Effects of Cholinomimetic Injection into the Brain Stem Reticular Formation on Halothane Anesthesia and Antinociception in Rats

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ABSTRACT

The brain stem reticular formation plays an important role in determining consciousness and arousal. Modulation of cholinergic neurotransmission in this region alters the sleep-wake cycle. In the present study, we examined the effect of the direct application of cholinergic agents into the pontine reticular nucleus on anesthetic requirements and recovery and antinociception in rats. Sprague-Dawley rats were implanted with 24-gauge guide cannulas 1.0 mm above the oral portion of pontine reticular nucleus (PnO) while under pentobarbital anesthesia with the use of a stereotaxic apparatus. After recovery from surgery, animals were randomly assigned to one of the following protocols: minimum alveolar concentration (MAC), recovery time, tail-flick latency, or motor blockade. All measurements were performed after carbachol microinjection into the PnO after pretreatment with atropine or mecamylamine. Carbachol injection into the PnO significantly reduced MAC of halothane and prolonged recovery in a dose-dependent manner. Pretreatment with atropine reversed MAC reduction by carbachol, and both atropine and mecamylamine shortened recovery time under carbachol. In anesthetized rats, carbachol produced antinociceptive effects as reflected by a change in tail-flick latency response. Atropine and mecamylamine inhibited antinociceptive effects of carbachol. These results suggest that cholinomimetic injection into the PnO modulates the anesthetic state produced by halothane, suggesting participation of this area in the mechanisms in the brain that generate the anesthetic state.

The brain stem reticular formation has been the target of much research in attempts to define consciousness and arousal (Meyer, 1970; Steriade et al., 1993; Steriade, 1996; Coenen, 1998). Progress in neuroscience in the past few decades has delineated that the arousal system originates in the upper brain stem reticular formation and projects to the cerebral cortex through synaptic relays in the thalamus (Meyer, 1970; Steriade et al., 1993, 1996; Coenen, 1998). The main neurotransmitters identified in the brain stem to cortex circuit include acetylcholine, norepinephrine, serotonin, and glutamate (Steriade et al., 1993; Steriade, 1996). In addition to generating arousal and different levels of awareness, the major functions of the reticular formation include cardiopulmonary control, control of somatic motor tone, and modulation of pain perception (Role and Kelly, 1991). Evidence suggests that the state of general anesthesia is generated in the anatomically distributed neuronal networks ranging from spinal to cerebral levels (Angel, 1993; Durieux, 1996; Antognini, 1997; Lydic and Baghdoyan, 1997). A cholinergic network in the brain stem reticular formation has been demonstrated to play a key role in generating some of the physiological characteristics observed during general anesthesia (Morales et al., 1987; Lydic et al., 1991; Keifer et al., 1996). For example, cholinomimetic injection into the pontine reticular formation inhibits spinal motoneuron excitability in cats (Morales et al., 1987). Cholinergic transmission in this region is also known to contribute to respiratory depression (Lydic et al., 1991). Furthermore, the direct application of cholinomimetics into the oral portion of pontine reticular nucleus (PnO) alters sleep-wake cycles and causes an increase in a rapid eye movement (REM) sleep-like state in rats (Gnadt and Pegram, 1986; Imeri et al., 1994; Bourgin et al., 1995). The blockade of muscarinic receptors in that region was shown to enhance wakefulness and decrease REM and slow wave sleep in rats (Imeri et al., 1994).

Although the role of cholinergic neurotransmission in general anesthesia has long been studied, the effect of cholinergic agents on minimum alveolar concentration (MAC) has been controversial (Horrigan, 1978; Zucker, 1991; Ishizawa et al., 1997). The i.p. injection of physostigmine, an acetylcholinesterase inhibitor, decreased halothane MAC in rats (Ishizawa et al., 1997), but another study showed that phy-

ABBREVIATIONS: PnO, oral portion of pontine reticular nucleus; MAC, minimum alveolar concentration; REM, rapid eye movement; MPE, maximum possible effect; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus; LC, locus ceruleus.
sostigmine increased isoflurane MAC in rats (Zucker, 1991). The effects of i.c.v. administration of cholinergic agents on MAC also were not consistent (Zucker, 1991). On the other hand, intrathecally administered cholinergic agonists are well known to consistently produce analgesia (Yaksh et al., 1985). Therefore, a study of the roles of discrete regions in the central nervous system in anesthesia could provide important information for further understanding cholinergic contribution to general anesthesia.

The present study was thus designed to detect whether directly administered cholinergic agents in the brain stem reticular formation change the state of anesthesia as well as antinociceptive responses in rats. The hypothesis tested in this study was that halothane requirements and recovery are modulated by microinjection of carbachol in the PnO in rats. We also tested the hypothesis that antinociceptive responses are altered by carbachol injection into the PnO using tail-flick latency in unanesthetized rats.

Materials and Methods

Animals. With approval of the animal care and use committee of our institution, studies were performed on male Sprague-Dawley rats weighing 250 g (8 weeks old). Rats were housed individually in a temperature-controlled (21–24°C) room with a 12-h light/dark cycle, and they were given free access to water and food. All experiments were performed between 10:00 AM and 6:00 PM. A total of 80 animals was used. Each animal was assigned to only one of the following protocols: MAC of halothane (the number of rats; n = 30), recovery time (n = 16), tail-flick latency (n = 20), or motor response (n = 14). Each animal was studied three or four times in the assigned protocol at an interval of 5 days between studies.

All surgical procedures were performed with the rats during anesthesia with 50 mg/kg i.p. pentobarbital. The rat was positioned in a stereotaxic apparatus (Narishige, Tokyo, Japan). A 24-gauge stainless steel guide cannula was unilaterally implanted 1.0 mm above the PnO using the following stereotaxic coordinates: with bregma as reference, 8.7 mm posterior, 1.0 mm lateral, and 6.4 mm ventral from the dura mater (Paxinos and Watson, 1998). Dental cement was applied to the skull around the guide cannula and around the screws fixed in the skull. The cannula was kept sealed, except during the injection. After surgery, the rats were again housed individually and allowed to recover for 5 days before any of the experiments described in the following sections. Experiments were carried out over the 4 weeks.

MAC of Halothane. Anesthesia was induced through inhalation of halothane in a transparent container (Fig. 1A). The rat’s trachea was intubated with a 16-gauge cannula, and the lungs were mechanically ventilated with halothane in oxygen and air (F1O2 0.5, rodent ventilator model 683; Harvard Apparatus, Holliston, MA). End-tidal carbon dioxide pressure was maintained at 35 to 40 mm Hg. Rectal temperature was continuously monitored and maintained at 37.5°C with a heating pad. Fifteen minutes after the initiation of halothane anesthesia, a 30-gauge internal cannula connected to polyethylene tubing was inserted into the guide cannula and positioned 1.0 mm below the tip. Atropine sulfate at 4.0 mg (19.6 mM) or 12.0 mg (59.0 mM), mecamylamine hydrochloride at 1.0 mg (163.3 mM) or 4.0 mg (65.3 mM), or saline was injected into the PnO in a volume of 0.3 μl over 90 s using a microinjection pump (CMA/100; Microdialysis, Acton, MA). Carbachol at 5.0 μg (136.9 mM), 10.0 μg (273.8 mM), or saline was injected into the PnO in a volume of 0.2 μl over 60 s at 15 min after pretreatment with antagonists or saline.

MAC was determined using the method described previously (Ishizawa et al., 1997). Briefly, the administration of halothane was adjusted in steps of 0.1%, and a stable end-tidal concentration for 15 min was obtained before stimulation. Noxious stimulation was applied with a 6-inch hemostat to the middle third of the tail for 60 s at the first ratchet position. The criteria for positive movements included purposeful movements of either the head or the four extremities. When the animal had a positive response, the halothane concentration was increased; when there was no response, the concentration was decreased until movement was observed. When the interval was bracketed by positive and negative responses, the midpoint of the interval was then tested. The MAC of halothane for each rat was defined as being midway between the highest end-tidal concentration with a positive response and the lowest end-tidal concentration with a negative response. End-tidal gas samples were obtained with an airtight glass syringe through a 26-gauge needle inserted to a tracheal tube during 15 expirations. Halothane concentrations were analyzed using an infrared analyzer (M1025B; Hewlett Packard). Calibration with the standard gas was performed before study. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Recovery Time of Halothane Anesthesia. While the rats were loosely restrained in a rodent restrainer (Braintree Scientific Inc., Braintree, MA), atropine sulfate (4.0 μg), mecamylamine hydrochloride (1.0 μg), or saline was injected into the PnO in a volume of 0.3 μl over 45 s (Fig. 1B). Anesthesia was induced by placing the rats in the airtight transparent container flowed with 2% halothane in air at 3 l/min through insufflating and draining tubes connected to the container. Fifteen minutes after the initiation of anesthesia, the rats were withdrawn from the anesthetic and carbachol (1.0 or 5.0 μg) or saline was immediately injected in a volume of 0.2 μl over 30 s into the PnO. The rats were promptly placed on their backs on the heating table at 30°C. As soon as they turned over so that all four feet contacted the surface, they were placed on their backs again. The second performance of this righting reflex was recorded, and the recovery time was defined as the time between withdrawal of halothane and the second performance of the righting reflex. The rate of breathing was measured during halothane anesthesia and the recovery.
Antinociceptive Testing. Nociceptive threshold was assessed using tail-flick latency test (Fig. 1C). A noxious somatic stimulus was measured by monitoring the latency to withdrawal from a high-intensity light focused on the dorsal surface of the tail (Thermal Analgesimeter model KN-205E; Natume, Tokyo, Japan). A cutoff time of 10 s was predetermined to minimize the risk of tissue damage. After baseline measurements for tail-flick latency had been obtained, each animal received atropine sulfate (4.0 μg), mecamylamine hydrochloride (1.0 μg), or saline injection into the PnO in a volume of 0.3 μl over 90 s. Fifteen minutes after the pretreatment, carbachol (1.0 or 5.0 μg) or saline was injected into the PnO, and tail-flick latencies were determined 5, 10, 15, 20, 30, 40, 50, and 60 min after microinjection of carbachol or saline. Data are expressed as maximum possible effect (MPE) according to the following formula: MPE (%) = [(postdrug latency) – (basal latency)] / (cutoff latency) – (basal latency)] × 100.

Motor blockade and righting reflex were evaluated 5, 10, 30, and 60 min after carbachol or saline injection after the pretreatment. These trials were performed in a separate group of the rats from those for tail-flick latency to keep the rats undisturbed during tail-flick tests. Motor blockade was graded according to the scale previously reported (Zeng et al., 1999) as follows: 0 indicates free movement of hindlimbs without limitation; 1, limited or asymmetrical movement of the hindlimbs to support the body and walk; 2, inability to support the back of the body on the hindlimbs, with detectable ability to move the limbs; and 3, total paralysis of the hindlimbs. When righting reflex was impaired in the rats, motor blockade was not graded because limb paralysis was difficult to evaluate properly.

Histological Localization of PnO Microinjection Sites. On completion of the experiments, animals were deeply anesthetized with pentobarbital, and 1.0 μl of 2.5% bromophenol blue was injected at the stereotaxic target. After euthanasia with an overdose of pentobarbital, the brains were immediately soak-fixed in 10% neutral formaldehyde. The brains were serially sectioned into 0.3- to 0.5-mm coronal slices. The microinjection sites were histologically localized with the use of the atlas of Paxinos and Watson (1998). Furthermore, the sections that contained injection sites were embedded in paraffin and sectioned at 20-μm thickness. The sections were stained with Luxol fast blue. The target area was defined as the area of the PnO at the level between 8.3 and 8.8 mm posterior from the bregma.

Statistical Analysis. Data are presented as mean ± S.E. Differences in MAC and recovery time were compared using ANOVA. Tail-flick latency data were compared by two-way ANOVA for repeated measurements. Comparisons of motor blockade and righting reflex data were performed at each time point by χ² test. The Student-Neuman-Keuls procedure or Bonferroni’s correction was used for post hoc comparisons. Probability levels of <.05 were considered significant.

Results

The region where drugs were microinjected in the PnO is shown in Fig. 2. A typical microinjection site is shown in Fig. 2A, and a schematic drawing shows microinjection sites in the rats studied in the two representative coronal sections of rat brain stem (Fig. 2B).

The effects of carbachol and antagonist pretreatment on MAC are illustrated in Fig. 3. MAC of halothane was significantly reduced by direct application of carbachol into the PnO in a dose-dependent manner. MAC of halothane in the control group (saline injection after saline pretreatment) was 0.95 ± 0.05%, which is in a good agreement with previously reported MAC values (White et al., 1974; Ishizawa et al., 1997). Carbachol at 5.0 and 10.0 μg with saline pretreatment decreased MAC of halothane by 29 and 52%, respectively. Pretreatment with 4.0 and 12.0 μg atropine inhibited MAC reduction by 5.0 μg carbachol. Atropine at 12.0 μg inhibited the effect of 10.0 μg carbachol, but atropine at 4.0 μg did not, suggesting a dose-dependent inhibitory effect of atropine. Mecamylamine did not cause significant changes in the effects of carbachol on MAC.

The effects of carbachol and antagonist pretreatment on the recovery time from halothane are illustrated in Fig. 4.
Carbachol at 1.0 and 5.0 μg microinjected into the PnO significantly prolonged recovery time in a dose-dependent manner. Pretreatment with 4.0 μg of atropine or 1.0 μg of mecamylamine significantly reduced prolonged recovery time by carbachol. Mean respiratory rates during anesthesia and recovery and the number of total breaths taken to recover are shown in Table 1. Neither preinjected atropine nor mecamylamine showed significant effects on the respiratory rates during halothane anesthesia. During recovery, the groups of saline with atropine pretreatment and saline with mecamylamine pretreatment showed significantly higher respiratory rates than carbachol with saline pretreatment. Carbachol significantly increased the number of total breaths taken to recover in a dose-dependent manner.

Figure 5 shows the time course of the tail-flick latency change. Carbachol at 1.0 and 5.0 μg microinjected into the PnO significantly prolonged tail-flick latency. The maximum increase in tail-flick latency, expressed as MPE, occurred significantly prolonged tail-flick latency. The maximum in the rats administered 5.0 μg of carbachol than 1.0 μg at 30, 40, 50, and 60 min, suggesting a dose-dependent effect of carbachol. Pretreatment with 4.0 μg of atropine or 1.0 μg of mecamylamine inhibited tail-flick latency prolongation by carbachol. Figure 6 summarizes the effects of carbachol and its antagonists on motor blockade and righting reflex in unanesthetized rats. A small number of the rats administered 1.0 or 5.0 μg of atropine showed loss of righting reflex in unanesthetized rats. Most of the effects became undetectable within 30 min after injection. Except as described above, the behavior of the rats was unremarkable.

**Discussion**

**Cholinergic Mechanisms in Brain Stem and General Anesthesia.** The present data demonstrate that cholinomimetic injection into the pontine reticular formation modulates halothane anesthesia through cholinergic mechanisms. Directly administered carbachol into the PnO markedly reduced anesthetic requirements of halothane through a muscarinic receptor-mediated mechanism. Recovery time from halothane anesthesia was also prolonged by carbachol injection into the PnO. The effect was inhibited by both muscarinic and nicotinic antagonists. Because carbachol also caused significant increases in the number of total breaths taken to recover along with prolongation of the recovery time, pharmacokinetic explanation are unlikely to explain the prolonged recovery by carbachol. These results thus suggest that cholinomimetic injection into the PnO facilitates the cholinergic mechanisms in the brain that contribute to the anesthetic state.

Cholinergic modulation of anesthesia has been extensively studied in the past decades (Durieux, 1996). However, the findings, particularly those of cholinergic effects on MAC, have been controversial. The i.c.v. injection of atropine did not alter MAC of isoflurane, but the i.c.v. injection of pancuronium decreased MAC in rats (Zucker, 1991). The i.c.v. injection of nicotine showed a biphasic effect on MAC, increasing it at a low concentration and decreasing it at a high concentration (Zucker, 1991). Furthermore, physostigmine, an acetylcholinesterase inhibitor, has been considered to decrease the depth of anesthesia by increasing brain acetylcholine (Saalens et al., 1973; Romano and Shih, 1983). However, systemic administration of physostigmine decreased halothane MAC in rats but induced waking patterns on electroencephalography at the same time (Ishizawa et al., 1997). Physostigmine was shown to increase isoflurane MAC in rats in another study (Zucker, 1991). The explanation of these inconsistencies is not clear. However, most of these studies did not use pharmacological blocking experiments with selective antagonists. In addition, cholinergic agents such as physostigmine show dose-dependent changes in their action

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**TABLE 1**

Respiratory rates during halothane anesthesia and recovery and total breaths taken to recover

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Carbachol</th>
<th>RR Anesthesia</th>
<th>RR Recovery</th>
<th>Total Breaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>111 ± 8</td>
<td>141 ± 11</td>
<td>774 ± 43</td>
</tr>
<tr>
<td>Saline</td>
<td>1.0</td>
<td>115 ± 4</td>
<td>125 ± 6</td>
<td>1288 ± 85***</td>
</tr>
<tr>
<td>Saline</td>
<td>5.0</td>
<td>112 ± 9</td>
<td>118 ± 4</td>
<td>1783 ± 181***</td>
</tr>
<tr>
<td>Atropine 4.0</td>
<td>0</td>
<td>126 ± 5</td>
<td>158 ± 5*</td>
<td>812 ± 56</td>
</tr>
<tr>
<td>Atropine 4.0</td>
<td>1.0</td>
<td>107 ± 6</td>
<td>141 ± 8</td>
<td>735 ± 39</td>
</tr>
<tr>
<td>Atropine 4.0</td>
<td>5.0</td>
<td>113 ± 6</td>
<td>136 ± 7</td>
<td>852 ± 94</td>
</tr>
<tr>
<td>Mecamylamine 1.0</td>
<td>0</td>
<td>118 ± 5</td>
<td>154 ± 10*</td>
<td>811 ± 69</td>
</tr>
<tr>
<td>Mecamylamine 1.0</td>
<td>1.0</td>
<td>109 ± 9</td>
<td>133 ± 5</td>
<td>864 ± 145</td>
</tr>
<tr>
<td>Mecamylamine 1.0</td>
<td>5.0</td>
<td>113 ± 4</td>
<td>132 ± 5</td>
<td>914 ± 99</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.; n (trials) = 6 for each group.

a Mean respiratory rates during halothane anesthesia.

b Mean respiratory rates during recovery time.

c The number of total breaths taken to recover.

**P < .05 versus 1.0 and 5.0 μg of carbachol with saline pretreatment.

PP P <.05 versus any other group.

***P < .05 versus 1.0 μg of carbachol with saline pretreatment. All one-way ANOVA followed by Student-Neuman-Keuls test.
at multiple sites (Pleuvry and Tobias, 1971; Schultz et al., 1993), and thus the effects of physostigmine observed in these previous studies may not occur only through cholinergic mechanisms. Finally, it might be possible that cholinergic effects on anesthesia will vary depending on the anesthetic drug per se.

In the present study, atropine, a nonselective muscarinic antagonist, inhibited the effects of carbachol on MAC and those on recovery time. This is consistent with the previous reports that showed muscarinic receptors play an important role in determining the level of consciousness (Fibiger, 1991; Durieux, 1996). In particular, muscarinic receptors in the PnO play a key role in sleep and wake states (Imeri et al., 1994). The microinjection of the selective M2 antagonist methoctramine into the PnO enhanced wakefulness and decreased REM sleep and slow wave sleep, but M1 or M3 antagonists had no effect on sleep-wake cycles in rats (Imeri et al., 1994). Carbachol microinjection was further shown to activate G proteins through muscarinic receptors in the PnO (Capece et al., 1998). Autoradiographic studies have confirmed the localization of M1, M2, and M3 subtypes in the pontine reticular formation in rats (Baghdoyan, 1997). In fact, the PnO is a cholinceptive area and has been demonstrated to receive cholinergic projections from the laterodorsal tegmental nucleus (LDT) and pedunculopontine tegmental nucleus (PPT) in the brain stem (Semba et al., 1990; Semba, 1993). On the other hand, the functional significance of nicotinic receptors in the brainstem arousal system is less well defined. Nicotine microinjected in the pontine reticular formation was reported to increase REM sleep in cats (Velazquez-Moctezuma et al., 1990). The expression of α4 and β2 nicotinic acetylcholine receptor subunits has been demonstrated in the PnO in rats (Wada et al., 1989), which gives a neuroanatomical basis for the present finding that mecamylamine inhibited the effect of carbachol on recovery time. The present results may suggest that neuronal nicotinic receptors in the PnO also play a role in the state of general anesthesia.

The present study was designed to address the effects of directly administered cholinergic agents in the PnO on halothane anesthesia and to confirm the effects were cholinergically mediated in the PnO. However, the present study was not designed to show whether cholinergic agents directly interact with halothane within the PnO. It is well understood that cholinergic neurons in the brainstem project to the thalamus and higher structures and receive sensory afferent input from the spinal cord (Role and Kelly, 1991; Durieux, 1996). In fact, cholinergic neurons in the LDT/PPT have been demonstrated to have simultaneous projections to the thalamus and to the PnO in rats (Semba et al., 1990), suggesting that the effect of cholinomimetic injection in the PnO could have direct influence on neurons in the thalamus. Furthermore, the brainstem reticular formation is a complex of distinct as well as interacting neuronal regions (Role and Kelly, 1991; Steriade et al., 1993; Steriade, 1996; Coenen, 1998). The PnO receives various inputs from these reticular neurons, including noradrenergic afferents from the locus ceruleus and serotoninergic afferents from the raphe nuclei (Semba, 1993). The LDT/PPT cholinergic neurons also project to the locus ceruleus and the raphe nuclei (Semba and Fibiger, 1992). These neuroanatomical findings suggest that cholinergic, serotoninergic, and noradrenergic inputs may act convergently on the neurons in the PnO. Therefore, modulation of halothane anesthesia observed in the present study might be the consequence of interactions of cholinomi-

![Fig. 6. Changes in righting reflex and motor function after antagonist pretreatment and carbachol injection into the PnO. There was no statistically significant difference in the number of the rats that showed loss of righting reflex or motor impairment between groups at each time point (χ² test) (n = 6 trials). No rat was tested more than once under the same condition. □, loss of righting reflex; □, motor blockade grade 1; □, normal righting reflex, no motor blockade.](image-url)
metic injection with halothane at any level of the cholinergic network in the central nervous system.

Cholinergic Modulation of Antinociception. Carbachol microinjected into the PnO induced significant and consistent prolongation of tail-flick latency in unanesthetized rats. This effect was nearly completely abolished by pretreatment with atropine or mecamylamine. Although many reticular neurons in the brain stem are known to respond preferentially to noxious stimuli and the reticular formation receives afferent input through the spinoreticular tract (Willis and Westlund, 1997), neurotransmitters involved in these neuronal pathways and possible neuronal connections in the brain stem are not well understood. However, the present results confirmed that the antinociceptive effect of carbachol injection into the PnO was cholinergically mediated. A recent study also showed that cholinomimetics injected into the pontine reticular formation in cats increased tail-flick latency (Kshatri et al., 1998). These data together strongly suggest that the pontine reticular formation plays a role in supraspinal antinociceptive behavior. Nicotinic was previously reported to produce antinociception through both presynaptic nicotinic and postsynaptic muscarinic receptors in the PPT in rats (Iwamoto, 1989). Because cholinergic neurons in the PPT project to the PnO, carbachol injection into the PnO could elicit the same mechanism of antinociception as nicotine in the PPT.

In the present study, carbachol appeared to affect righting reflex in a small number of the rats tested. It is possible that the loss of righting reflex influenced the observed tail-flick latency prolongation if it was caused by generalized motor weakness. On the other hand, the observed motor blockade was very mild and therefore unlikely to cause significant prolongation of the tail-flick latency. In addition, the time course and consistency of the tail-flick response in the rats administered carbachol confirmed that contribution of the motor impairment to the tail-flick latency prolongation was minimal. However, further studies on neurobehavioral effects of cholinergic agent injection in the PnO will be needed to fully define a wide spectrum of the cholinergic roles in the brain stem reticular formation.

Limitations and Conclusions. In the present study, both muscarinic and nicotinic antagonists inhibited the effects of carbachol on recovery time as well as on antinociception. However, only the muscarinic antagonist showed reversibility to noxious stimuli and the reticular formation receives afferent input through the spinoreticular tract (Willis and Westlund, 1997), neurotransmitters involved in these neuronal pathways and possible neuronal connections in the brain stem are not well understood. However, the present results confirmed that the antinociceptive effect of carbachol injection into the PnO was cholinergically mediated. A recent study also showed that cholinomimetics injected into the pontine reticular formation in cats increased tail-flick latency (Kshatri et al., 1998). These data together strongly suggest that the pontine reticular formation plays a role in supraspinal antinociceptive behavior. Nicotinic was previously reported to produce antinociception through both presynaptic nicotinic and postsynaptic muscarinic receptors in the PPT in rats (Iwamoto, 1989). Because cholinergic neurons in the PPT project to the PnO, carbachol injection into the PnO could elicit the same mechanism of antinociception as nicotine in the PPT.

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