Pharmacological Analysis of the Enhanced Pressor Response to Central Cholinergic Stimulation in Spontaneously Hypertensive Rats

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Abstract—Central cholinergic pressor neurons exist at several levels of the neuraxis. Activation of these neurons in spontaneously hypertensive rats (SHR) can lead to an exaggerated hypertensive response when compared with normotensive control animals (WKY). Our earlier studies demonstrated that intravenous injection of the indirect acting agonist physostigmine, but not the direct agonist arecoline, resulted in an exaggerated pressor response in SHR. The purpose of this study was to determine whether discrete injection of cholinergic agonists directly into the CNS would also result in an exaggerated pressor response in SHR. Cholinergic pressor neurons in the posterior hypothalamus may be activated following injection of cholinergic agonists into the lateral cerebral ventricles (I.c.v. injection). Cholinergic pressor neurons in the medulla may be activated following injection of agonists into the cisterna magna (i.c. injection). I.c.v. injection of arecoline in freely-moving animals evoked a pressor response in both SHR and WKY rats that was not significantly different between the two strains. Prior depletion of brain acetylcholine with hemicholinium-3 did not affect the pressor response to I.c.v. arecoline, confirming the postsynaptic site of action of the agonist. Surprisingly, I.c.v. injection of physostigmine also did not result in an exaggerated pressor response in SHR. In contrast, injection of physostigmine into the cisterna magna did produce an enhanced hypertensive response in SHR compared to WKY rats. These results are consistent with the presence of two cholinergic pressor systems; however, only the medullary cholinergic system appears to play a prominent role in spontaneous hypertension. Also the hypothalamic (rostral) cholinergic system does not appear to directly interact with the caudal system.

Since the introduction of the spontaneously hypertensive rat (SHR) strain in the early 1970’s, it has been recognized that certain cholinergic neurons may be abnormal and, in fact, participate in the development and maintenance of hypertension in this strain (1; for review, see ref. 2). Although activation of central cholinergic neurons results in elevated blood pressure in several species, including humans (for review 3), SHR and other rat models of hypertension are known to be particularly sensitive to central cholinergic stimulation (4–7). Such sensitivity is not due to the known heightened vascular reactivity (4) in hypertension. Sensitivity to central cholinergic stimulation is easily demonstrated following intravenous (i.v.) injection of the centrally-acting cholinesterase inhibitor physostigmine, which evokes an enhanced hypertensive response in SHR (4, 5, 7). Recent studies in this laboratory have demonstrated that this exaggerated pressor response to physostigmine is not mirrored following i.v.
injection of the direct acting agonist arecoline (7). While arecoline is capable of stimulating central muscarinic receptors, it does not require (as does physostigmine) the participation of endogenous acetylcholine release to evoke its hypertensive response (7). We concluded, therefore, that the exaggerated hypertensive response observed in SHR following i.v. injection of physostigmine is due to enhanced release of brain acetylcholine, rather than muscarinic receptor supersensitivity. This hypothesis is strengthened through our recent finding of enhanced cholinergic activity in the medulla of SHR as measured biochemically (8).

While i.v. injection of physostigmine might be expected to act at all brain cholinergic synapses, in fact, the pressor response has been demonstrated to be mediated almost exclusively through a pathway within the rostral ventrolateral medulla (9, 10). In contrast, the pressor response to injection of cholinesterase inhibitors into the lateral cerebral ventricle (i.c.v.) appears to be mediated primarily through a pathway within the posterior hypothalamus (11). That the pathways mediating the hypertensive response to physostigmine administered through i.v. or i.c.v. routes may be different has been known for several years (12). One limitation of the i.v. physostigmine studies mentioned above is that they were performed in normotensive, anesthetized rats (9, 10). Under these conditions, the selectivity could be misleading if alternate cholinergic sites were affected by anesthesia. Also, the present study is concerned with the enhanced pressor response to central cholinergic stimulation in the hypertensive animal. Therefore, in an effort to provide additional evidence for the site and specificity of the enhanced response to physostigmine, the cholinergic sites in the medulla were activated following intracisternal (i.c.) rather than i.v. injection of physostigmine.

The purpose of this study was to extend our earlier findings (7) concerning the involvement of presynaptic cholinergic mechanisms involved in spontaneous hypertension examining the roles of two brain regions in mediating the enhanced pressor to cholinergic activation in SHR. While it would have been preferable to perform bilateral injections of agonists directly into specific brain nuclei, this technique is extremely difficult in freely-moving rats, particularly for studies in the hindbrain. As a first approximation, however, and because of previous work (see above) establishing the primary site of action for i.c.v. and i.v. injection of agonists, we employed the administration of agonists via the rostral and caudal ventricular systems. The marked differences observed following these routes of administration (see below) for SHR vs. WKY rats appear to have justified this approach.

Materials and Methods

This study was performed using conscious, freely, moving rats in their home cage environment. A total of 45 male, spontaneously hypertensive rats (SHR) and 45 aged-matched normotensive Wistar Kyoto (WKY) rats were obtained from Harlan-Sprague Dawley, Indianapolis, IN and housed in an environmentally controlled room having a 12 hr light-12 hr dark cycle. Standard rat chow (Wayne Rodent Blox) and tap water were supplied on an unlimited basis. The animals were 16–20 weeks of age at the time of the experiment.

Lateral cerebroventricular (i.c.v.) cannula guide: Rats (30 SHR and 30 WKY) were anesthetized with methohexital and placed in a stereotaxic instrument in a flat skull orientation (13). A 10-mm, 23-gauge stainless steel cannula guide was placed 1.5-mm below the cortical surface so that its tip was directly above the surface of the left lateral cerebral ventricle (0.8 mm caudal and 1.4 mm lateral to bregma). The guide was anchored in place via a stainless steel screw and covered with acrylic cement and plugged with 30-gauge wire. Rats were allowed to recover for at least 5 days prior to the next procedure. For i.c.v. injections in freely-moving rats, a 30-gauge stainless steel injection cannula was connected to a 50-μl syringe using polyethylene tubing. The cannula was lowered through the guide (4.5 mm below cortex) to rest in the ventricle. Drug solutions or vehicle (sterile saline) in a volume of 5 μl were delivered via a 50-μl syringe over 15 sec using a constant speed syringe pump. Upon completion of the experiments, cannula place-
ment was confirmed by dye injection.

**Indwelling intracisternal (i.c.) catheters:**
In a separate group of animals, 15 SHR and 15 WKY were anesthetized with 65 mg/kg i.p., of sodium methohexital and placed in a stereotaxic frame. For intracisternal injection, a catheter was permanently placed in the cisterna magna according to a procedure modified from Kiser (14). Rats were placed in a stereotaxic frame with the surface of the skull in a horizontal position. A midline incision was made to expose the posterior part of the skull and a burr hole placed at the sagittal midline approximately 0.5 mm rostral to the suture between the interparietal and supraoccipital bones. A loose knot was placed in a length of PE 10 tubing. The distal end of the tubing was lowered freehand 6 mm below the surface of the skull. The proximal end of the tubing was stabilized to a stainless steel screw (previously mounted in the skull) using dental acrylic. The tubing was plugged and tunneled subcutaneously to emerge at the nape of the neck. Intracisternal injections (5 μl) were delivered via a 50-μl syringe over 15 sec using a constant speed syringe pump. The injection was followed with a 10-μl saline flush to clear the catheter of drug. At the completion of the experiment, dye administration revealed distribution to the dorsal and ventral surface of the medulla and the posterior portion of the cerebellum, with little distribution to the cervical spinal cord.

**Preparation for arterial blood pressure recording:** Rats were anesthetized with methohexital and a midline abdominal incision made to expose the left iliac artery. A polyethylene (PE 50) catheter, filled with heparinized (20 units/ml) saline, was inserted so that the tip of the catheter terminated in the base of the abdominal aorta, below the origin of the renal arteries. The opposite end of the catheter was plugged and tunneled subcutaneously to emerge at the nape of the neck. Intracisternal injections (5 μl) were delivered via a 50-μl syringe over 15 sec using a constant speed syringe pump. The injection was followed with a 10-μl saline flush to clear the catheter of drug. At the completion of the experiment, dye administration revealed distribution to the dorsal and ventral surface of the medulla and the posterior portion of the cerebellum, with little distribution to the cervical spinal cord.

**Pressor response to central injection of arecoline:** L.c.v. injection of 10 μg of arecoline in WKY rats resulted in an immediate increase in blood pressure which peaked within 2 min and returned to baseline within 20 min. Pretreatment with 20 μg, I.c.v., of HC-3 did not significantly alter blood pressure over the 60 min period. This dose and pretreatment time were selected to allow for maximal depletion of brain acetylcholine (15). Subsequent injection of arecoline resulted in a pressor response not significantly different in magnitude from that produced in untreated animals (Fig. 1). If anything, there was a tendency for the pressor response to be slightly greater in the HC-3 pretreated rats. These findings confirm the fact that, unlike physostigmine, arecoline produces its hypertensive response through stimulation of muscarinic receptors, independent of the release of endogenous acetylcholine. L.c.v. injection of arecoline at 0.5 to 25 μg increased arterial pressure, although the response appeared to be maximal following the 10 μg dose (Tables 1 and 2). Changes in systolic pressure were larger than changes in diastolic pressure. The profile of blood pres-
sure changes, however, were not significantly different from those obtained in WKY, although for each dose, the change was slightly greater for SHR.

Pressor response to central injection of physostigmine: Thus far, the results with arecoline were similar to those observed during our earlier study in which the intravenous injection of cholinergic agonists was employed (7); that is, central depletion of acetylcholine did not affect the pressor response to i.v. arecoline, and the pressor response to i.v. arecoline was not significantly different between SHR and normotensive rats. As with our earlier study, in which i.v. physostigmine produced an exaggerated response in SHR, we expected i.c.v. injection of the cholinesterase inhibitor to do the same. In the present study, i.c.v. injection of physostigmine (1–35 µg) produced a dose-related increase in arterial blood pressure in WKY rats (Tables 1 and 2). In this case, the response became maximal between 2 and 5 min after injection, and it returned to pre-injection

![Graph](https://via.placeholder.com/150)

Table 1. The pressor response to central injection of cholinergic agonists in WKY and SHR (Diastolic pressure)

| Treatmenta | Maximal change in diastolic pressure (mmHg) |
|------------|-------------------------------------------|
|            | 0.5 µg | 10 µg | 25 µg |
| Arecoline   |         |       |       |
| l.c.v.      | WKY     | 20±3  | 33±3  | 30±4  |
|             | SHR     | 31±4  | 38±4  | 39±4  |
| Physostigmine|        |       |       |
| l.c.v.      | WKY     | 13±4  | 21±2  | 34±5  |
|             | SHR     | 23±3  | 28±3  | 37±7  |
| Physostigmine|        |       |       |
| i.c.        | WKY     | 11±3  | 15±3  | 24±4  |
|             | SHR     | 27±7* | 43±5* | 43±3* |

a: l.c.v.=intracerebroventricular, i.c.=intracisternal injection. *significantly different from the WKY value, P<0.05. Each value represents the mean±S.E. of 5–8 experiments.
levels within 30–40 min. In contrast to our earlier study employing i.v. injection of physostigmine, however, the pressor response in SHR was not significantly different from WKY animals (except for a greater change in systolic pressure for the lowest dose). As with arecoline, the responses in SHR were always numerically higher than those in WKY rats. Consistent with the data for maximal increases, there was no significant difference between the entire profile of systolic pressure responses between SHR and WKY to 35 µg of physostigmine, i.c.v. (Fig. 2).

Physostigmine administered through the i.c. route in WKY rats produced a dose-related increase in arterial pressure which was similar in profile to i.c.v. injection. However, the two routes of injection resulted in responses that were different in three ways: 1) Much higher doses were required through the i.c. route to produce changes in blood pressure similar to those following i.c.v. injection; 2) Increases in pulse pressure were greater for i.c. compared with i.c.v. injection (combining values for the two higher doses, and including both strains, these differences

### Table 2. The pressor response to central injection of cholinergic agonists in WKY and SHR (Systolic pressure)

| Treatment  | Maximal change in systolic pressure (mmHg) |
|------------|-------------------------------------------|
|            | 0.5 µg | 10 µg | 25 µg |
| Arecoline   |        |       |       |
| WKY        | 30±5   | 41±5  | 35±5  |
| SHR        | 40±3   | 48±5  | 43±5  |
| Physostigmine |      |       |       |
| WKY        | 18±5   | 33±5  | 46±10 |
| SHR        | 35±5*  | 43±5  | 51±10 |
| Physostigmine |      |       |       |
| WKY        | 17±4   | 29±2  | 42±5  |
| SHR        | 33±6*  | 65±6* | 61±6* |

a: i.c.v. = intracerebroventricular, i.c. = intracisternal injection. *significantly different from the WKY value, P<0.05. Each value represents the mean±S.E. of 5–8 experiments.

**Fig. 2.** Time course of the change in systolic pressure following lateral cerebroventricular (i.c.v.) injection of physostigmine in SHR and WKY rats. There was no significant difference between groups by ANOVA for repeated measures.
following each route of injection averaged 13 and 18 mmHg, respectively); 3) The pressor response to i.c. injection of physostigmine in SHR was significantly greater than that observed for normotensive controls (Tables 1 and 2). This difference between strains also was apparent when the data are expressed over the entire time course of the response (Fig. 3). In fact, both the magnitude and the duration of the pressor response to i.c. injection of physostigmine in SHR were greater than in WKY rats.

**Discussion**

Over the last several years central cholinergic neurons have been implicated in mediating the development and/or maintenance of hypertension in several rat models (2, 7, 8, 16, 17). Clearly, powerful pressor responses may be obtained following injection of central muscarinic agonists at several levels of the neuraxis (3, 18). Enhanced pressor response to i.v. injection of indirect, but not direct-acting agonists, has been interpreted by us to indicate that the primary defect in spontaneous hypertension is related to accentuated acetylcholine release or turnover, as opposed to increased muscarinic receptor sensitivity (7). This is in keeping with the finding of enhanced synaptosomal high affinity choline uptake (the rate-limiting step in acetylcholine synthesis) in tissues derived from the medulla and hypothalamus of SHR (8). The results of the present study confirm our experience with i.v. arecoline in that direct central receptor stimulation was not associated with an enhanced hypertensive response in SHR. More surprising was the fact that i.c.v. injection of physostigmine was not as effective as i.v. injection (7) in producing an exaggerated pressor response in SHR. We had originally expected that most cholinergic centers of the brain would be activated following i.c.v. administration. However, since we began this study, and in keeping with our present results, Xiao and Brezenoff (11) recently demonstrated that at least 50% of the pressor response to i.c.v. injection of a cholinesterase inhibition in normotensive rats was mediated through the posterior hypothalamic nucleus. Likewise, while one might expect i.v. injection of physostigmine to affect most central cholinergic centers, the pressor response following i.v. administration is mediated primarily within the medulla (9, 10). To selectively activate this region, we employed intracisternal injection of the agonist. The limited distribution of dye injection following i.c. injection (see Methods), and differences in the dose response and changes in systolic vs. diastolic pressure between the I.c.v. and i.c. routes (see Results), indicate that two different cholinergic systems were activated following I.c.v. and i.c. injection of
physostigmine. The most direct evidence for differences between i.c.v. and i.c. injection is that only the i.c. route was associated with a significantly enhanced pressor response to physostigmine in SHR. This finding is consistent with our earlier observation that increased high affinity choline uptake in SHR was much greater in tissues derived from the medulla than from the hypothalamus (8). In fact, the increase in the capacity of the high affinity carrier was correlated with the level of resting systolic blood pressure in both SHR and normotensive rats. This was interpreted as biochemical evidence for enhanced activity of cholinergic neurons in the medulla of the SHR. This enhanced activity could be responsible for the exaggerated pressor response to i.v. or i.c. injection of physostigmine in the hypertensive strain. In the present study, i.c.v. injection of both arecoline and physostigmine was always associated with a numerically greater, but a non-statistically significant increase in the pressor response in SHR (except for the lowest dose of i.c.v. physostigmine on systolic pressure which was significantly greater in the SHR; see Table 2). This may be explained by the peripheral vascular hyper-responsiveness known to occur in adult SHR. Another possibility is that there may be some degree of elevated muscarinic receptor sensitivity in the hypothalamus as has been reported previously (19). Also, in our earlier choline uptake study, we discovered a significant (albeit weaker than for the medulla) correlation between hypothalamic choline uptake and resting systolic pressure (8). Nevertheless, based upon our biochemical (8) and current pharmacological studies, it appears that the primary site in which cholinergic neurons participate both in the maintenance of hypertension and in the exaggerated pressor response to physostigmine in the SHR is the medulla.

The diagram in Fig. 4 summarizes our findings and presents a model for the cholinergic interactions involved in spontaneous hypertension. Illustrated are some of the potential cholinergic pathways and their interactions in mediating the pressor response to cholinergic agonists in normotensive rats and for the exaggerated pressor response to i.c. injection of physostigmine in SHR. Pathways A, B and C are not correct since they require that the rostral (hypothalamic) pathway be the site of enhanced cholinergic activity in SHR and/or that it directly interacts with the enhanced medullary pathway. Also, B and C are inconsistent with the lack of ability of HC-3 induced brain acetylcholine depletion to inhibit the pressor response to i.c.v. injection of arecoline. Pathway D is consistent with the observations that 1) the medullary site exhibits enhanced cholinergic function in SHR; 2) the rostral pathway does not exhibit enhanced cholinergic function in SHR; and 3) the rostral pathway is independent of the medullary pathway. The latter possibility is consistent with known direct anatomic connections between the posterior hypothalamus and the spinal sympathetic neurons (20, 21). Finally, the potential configurations depicted in Fig. 4 as A, B or C cannot be ruled out in...
terms of cholinergic involvement in blood pressure regulation in normotension or other disease processes by these data.

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