning it may not exert tonic activity, it may be activated when homeostatic demands (e.g. baroreceptor reflexes) or pathological processes (e.g. hypertension) supervene.

CONCLUSION

Intravenous or central administration of various direct-acting (e.g. carbachol, oxotremorine) or indirect-acting (e.g. physostigmine, sarin) cholinergic agonists causes an increase in arterial pressure in conscious or anesthetized dogs, cats, and rats. When AChE inhibitors are administered, the pressor response requires functional brain AChE and endogenous brain ACh.

The pressor effect of these substances injected systemically is a consequence primarily of enhanced sympathetic outflow resulting from activation of muscarinic receptors in the midbrain and/or brainstem. Atropine, but not methylatropine, specifically antagonizes the cholinergically-induced pressure increase. The rise in arterial pressure evoked by intraventricularly administered carbachol may involve ADH release as well as increased sympathetic activity. Muscarinic receptor activation in the hypothalamus elevates BP and potentiates the CAOR, probably via independent mechanisms. Similar receptor sites, possibly in the brainstem, also participate in baroreceptor reflex cardiac activity. Anesthetics can alter both qualitatively and quantitatively the CV responses to central cholinergic activation.

Central pathways involved in the muscarinic activated pressor response require intact adrenergic neurons, suggesting at least one cholinergic-CCA link in the pressor mechanism. Such cholinergic-CCA interactions may be of significance in the antihypertensive action of certain drugs, e.g. clonidine. Recent evidence from several laboratories also suggests that brain ACh may be important in the pathogenesis of animal models of hypertension. Under conditions of enhanced central cholinergic activity associated with the SH, DOCA-salt, and Grollman renal models of the disease, depletion of brain ACh stores (by icv HC-3) significantly lowers arterial pressure. Systemically administered atropine also significantly reduces BP in the SH rat.

It is hoped that this review has served to consolidate the heretofore scattered literature on central muscarinic participation in CV control. The relationship of brain ACh to those receptors involved in the physiological regulation of CV function and in the etiology of hypertension is clearly worthy of further investigation. Additional studies offer the potential for exploitation, and could lead to the development of novel classes of compounds for the treatment of hypertension in man.

ACKNOWLEDGMENTS

We wish to acknowledge our debt to Kathleen Carney for her attentive editorial assistance, to Karen Bauer for her skillful preparation of the manuscript, and to the U.S. Army Research Office for supporting our research.

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