Laboratory Studies

Role of Vasopressin in Cardiovascular Response to Central Cholinergic Stimulation in Rats

Yutaka Imai, Keishi Abe, Shuichi Sasaki, Naoyoshi Minami, Masanori Munakata, Shigeu Yumita, Toshima Nobunaga, Hiroshi Sekino, and Kaoru Yoshinaga

The cardiovascular effects of centrally administered cholinomimetics were examined in conscious Long-Evans and Brattleboro rats. Carbachol (1 μg/kg) or physostigmine (50 μg/kg) induced a long-lasting increase in blood pressure and a decrease in heart rate in Long-Evans rats whereas no bradycardia was observed in Brattleboro rats, and the pressor response was significantly less than that in Long-Evans rats. The cardiovascular responses to nicotine (30 μg/kg) in Brattleboro rats were not different from those in Long-Evans rats. Intravenous vasopressin antagonist, d(CH2)5Tyr(Me) arginine vasopressin, significantly attenuated the pressor response and eliminated the bradycardic response to carbachol in Long-Evans rats. However, the pressor response to carbachol in Brattleboro rats was still significantly less than that in Long-Evans rats treated with vasopressin antagonist. Intravenous phentolamine partially inhibited the pressor response to carbachol in Long-Evans rats and completely eliminated it in Brattleboro rats. Combined intravenous treatment with phentolamine and vasopressin antagonist completely eliminated the pressor response to carbachol in Long-Evans rats. Centrally administered methylatropine eliminated either the hypertensive or bradycardic response to carbachol in normal rats. These results indicate that the pressor and bradycardic response to carbachol is mediated by the vasopressin release.

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It is well known that central cholinergic stimulation evokes hypertension and bradycardia in several species of animal and in humans (see References 1 and 2 for review). The mechanisms responsible for the cardiovascular responses to central cholinergic stimulation are controversial. Some previous studies have emphasized that the cardiovascular responses to central cholinergic stimulation are mediated by a change in sympathetic outflow from the central nervous system (CNS).2-3 There are also reports that cholinergic agents act centrally to stimulate vasopressin release.4-6 Hoffman and Phillips7 and Hoffman6 observed that the hypertensive response to central cholinergic stimulation is mediated, at least in part, by the release of vasopressin. It has been reported that some physiological and pharmacological stimuli elevate blood pressure and circulating vasopressin levels simultaneously. However, the role of the raised vasopressin levels in the pressor response to such stimuli is uncertain.9-12 In the present study we have examined qualitatively and quantitatively the contribution of circulating vasopressin to the hypertensive and bradycardic responses to centrally administered cholinomimetics. To do this we have used a vasopressin antagonist as well as autonomic blockers in normal Long-Evans rats (LE) and in Brattleboro (DI) rats; endogenous vasopressin is lacking in the latter.13

Materials and Methods

Male rats weighing 250–390 g (22–27 weeks old) and male homozygous DI rats weighing 200–340 g
(21–27 weeks old) were used. The lateral cerebroventricle was cannulated under pentobarbital anesthesia (Nembutal, Abbott Laboratories, North Chicago, Illinois) by means of PE 20 polyethylene tubing (Clay Adams, Parsippany, New Jersey). Coordinates for the cannulation with respect to the bregma were 1.0 mm posterior, 1.5 mm lateral, and 5 mm deep. The cannulae were filled with artificial cerebrospinal fluid (ACSF) (composition [mmol/l]: Na 148.4, K 3.0, Mg 1.0, Ca 2.5, HPO\(_4\) 1.5, Cl 156.9, and glucose 3.0) and fixed in place with stainless steel anchoring screws and orthopedic bone cement.

A week after the cerebroventricular cannulation, the left femoral artery and vein were cannulated by means of polyethylene tubing (tapered PE-100 tubing and PE-20 tubing, respectively) under ether anesthesia. The catheters were led subcutaneously and exteriorized at the nape of the neck. During surgery, 15 mg aminobenzyl penicillin was administered. The rats were housed in individual plastic metabolic cages in a room with constant temperature (23° C) and humidity that was lit from 8:00 AM to 8:00 PM daily. The arterial catheters were connected to a hydraulic swivel tethering system. The rats were allowed to recover for at least 24 hours after surgery and were conscious and unrestrained during the studies. Blood pressure was recorded from the femoral artery catheter with a P231b transducer (Gould-Statham, Oxnard, California) and strain-gauge amplifier (model 1321, NEC-San-ei, Tokyo, Japan). Heart rate was counted from the phasic pressure wave by a cardiotachometer (model Rectigraph-8K, NEC-San-ei). Phasic arterial blood pressure, mean arterial blood pressure (MBP), and heart rate were recorded continuously on a rectiorder.

To keep the arterial catheter patent, heparin saline solution (100 unit/ml) was continuously infused through the arterial catheter at a rate of 80 μl/hr. All drugs for intracerebroventricular administration were dissolved in ACSF or physiological saline (0.9%), respectively. Injections were given at a volume of 150 μl i.v. Drug solution for intracerebroventricular injection was pumped into a PE-20 tubing that was connected with microsyringe (Hamilton, Reno, Nevada) containing ACSF. In the PE-20 tubing, drug solution was insufflated from ACSF by air (0.1–0.2 μl). A total injection volume of 5 μl i.c.v. was given; 2 μl or less ACSF was contained in the dead space of the intracerebroventricular cannula followed by 3 μl or less of drug solution, which was pushed by ACSF of the injection system (PE-20 tubing and microsyringe). Drug solutions were delivered over a period of 5 seconds. The drugs used in the present study were: carbachol (K&K Laboratories, Plainview, New York), physostigmine (Nakarai Chemicals, Tokyo, Japan), nicotine hydrogen tartrate salts (Sigma Chemical Co., St. Louis, Missouri), phenolamine melycate (CIBA-GEIGY, Summit, New Jersey), methylatropine bromide (Takeda, Tokyo, Japan), propranolol-HCl (ICI, London, UK), phenylephrine-HCl (Sigma Chemical Co.), 1-(β-mercaptop-β, cyclopentamethylen propionic acid), 2-[(O-methyl) tyrosine arginine vasopressin (d(CH\(_3\))\(_2\)Tyr(Me)AVP; vasopressin antagonist, Peninsula, San Carlos, California, and 1-(3-mercaptopropionic acid)-8-O-arginine vasopressin (DDAVP, Ferring, Malmo, Sweden).

Post mortem examination of the brain was performed to verify the position of the intracerebroventricular cannulas.

**Experimental Protocol**

Experiments were carried out as described below.

**Cardiovascular effects of intravenous and intracerebroventricular cholinomimetics in Long-Evans and Brattleboro rats.** The dose–response relation for the cardiovascular response to carbachol (0.1, 0.3, and 1.0 μg/kg i.c.v.; in eight LE and eight DI rats) was examined. Thirty minutes were allowed for recovery after the 0.1 μg/kg dose and 1 hour after the 0.3 μg/kg dose. The time courses of the response to carbachol (1 μg/kg i.c.v.; 11 LE and 22 DI rats), carbachol (3 μg/kg i.v.; seven LE and seven DI rats), and physostigmine (50 μg/kg i.c.v.; six LE and six DI rats) were examined. In five LE rats the reproducibility of the cardiovascular response to carbachol (1 μg/kg i.c.v.) was examined, allowing intervals of 2 hours and 24 hours.

The cardiovascular responses to nicotine (30 μg/kg i.c.v.) were examined in seven LE and DI rats. **Modification of the cardiovascular effect of intracerebroventricular carbachol in Long-Evans rats by vasopressin analogues.** In the experiment described below, carbachol in a dose of 1 μg/kg i.c.v. was used to produce a "standard" cardiovascular response to carbachol.

The cardiovascular effects of intracerebroventricular carbachol before and during treatment with a vasopressin-specific vascular receptor antagonist (VPVRA), d(CH\(_3\))\(_2\)Tyr(Me)AVP, were examined in five LE rats. Two hours after the control experiment, the vasopressin antagonist was administered in a bolus dose of 1 μg/kg i.v. followed by an infusion of 5 μg/kg/hr for 1 hour. The cardiovascular effect of intracerebroventricular carbachol was reexamined during the infusion of the antagonist. The control response to the vasopressin antagonist given alone (i.v.) was also obtained. To determine the degree of vasopressin blockade by the antagonist, the pressor effect of vasopressin (100 ng/kg i.v.) was examined before and during infusion of the antagonist in five LE rats.

The cardiovascular effects of intracerebroventricular carbachol before and during treatment with DDAVP, a vasopressin antidiuretic agonist, were examined in five DI rats. After the control experiment, DDAVP was infused at a rate of 0.2 ng/kg/min for 24 hours. The cardiovascular effect of intracerebroventricular carbachol was reexamined during the infusion of DDAVP. To determine the antidiuretic effect of DDAVP, 24-hour urinary pro-
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Figure 1. Line graphs of effects of carbachol (1 μg/kg i.c.v.) on mean arterial pressure (MAP) and heart rate (HR) in Long-Evans (LE) and Brattleboro (DI) rats. In some DI rats a slight decrease in blood pressure was observed after hypertension. Modification of the effects of carbachol (1 μg/kg i.c.v.) on MAP and HR by a vasopressin-specific vascular receptor antagonist (VPVRA), d(CH₃)₂Tyr(Me)AVP, in LE rats is also illustrated in the figure.

Modification of cardiovascular effect of intracerebroventricular carbachol by autonomic blockade.

The cardiovascular effect of intracerebroventricular carbachol was examined before and after administration of phentolamine (3 mg/kg i.v. in six LE rats and five DI rats), methylatropine (1 mg/kg i.v. in five LE rats or 3 μg/kg i.c.v. in five LE rats), or propranolol (2 mg/kg i.v. in five LE rats). Two hours after the control experiment, autonomic blockers were administered. Five minutes after intracerebroventricular or 15 minutes after intravenous treatment with the autonomic blocker, carbachol was administered intracerebroventricularly. Twenty-four hours before or after the experiment, the control response to the appropriate blocker alone was examined together with the degree of autonomic blockade. In five LE rats the cardiovascular response to intracerebroventricular carbachol was examined before and 15 minutes after combined treatment with the vasopressin antagonist (5 μg/kg i.v.) and phentolamine (3 mg/kg i.v.). Twenty-four hours later, the control response of combined treatment with the two antagonists was examined.

Statistical Method

All values reported are the mean±SEM, unless otherwise stated. Dose–response curves and the time course of change in cardiovascular parameters were compared by means of two-way analysis of variance (ANOVA) for the repeated measurements. In comparing the three time courses, a two-way ANOVA that included all three groups was performed first. Comparison between any two groups was done by the Bonferroni correction for multiple comparison. One-way ANOVA and Student’s t test were applied as the statistical methods to other results.

Results

In some of the experiments, some rats were used twice for different experimental protocols, allowing a recovery interval of 24 hours between experiments. In five DI rats used twice for different experimental protocols, 24-hour urinary volume after the experiment (315±25 ml/24 hr) was not different from that preceding the experiment (326±17 ml/24 hrs). Body weight fell slightly but significantly 24 hours after the experiment (295±5 vs. 287±4 g, n=5 LE rats); 273±3 vs. 268±4 g (n=5 DI rats).

Cardiovascular Effect of Carbachol

Figure 1 shows the cardiovascular response to carbachol, 1 μg/kg i.c.v., in LE and DI rats. Intracerebroventricular carbachol induced a long lasting increase in blood pressure in both strains. In some DI rats, a slight decrease in blood pressure was observed after the hypertension. As shown in the figure, the hypertensive response to intracerebroventricular carbachol in DI rats was significantly less than that in LE rats throughout the period of the response (F₃,1₁₀=231.5, p<0.001). Intracerebroventricular carbachol elicited an initial transient tachycardia followed by prolonged bradycardia in the LE rats, whereas in the DI rats only tachycardia was observed (F₃,1₁₀=39.4, p<0.001). The maximum changes in blood pressure and heart rate were used to construct dose–response curves (Figure 2). Intracerebroventricular carbachol caused dose-dependent increases in blood pressure and decreases in heart rate in LE rats, whereas in DI rats the responses were not dose dependent. Overall, the hypertensive and bradycardic responses to intracerebroventricular-
ular carbachol in the LE rats were significantly greater than those in the DI rats ($F_{1,44}=65.3, p<0.001$ in blood pressure; $F_{1,44}=23.9, p<0.001$ in heart rate).

Carbachol (3 $\mu$g/kg i.v.) induced a short-lasting and steep decrease in blood pressure that returned to the initial level within 1 minute in both strains. The magnitude of the accompanying tachycardia was not significantly different between the two strains, although it was more persistent in the DI rats (Figure 3; $F_{1,90}=5.0, p<0.03$).

Reproducibility of the cardiovascular response to intracerebroventricular carbachol was examined, allowing 2-hour and 24-hour intervals, and was excellent (Figure 4) (blood pressure for 24 hours: $F_{1,80}=0.66$, for 2 hours: $F_{1,80}=0.92$; heart rate for 24 hours: $F_{1,80}=0.42$, for 2 hours: $F_{1,80}=0.23$).

Cardiovascular Effect of Physostigmine

The cardiovascular response to intracerebroventricular physostigmine was essentially the same as observed in intracerebroventricular carbachol. Pressor responses to intracerebroventricular physostigmine in DI rats (19±2 mm Hg, 6 minutes after injection) was significantly less than that in LE rats (36±4 mm Hg, 6 minutes after injection, $F_{1,106}=136.3, p<0.001$). Intracerebroventricular physostigmine induced a transient tachycardia (57±17 beats/min, 3 minutes after injection) followed by a long-lasting bradycardia in the LE rats (−38±17 beats/min, 10 minutes after injection), whereas bradycardia was scarcely observed in the DI rats (43±18 beats/min, 3 minutes after injection and 13±15 beats/min, 10 minutes after injection) (tachycardic phase, 0–6 minutes, $F_{1,60}=0.2$; bradycardic phase, 6–20 minutes, $F_{1,70}=12.8, p<0.001$).

Cardiovascular Effect of Nicotine

Intracerebroventricular nicotine induced an initial, transient rise in blood pressure and heart rate (LE, 16±3 mm Hg and 35±11 beats/min; DI, 20±11 mm Hg and 37±16 beats/min) followed by a decrease in blood pressure (LE, −7±1 mm Hg; DI, −6±3 mm Hg) accompanied by bradycardia (LE, −41±8 beats/min; DI, −23±11 beats/min). The hypertensive and bradycardic responses to intracerebroventricular nicotine in the two strains were not different ($p>0.1$).

Modification of Cardiovascular Effect of Carbachol by Vasopressin-Antagonist

Vasopressin antagonist (VPVRA) alone did not cause any changes in blood pressure and heart rate in LE rats. As shown in Figure 1, the three time courses of change in blood pressure, that is, the responses to intracerebroventricular carbachol in LE rats, in DI rats, or in LE rats during vasopressin antagonist, were significantly different ($F_{2,90}=6.94, p<0.001$). The Bonferroni correction for multiple comparison revealed that the responses between any two groups were significantly different ($p<0.05$).
indicating that the pressor response to intracerebroventricular carbachol was significantly attenuated by the vasopressin antagonist. The pressor response to intracerebroventricular carbachol during administration of vasopressin antagonist in the LE rats was still significantly greater than that in the DI rat. The bradycardic response to intracerebroventricular carbachol was eliminated almost completely by the vasopressin antagonist ($F_{1,30}=8.1$, $p<0.01$).

The pressor effect of vasopressin at a dose of 100 ng/kg i.v. (51.7±2.8 mm Hg) was completely inhibited by the treatment with the vasopressin antagonist.

**Effect of Vasopressin Antidiuretic Agonist on Cardiovascular Response to Carbachol**

Twenty-four hours later, DDAVP infusion had little effect on blood pressure (121±9 vs. 118±11 mm Hg, mean±SD) and heart rate (388±29 vs. 380±35 beats/min, mean±SD) in DI rats. DDAVP decreased urinary volume significantly (298±25 vs. 18±4 ml/24 hr, mean±SD). Cardiovascular responses to intracerebroventricular carbachol during DDAVP treatment were not different from those in the controls (blood pressure, $F_{1,30}=0.54$; heart rate, $F_{1,30}=0.36$).

**Modification of Cardiovascular Effect of Carbachol by an Autonomic Blocker**

Phentolamine (3 mg/kg i.v.) alone in LE rats (70±10 mm Hg and 490±19 beats/min, mean±SD, 15 minutes after blockade) and in DI rats (66±9 mm Hg and 491±15 beats/min, mean±SD, 15 minutes after blockade) or combined with vasopressin antagonist (5 µg/kg i.v.) in LE rats (72±15 mm Hg and 495±43 beats/min, mean±SD, 15 minutes after blockade) produced extreme hypotension and tachycardia (Table 1). The doses of phentolamine and vasopressin antagonist used completely inhibited the pressor effect of phenylephrine (10 µg/kg i.v., 51.0±3.5 mm Hg) and vasopressin (100 ng/kg i.v., 48.2±2.5 mm Hg) 45 minutes after the intravenous injection. As shown in Figure 5, the three time courses of change in blood pressure (i.e., the response to intracerebroventricular carbachol in LE rats, the response to intracerebroventricular carbachol in LE rats + phentolamine, and LE rats + phentolamine + time control) demonstrate the complete inhibition of the pressor effect by phentolamine.
TABLE 1. Basal Arterial Blood Pressure and Heart Rate in Each Experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rat</th>
<th>n</th>
<th>Basal MBP (mm Hg)</th>
<th>Basal HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.c.v. carbachol</td>
<td>LE</td>
<td>11</td>
<td>109±14</td>
<td>427±56</td>
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<tr>
<td></td>
<td>DI</td>
<td>22</td>
<td>118±17</td>
<td>417±61</td>
</tr>
<tr>
<td>i.v. carbachol</td>
<td>LE</td>
<td>7</td>
<td>101±21</td>
<td>406±58</td>
</tr>
<tr>
<td></td>
<td>DI</td>
<td>7</td>
<td>123±15</td>
<td>385±33</td>
</tr>
<tr>
<td>i.c.v. carbachol dose response</td>
<td>LE</td>
<td>8</td>
<td>109±15</td>
<td>373±47</td>
</tr>
<tr>
<td></td>
<td>DI</td>
<td>8</td>
<td>119±14</td>
<td>393±34</td>
</tr>
<tr>
<td>i.c.v. nicotine</td>
<td>LE</td>
<td>7</td>
<td>110±16</td>
<td>380±63</td>
</tr>
<tr>
<td></td>
<td>DI</td>
<td>7</td>
<td>113±21</td>
<td>389±40</td>
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<tr>
<td>i.c.v. physostigmine</td>
<td>LE</td>
<td>6</td>
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<td>378±30</td>
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<tr>
<td></td>
<td>DI</td>
<td>6</td>
<td>110±16</td>
<td>395±50</td>
</tr>
<tr>
<td>i.v. VPVRA+i.c.v. carbachol</td>
<td>LE</td>
<td>5</td>
<td>111±20</td>
<td>379±52</td>
</tr>
<tr>
<td>i.v. phenolamine+i.c.v. carbachol</td>
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<td>6</td>
<td>112±12</td>
<td>386±30</td>
</tr>
<tr>
<td></td>
<td>DI</td>
<td>6</td>
<td>116±14</td>
<td>379±25</td>
</tr>
<tr>
<td>i.v. phentolamine+i.v. VPVRA+i.c.v. carbachol</td>
<td>LE</td>
<td>5</td>
<td>122±14</td>
<td>334±19</td>
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<tr>
<td>i.v. methylatropine+i.c.v. carbachol</td>
<td>LE</td>
<td>5</td>
<td>115±17</td>
<td>354±55</td>
</tr>
<tr>
<td>i.c.v. methylatropine+i.c.v. carbachol</td>
<td>LE</td>
<td>5</td>
<td>115±16</td>
<td>360±45</td>
</tr>
<tr>
<td>i.v. propranolol+i.c.v. carbachol</td>
<td>LE</td>
<td>5</td>
<td>122±10</td>
<td>368±41</td>
</tr>
</tbody>
</table>

Values expressed are mean±SD. MBP, mean arterial blood pressure; DI, Brattleboro rats; HR, heart rate; i.c.v., intracerebroventricular; LE, Long-Evans rats; i.v., intravenous; VPVRA, vasopressin-specific vascular receptor antagonist.

Combined treatment with intravenous phentolamine and intravenous vasopressin antagonist in LE rats completely inhibited the pressor and bradycardic responses to intracerebroventricular carbachol (Table 2). Methylatropine (3 µg/kg i.c.v.) alone in LE rats had little effect on blood pressure (114±13 mm Hg, mean±SD, 15 minutes after administration) but caused a small increase in heart rate (370±43 beats/min, mean±SD, 15 minutes after administration) (Table 1). Intracerebroventricular methylatropine, however, abolished the heart rate response almost completely and markedly attenuated the blood pressure response to intracerebroventricular carbachol in LE rats (Table 2). Intracerebroventricular methylatropine in LE rats slightly attenuated the depressor effect of carbachol (3 µg/kg i.v.) (-24.6±1.3 vs. -19.2±2.5 mm Hg, p<0.05) 30 minutes after treatment.

TABLE 2. Modification of Cardiovascular Effect of Intracerebroventricular Carbachol

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rat</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MBP (mm Hg)</td>
<td></td>
<td>HR (beats/min)</td>
<td></td>
</tr>
<tr>
<td>i.v. phenolamine</td>
<td>DI</td>
<td>12±1</td>
<td>2±2*</td>
<td>-49±6</td>
<td>1±4*</td>
</tr>
<tr>
<td></td>
<td>LE</td>
<td>39±2</td>
<td>17±6†</td>
<td>-25±9</td>
<td>-3±4*</td>
</tr>
<tr>
<td>i.v. phenolamine+i.v. VPVRA</td>
<td>LE</td>
<td>32±5</td>
<td>2±3*</td>
<td>-55±4</td>
<td>-2±6*</td>
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<tr>
<td>i.c.v. methylatropine</td>
<td>LE</td>
<td>37±4</td>
<td>8±2*</td>
<td>-58±7</td>
<td>-4±3*</td>
</tr>
<tr>
<td>i.v. methylatropine</td>
<td>LE</td>
<td>36±2</td>
<td>4±4*</td>
<td>-61±5</td>
<td>2±5*</td>
</tr>
<tr>
<td>i.v. propranolol</td>
<td>LE</td>
<td>44±6</td>
<td>52±5†</td>
<td>-45±3</td>
<td>-24±4*</td>
</tr>
</tbody>
</table>

Values expressed are mean±SEM. After treatment, 5 minutes after administration of an autonomic blocker or vasopressin antagonist, or both. Changes in mean blood pressure (MBP) and heart rate (HR) after treatment were obtained as a difference from the time-control experiment, i.v., intravenous; DI, Brattleboro rats; LE, Long-Evans rats; VPVRA, vasopressin antagonist; i.c.v., intracerebroventricular.

*p<0.001; †p<0.01 compared with before treatment values.
Methylatropine in LE rats (1 mg/kg i.v.) produced pronounced tachycardia (492±43 beats/min, mean±SD, 15 minutes after administration) but had little effect on blood pressure (116±15 mm Hg, mean±SD, 15 minutes after administration). It abolished both the hypertensive and bradycardic responses to intracerebroventricular carbachol (Table 2).

Propranolol in LE rats (2 mg/kg i.v.) induced transient hypertension and long-lasting bradycardia (300±14 beats/min, mean±SD, 15 minutes after administration); the former recovered to the initial level 15 minutes after the treatment (124±13 mm Hg, mean±SD). Intravenous propranolol significantly potentiated the pressor response and attenuated the bradycardic response to intracerebroventricular carbachol (Table 2).

**Discussion**

In the current study, centrally administered cholinomimetics elevated blood pressure and decreased heart rate in normal conscious rats. The distinct and different blood pressure and heart rate responses to central cholinergic stimulation suggest a possible differential effect of the drug on the central autonomic centers governing the blood pressure and heart rate. It is concluded that the response to intracerebroventricular carbachol is elicited by a central mechanism, since intravenous carbachol did not cause hypertension and intracerebroventricular carbachol caused bradycardia in LE but not DI rats. Intravenous carbachol, on the other hand, produced tachycardia in both strains. Centrally administered methylatropine had little, if any, influence on the cardiovascular response to intravenous carbachol, although the pressor and bradycardic effects of intracerebroventricular carbachol were greatly reduced.

The pressor response to intracerebroventricular carbachol in DI rats was comparatively less than that in normal LE rats. This difference was also observed when physostigmine was administered centrally. Since the effect of physostigmine is mediated by the endogenous cholinergic system, the difference of pressor response to cholinergic stimulation between LE and DI rats may be physiologically significant.

It is well known that central cholinergic stimulation elicits a hypertensive response in several species of animal as well as in humans, and it is hypothesized that the pressor effect is mainly mediated by central muscarinic receptor mechanisms.3,6,14-16 We also have confirmed that the pressor effect of intracerebroventricular carbachol in rats is mediated by a central muscarinic receptor mechanism, because methylatropine administered centrally inhibited the pressor response to intracerebroventricular carbachol. Central regions for which the cholinergic stimulation evokes elevation of blood pressure have been identified in several central loci.3,17-19 The mechanism responsible for the pressor response to central cholinergic stimulation is controversial (see above). In the present study the pressor response to intracerebroventricular carbachol in DI rats was completely eliminated by an intravenous α-adrenergic receptor antagonist, suggesting that the response is solely mediated by an increase in sympathetic outflow. In LE rats, however, either the α-adrenergic receptor antagonist or vasopressin antagonist partially attenuated the pressor response to intracerebroventricular carbachol, and combined treatment with both antagonists completely inhibited it, indicating that in normal rats the pressor response to central cholinergic stimulation is mediated by both an activation of the sympathetic nervous system and an increase in circulating vasopressin. This conclusion is also supported by the recent report by Litake et al,6 who demonstrated the high plasma vasopressin level (approximately 80 pg/ml) produced by carbachol at a dose of 250 ng i.c.v. per rat; this dose is almost equivalent to the one used in the present study (1 μg/kg). The plasma vasopressin level reported by these authors might be sufficient to elevate blood pressure in conscious rats.20,21

If the pressor response to intracerebroventricular carbachol in LE rats is the product of increases in sympathetic outflow and circulating vasopressin, the response after the vasopressin antagonist might be expected to be identical with that in DI rats. However, the magnitude of the pressor response to intracerebroventricular carbachol in LE rats after vasopressin antagonist was still apparently greater than that in DI rats. This may indicate that the sympathoexcitatory response to centrally administered carbachol in DI rats is attenuated when compared with that in LE rats.

The loss of peripheral interaction between circulating vasopressin and sympathetic outflow may also explain the attenuated pressor response in DI rats. However, this possibility is unlikely since the pressor response in DI rats to an α-agonist was not different from that in normal LE rats.20

Cholinergic depressor responses, which are believed to be mediated mainly by nicotinic mechanisms,22,23 have also been demonstrated from stimulation of the brainstem area. It is possible that in DI rats the nicotinic depressor effect by carbachol is more prominent than that in LE rats, resulting in a smaller pressor response to the drug in the DI rats. However, this possibility may be excluded since the depressor effect of intracerebroventricular nicotine in DI rats was not different from that in LE rats.

It has been reported that centrally administered cholinomimetics cause variable heart rate changes.13,15 In the present study, intracerebroventricular carbachol induced bradycardia in normal LE rats, but no such effect in DI rats, suggesting that the bradycardia was mediated directly or indirectly, or both, by endogenous vasopressin. Actually, in the present study, a vasopressin vascular receptor antagonist administered peripherally inhib-
imited the bradycardia in response to intracerebroventricular carbachol almost completely. This also suggests that the bradycardia was mediated by vasopressin. This conclusion is supported by previous work in which the bradycardic response to intravenous vasopressin was eliminated or converted to tachycardia by treatment with a vasopressin vascular-receptor antagonist.24-26 Vasopressin may evoke bradycardia through its direct cardiac action or its effect on the autonomic nervous system. Modulatory effects of vasopressin on the baroreceptor reflex have also been well documented. Vasopressin augmented the baroreceptor reflex through both central and peripheral mechanisms.20,27-30 In the present study intravenous methylatropine eliminated the bradycardia almost completely, suggesting that the bradycardia is mediated mainly by stimulation of the parasympathetic nervous system. Taken together, it is concluded that the increase in circulating vasopressin, in response to central cholinergic stimulation evokes bradycardia mainly through its effect on baroreceptor reflex-dependent or reflex-independent autonomic neural pathways. However, in the present study it has also been confirmed that intravenous propranolol administered to LE rats attenuated the bradycardia slightly but significantly, suggesting that the bradycardia is mediated partly by inhibition of the sympathetic nervous system. This may indicate the differential effect of carbachol on the central sympathetic centers governing blood pressure and heart rate or that the bradycardia is mediated at least in part by inhibition of cardiac sympathetic tone, which is reflexly induced by pressor effect of intracerebroventricular carbachol.

In the present study, intravenous phentolamine blocked the bradycardic response to centrally administered carbachol. However, the phentolamine also caused a pronounced reduction in arterial pressure, which in turn probably caused a major release of vasopressin and a large increase in the plasma vasopressin concentration. In this circumstance it is uncertain whether the subsequent administration of carbachol would have any further effect on vasopressin release or heart rate.

It is believed that methylatropine administered peripherally does not affect the central cholinergic system.31 However, in the present study methylatropine in a 1 mg/kg i.v. dose abolished the cardiovascular response to intracerebroventricular carbachol, suggesting that methylatropine administered peripherally may block the central muscarinic receptor to release vasopressin. Therefore, in the present study it was difficult to estimate qualitatively or quantitatively the role of peripheral parasympathetic nervous system for the cardiovascular response to centrally administered carbachol. Thus, the possibility remains that elevated circulating vasopressin acts directly on the heart to evoke bradycardia.

As a remaining explanation for the differential cardiovascular responses to intracerebroventricular carbachol seen in LE and DI rats, differential volume and level of hydration between the two strains may be postulated. However, this possibility is unlikely since DDAVP did not modulate the cardiovascular responses to intracerebroventricular carbachol, whereas DDAVP restored the urinary production in the DI rats to within the range in the LE rats.

In summary, it is concluded that central cholinergic muscarinic receptor mechanism contributes to cardiovascular regulation through the effect on vasopressin release as well as on the autonomic nervous system.

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References


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