# Arecoline Excites Rat Locus Coeruleus Neurons by Activating the $M_2$-Muscarinic Receptor

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## Abstract

**The action of arecoline on rat locus coeruleus neurons was studied by intracellular recording from the in vitro brain slice preparation. Superfusion of arecoline (0.1-100 μM) caused two dose-related effects, an increased firing rate and, in neurons previously hyperpolarized to a constant potential by passing a steady hyperpolarizing current across the membrane, depolarization. Both effects were associated with a reduction in membrane input resistance. Moreover, the arecoline-induced excitatory effects were antagonized by the muscarinic receptor antagonist, atropine, but not by the nicotinic receptor antagonist, hexamethonium. Methoctramine, a selective $M_2$-muscarinic receptor antagonist, was also effective in reversing the arecoline-induced effects, with a dissociation equilibrium constant of 14.2±1.2 nM (n=6). These results therefore suggest that arecoline exerts its excitatory actions by binding to $M_2$-muscarinic receptors on the cell membrane of neurons of the locus coeruleus.**

**Key Words: arecoline, hexamethonium, atropine, methoctramine, locus coeruleus**

## Introduction

It is estimated that between 10 % and 25 % of the world's population chews betel (29). Betel chewing is as prevalent as the use of tobacco, and has an enormous influence on medical, social, and public health issues in Taiwan; however, the long-term health effects of continuous betel chewing remain controversial. Betel is said to produce feelings of euphoria and a mild state of intoxication similar to that produced by ethanol. Arecoline, a cholinergic alkaloid and a major constituent of betel nut, is responsible for the euphoric effects. It readily penetrates the blood-brain barrier (33) and is a nonselective muscarinic receptor agonist (23, 26, 34). It is reported to attenuate specific cholinergic cognitive impairment in both rodents and the marmoset (15, 28), improve age-related deficits in a delayed-recall task in primates (11), and enhance memory in healthy human subjects (36). Moreover, intravenous administration of arecoline reproducibly improves memory in some subjects with Alzheimer's disease (31, 32, 37). Recently, Raffaele et al. (30) reported that, in subjects with Alzheimer's disease, verbal ability tends to improve at low doses of arecoline, whereas attention and visuospatial ability tend to improve at higher doses of arecoline. Although arecoline is known to stimulate muscarinic cholinergic activity, its cellular mechanism or the site of action on the central nervous system involved in improving cognitive function has not yet been entirely elucidated.

The central norepinephrine system appears to play an important role in cognitive function. The locus coeruleus (LC) is a compact group of norepinephrine-containing cell bodies located near the floor of the fourth ventricle at the upper border of the pons, and neurons from this small pontine nucleus give rise to more than half the noradrenergic projections in the brain (4). The widespread nature of the coeruleo-cortical projection indicates that

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Received February 1, 2000; Revised February 19, 2000; Accepted February 20, 2000.

activation of this noradrenergic cell group will have pervasive effects in diverse terminal regions. Thus, it is perhaps no surprise that LC neurons may play a key role in the regulation of vigilance, attention, learning, memory, and anxiety, and are affected in neurodegenerative disorders (35). Additionally, it has been demonstrated that the LC receives cholinergic input from cells in the neighboring pontine reticular formation (pedunculopontine tegmental nucleus and laterodorsal tegmental nucleus, see ref. 5) and that the LC is rich in acetylcholinesterase (3). The noradrenergic neurons of the LC contain both nicotinic and muscarinic receptors (18, 21). Electrophysiological studies indicate that cholinergic agonists, acting via nicotinic and/or muscarinic receptors, markedly increase the firing rate of LC neurons (1, 21). The aims of the present study were therefore to investigate the effects of arecoline on LC neurons, using intracellular recording techniques, and to characterize the receptor subtype involved in the arecoline-induced effects.

## Materials and Methods

*Preparation and Maintenance of LC Slices*

The methods used to prepare and maintain LC slices from the rat were similar to those previously described (17, 40, 41). Male Sprague-Dawley rats (120-200 g) were sacrificed and their brains rapidly removed. A block of tissue containing the pons was excised and attached to a small Plexiglass stage with cyanoacrylate glue, an agar block, placed next to the tissue, acting as a support during sectioning. The tissue was then submerged in oxygenated artificial cerebrospinal fluid (aCSF), maintained at 3-5°C, in the well of a Lancer 1000 vibratome. Several 300-350 μm thick coronal sections of pons were cut, then a slice containing a cross-section through the caudal end of the LC was mounted in the recording chamber and allowed to equilibrate for 1 hr. The slice was then completely submerged in a heated (33-34°C) flowing (2.3 ml/min) solution with the following millimolar composition : NaCl 126, KCl 2.5, $NaH_2PO_4$ 1.2, $NaHCO_3$ 26.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, glucose 11.1, gassed with 95% $O_2$/5% $CO_2$. The slice was viewed from above using a dissection microscope; in the trans-illuminated slice, the LC was seen as a translucent area lying on the lateral aspect of the periventricular gray, below the fourth ventricle.

*Intracellular Recording*

Intracellular recording from LC neurons was performed using sharp microelectrodes, filled with 2M KCl, with a d.c. tip resistance of 40-70 MΩ. The recording microelectrodes were inserted into the LC under visual control. Intracellular potentials were recorded using an amplifier with an active bridge circuit, permitting current injection through the recording electrode (WPI M707). Current and voltage traces were displayed on a storage oscilloscope (Textronix 5113) and a rectilinear pen recorder (Gould 2400). Input resistance was measured by passing hyperpolarizing constant current pulses of sufficient duration to fully charge the membrane capacitance and reach a steady-state voltage deflection.

*Perfusion of Solutions and Drugs*

A valve system was employed for switching the solution superfusing the preparation between control and drug-containing aCSF. The period required for test solutions to reach the chamber was known, and ranged from 25-35 sec. The drugs used in the present experiments were arecoline, hexamethonium bromide, atropine (Sigma), and methoctramine (Research Biochemicals Inc.) and were administered for a long enough duration to attain a steady-state response (5 min or longer).

*Data Analysis*

Numerical data are expressed as the mean±the standard error of the mean (S.E.M.). Paired or unpaired Student's *t* tests were used to determine whether the differences in the means were statistically significant. p-Values equal to, or less than, 0.05 were judged to be statistically significant.

## Results

*Membrane Properties of LC Neurons*

Electrophysiological properties were examined in a total of 88 LC neurons by stable intracellular impalement. All the LC neurons included in this study showed spontaneous activity, the frequency of spontaneous firing ranging from 0.2 to 4.2 Hz (1.9± 0.1 Hz, n=88). The pattern of LC neuron spontaneous firing recorded in each slice was very regular, i.e. the interspike interval was remarkably uniform. The neurons had resting membrane potentials of -49 to -70 mV (-57.8±0.5 mV, n=88) and apparent input resistances of 125 to 416 MΩ(184±7 MΩ, n=88). These data are similar to those obtained in our previous studies (17, 40, 41).

*Effects of Arecoline*

As shown in Fig. 1 (A & B), arecoline (0.1-100 μM) reversibly increased the firing rate and decreased

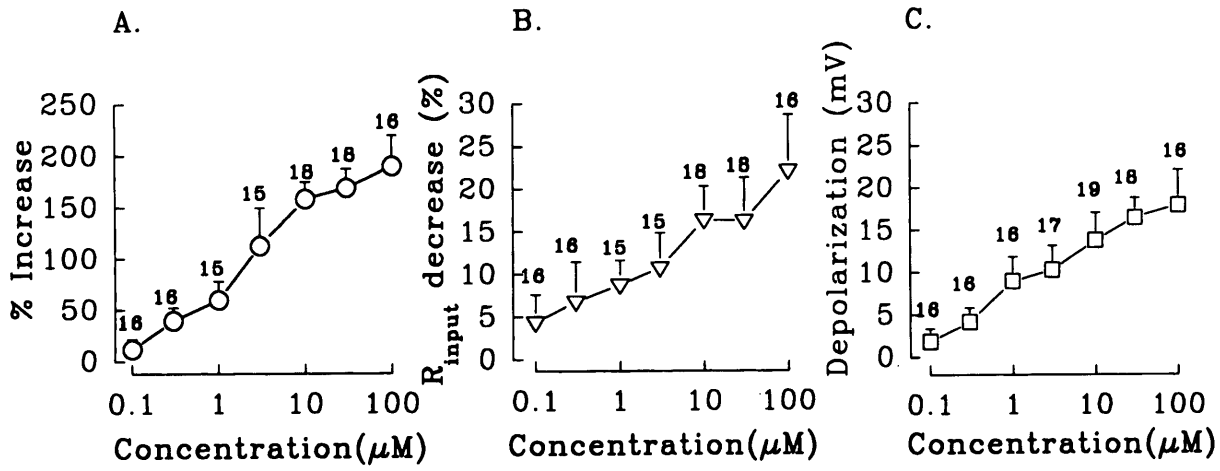


Fig. 1. Dose-dependent effects of arecoline on the firing rate (A), input resistance (B), and membrane potential (C) of neurons of the locus coeruleus. In (C), constant current was passed through the electrode to hyperpolarize the neurons by about 25 mV. The vertical bars represent the S.E.M. for the number of neurons indicated.

the input resistance of all LC neurons tested. Although neurons varied in their sensitivity to arecoline, the increase in firing rate and reduction in input resistance depended on the concentration. At concentrations of 0.1 or 1 μM arecoline caused an increase in neuronal firing rate of 11.8 % (n=16) or 59.7 % (n=15), respectively, with only a small reduction in input resistance, while, at higher concentrations (10 or 100 μM), a greater increase in firing rate was seen, together with a decrease in input resistance. At a given concentration of arecoline, considerable inter-neuron variation was seen in the increase in firing and decrease in input resistance. For example, in response to 100 μM arecoline, the increase in firing rate and decrease in input resistance ranged from 159.1 to 225.9 % (190.3±30.9 %, n=16) and from 15.7 to 29.3 % (22.2±6.5 %, n=16), respectively. In another set of experiments, shown in Figure 1C, in cells previously hyperpolarized to a constant potential (-70 – -75 mV) by passing a steady hyperpolarizing current across the membrane, arecoline caused a concentration-dependent depolarization, which was accompanied by a fall in input resistance (not shown).

**Table 1. Effects of Nicotinic and Muscarinic Receptor Antagonists on Arecoline-Induced Changes in Firing Rate and Input Resistance**

| | n | Increase in firing rate (%) | Decrease in $R_{input}$ (%) |
|---|---|---|---|
| Arecoline (0.3 μM) | 6 | 38.5±4.7 | 6.8±2.3 |
| Arecoline (0.3 μM) + Hexamethonium (400 μM) | 6 | 36.7±3.1 | 7.1±1.2 |
| | | p > 0.05 | p > 0.05 |
| Arecoline (100 μM) | 8 | 202.2±24.5 | 24.1±3.0 |
| Arecoline (100 μM) + Atropine (10 nM) | 8 | 3.1±0.8 | 0.7±0.1 |
| | | p < 0.005 | p < 0.005 |
| Arecoline (0.3 μM) | 6 | 36.9±6.7 | 7.8±2.6 |
| Arecoline (0.3 μM) + Methoctramine (30 μM) | 6 | 2.1±0.7 | 1.2±0.3 |
| | | p < 0.005 | p < 0.005 |

$R_{input}$: Input resistance

*Interaction of Arecoline with Atropine and Hexamethonium*

To study the effects of a muscarinic receptor antagonist (atropine) and a nicotinic receptor antagonist (hexamethonium), a single concentration of atropine (10 nM) or hexamethonium (400 μM) was first applied for 15 minutes before the perfusate was changed to atropine (10 nM) plus arecoline (100 μM) or hexamethonium (400 μM) plus arecoline (0.3 μM). Atropine alone had no significant effect on firing rate, membrane potential, or input resistance, but drastically modified the arecoline-induced responses (Tables 1 and 2). On average, atropine (10 nM) produced a 98.5 % and 97.1 %, respectively, reduction in the increase in firing rate and decrease in input resistance induced by arecoline (100 μM). In cells previously hyperpolarized by about 25 mV, atropine reduced the arecoline-induced depolarization and decrease in input resistance by 92.7 % and 96.6 %, respectively. In contrast, the excitatory effects resulting from

**Table 2. Effects of Nicotinic and Muscarinic Receptor Antagonists on Arecoline-Induced Changes in Membrane Potential and Input Resistance While Passing a Steady Hyperpolarizing Current to Hyperpolarize the Neurons by about 25 mV**

| | n | Depolarization (mV) | Decrease in $R_{input}$ (%) |
|---|---|---|---|
| Arecoline (0.3 μM) | 6 | 4.5±1.3 | 6.9±1.4 |
| Arecoline (0.3 μM) + Hexamethonium (400 μM) | 6 | 4.1±1.8 | 5.7±1.5 |
| | | p > 0.05 | p > 0.05 |
| Arecoline (100 μM) | 6 | 16.5±2.7 | 20.8±2.1 |
| Arecoline (100 μM) + Atropine (10 nM) | 6 | 1.2±0.3 | 0.7±0.1 |
| | | p < 0.005 | p < 0.005 |
| Arecoline (0.3 μM) | 8 | 4.2±1.2 | 6.3±1.6 |
| Arecoline (0.3 μM) + Methoctramine (30 μM) | 8 | 0.3±0.1 | 0.8±0.1 |
| | | p < 0.005 | p < 0.005 |

$R_{input}$: Input resistance

superfusion of arecoline (0.3 μM) were not significantly affected by concurrent superfusion of hexamethonium (400 μM) (Tables 1 and 2).

*Effect of Methoctramine on the Arecoline-Evoked Excitatory Responses*

In this study, the receptor subtype involved in the arecoline-induced effects was characterized by the use of a selective $M_2$-muscarinic receptor antagonist, methoctramine. Pretreatment with methoctramine (30 μM) before applying a mixture of methoctramine (30 μM) plus arecoline (0.3 μM) resulted in almost complete block of the effects of arecoline on firing rate, membrane potential, and input resistance (Tables 1 and 2). In another six neurons, the concentration-response relationship for arecoline, constructed in the presence or absence of increasing methoctramine concentrations while recording from a single neuron, was used to determine the dissociation equilibrium constant ($K_d$) for the antagonist; an example is shown in Fig. 2. Methoctramine (30-300 nM) produced a parallel, dose-related shift to the right of the arecoline concentration-response curve (Fig. 2A). To determine the $K_d$ for methoctramine, we calculated the dose-ratios by dividing the concentration of arecoline required to

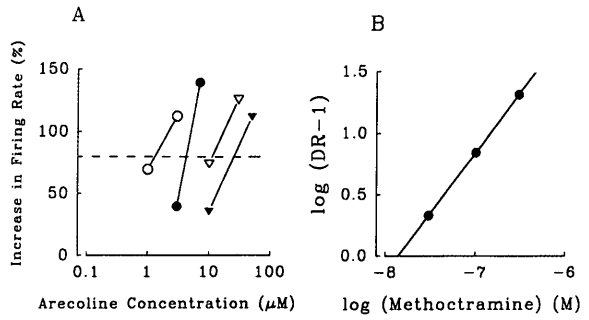


Fig. 2. Methoctramine antagonism of the arecoline-induced increase in firing rate. (A) Dose-response curves for arecoline in the presence of 0 (○), 30 (●), 100 (▽), or 300 nM (▼) methoctramine. The data were obtained by recording from a single neuron. (B) Schild plot of the data from (A) at a response level of 80 %. The dissociation equilibrium constant was 14.1 nM in this neuron.

produce a given increase in firing rate in the presence of methoctramine by that required to produce the same increase in firing rate in the absence of methoctramine; these dose-ratios were then used to construct the Schild plots (Fig. 2B). These experiments yielded a $K_d$ value of 14.2±0.9 nM (n=6) for methoctramine antagonism of the arecoline-induced activation.

## Discussion

Our present investigation provides evidence that, in LC neurons, arecoline can increase the spontaneous firing rate, reduce the input resistance, and depolarize the membrane potential. Previous studies on animals or using brain slice preparations have shown that neurons in the LC are excited by cholinergic agonists (1, 20, 21). Using electrophysiological methods, arecoline has been shown to induce dose-dependent depolarization of the fast coxal depressor motor neuron in the metathoracic ganglion of the cockroach, Periplaneta americana (9). Kurihara et al. (24) also demonstrated that bath-applied arecoline produced inhibition of the mono-synaptic reflex and depolarization of motoneurons. The present results are compatible with these findings. The functional significance of the excitatory effects would be to decrease the threshold of impulse initiation and thus to increase the probability of a cell discharge in response to a given excitatory synaptic input. Furthermore, the LC is the major norepinephrine-containing cell group in the central nervous system (22) and has been implicated in attention, learning, and memory processes (35). Electrophysiological and behavioral studies have shown that corticotropin-releasing factor or yohimbine increases the firing rate of LC neurons (2, 38) and significantly improves retention of the passive avoidance responses in rats

after infusion into the LC (16). Our present results show that arecoline has a similar effect in exciting LC neurons. Several studies have shown that arecoline may improve cognitive function (see Introduction section). Taken together, the present data imply that the effect of arecoline to improve cognitive function is probably mediated in part through LC activation. However, more studies are required to prove this.

Regarding the receptor subtype involved in the arecoline-induced effects, autoradiographic and electrophysiological studies have shown that LC neurons possess both muscarinic and nicotinic receptors (18, 21). In the present investigation the receptor subtype involved in the action of arecoline on rat LC neurons was characterized. The effect is mediated through muscarinic receptors, since the excitatory effect of arecoline on LC neurons was blocked by atropine, a muscarinic receptor antagonist, but not by hexamethonium, a nicotinic receptor antagonist. Three distinct muscarinic receptor subtypes, $M_1$, $M_2$, and $M_3$, have been identified by functional and binding studies (13), and cloning studies have identified five genetically distinct muscarinic receptor subtypes, $m_1$-$m_5$ (12). The antagonist binding properties and tissue distribution of the cloned $m_1$, $m_2$, and $m_3$ receptor proteins correspond closely to those of the pharmacologically defined subtypes, $M_1$, $M_2$, and $M_3$ (14, 25). This study showed that the selective $M_2$-muscarinic receptor antagonist, methoctramine, was able to cause almost complete block of the excitatory effect of arecoline on LC neurons, with a dissociation equilibrium constant of about 14.2 nM, consistent with the previously described methoctramine $K_d$ range for the $M_2$ subtype (4-47 nM) (19, 27). Based on our findings, it is reasonable to conclude that the muscarinic receptor mediating the excitatory effects of arecoline in these LC neurons is the $M_2$ subtype, although we do not definititely rule out the involvement of other muscarinic receptor subtypes. This is in agreement with results obtained in previous studies. Using an in vitro autoradiographic technique, the rat and cat pons, including the LC, was found to contain a relatively high density of $M_2$ binding sites (7, 8). Vanderheyden et al. (39) also indicated that the majority of $M_2$-type muscarinic receptors are found in the pons. In addition, an electrophysiological study has revealed that excitation of LC neurons by the muscarinic receptor agonists, muscarine, methylfurmethide, and oxotremorine, is mediated by the $M_2$-type muscarinic receptor (20).

In conclusion, our data suggest that arecoline binds to $M_2$-muscarinic receptors on the cell membrane of LC neurons, resulting in the observed excitatory effects. Furthermore, based on the present and previous findings, it is reasonable to hypothesize that arecoline, by activating LC neurons, increases their firing rate and consequently increases norepinephrine release from axon terminals in diverse regions such as the neocortex, hippocampus, and amygdala, thus improving cognitive functions. Recently, several studies have been designed to investigate the clinical pharmacokinetics and therapeutic efficacy of arecoline in subjects with Alzheimer's disease (6, 10, 30). We believe that the data from the present investigation could provide important information for the design of further studies aimed at understanding the mechanism of cognitive enhancement by arecoline in patients with Alzheimer's disease.

## Acknowledgements

This work was supported in part by the National Science Council (to T. H. C.),Repubic of China.

## References

1. Adams, L. M. and Foote, S. L. Effects of locally infused pharmacological agents on spontaneous and sensory-evoked activity of locus coeruleus neurons. *Brain Res. Bull.* 21: 395-400, 1988.
2. Aghajanian, G. K., Cedarbaum, J. M. and Wang, R. Y. Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. *Brain Res.* 136: 570-577, 1977.
3. Albanese, A. and Butcher, L. L. Acetylcholinesterase and catecholamine distribution in the locus coeruleus of the rat. *Brain Res. Bull.* 5: 127-134, 1980.
4. Amaral, D. G. and Sinnamon, H. M. The locus coeruleus: neurobiology of a central noradrenergic nucleus. *Prog. Neurobiol.* 9: 147-196, 1977.
5. Armstrong, D. M., Saper, C. B., Levey, A. I., Wainer, B. H. and Terry, R. D. Distribution of cholinergic neurons in rat brain: demonstrated by the immunocytochemical localization of choline acetyltransferase. *J. Comp. Neurol.* 216: 53-68, 1983.
6. Asthana, S., Greig, N. H., Holloway, H. W., Raffaele, K. C., Berardi, A., Schapiro, M. B., Rapoport, S. I. and Soncrant, T. T. Clinical pharmacokinetics of arecoline in subjects with Alzheimer's disease. *Clin. Pharmacol. Ther.* 60: 276-282, 1996.
7. Baghdoyan, H. A. Location and quantification of muscarinic receptor subtypes in rat pons: implications for REM sleep generation. *Am. J. Physiol.* 273: R896-904, 1997.
8. Baghdoyan, H. A., Mallios, V. J., Duckrow, R. B. and Mash, D. C. Localization of muscarinic receptor subtypes in brain stem areas regulating sleep. *Neuroreport* 5: 1631-1634, 1994.
9. Bai, D. and Sattelle, D. B. Muscarinic acetylcholine receptors on an identified motor neurone in the cockroach, Periplaneta americana. *Neurosci. Lett.* 175: 161-165, 1994.
10. Bartolomeo, A. C., Morris, H. and Boast, C. A. Arecoline via miniosmotic pump improves AF64A-impaired radial maze performance in rats: a possible model of Alzheimer's disease. *Neurobiol. Learn. Mem.* 68: 333-342, 1997.
11. Bartus, R. T., Dean, R. L. and Beer, B. Memory deficits in aged cebus monkeys and facilitation with central cholinomimetics. *Neurobiol. Aging* 1: 145-152, 1980.
12. Bonner, T. I. The molecular basis of muscarinic receptor diversity. *Trends Neurosci.* 12: 148-151, 1989.
13. Bonner, T. I. New subtypes of muscarinic acetylcholine receptors. *Trends Pharmacol. Sci. Suppl.*: 11-15, 1989.
14. Buckley, N. J., Bonner, T. I., Buckley, C. M. and Brann, M. R. Antagonist binding properties of five cloned muscarinic receptors

expressed in CHO-K1 cells. *Mol. Pharmacol.* 35: 469-476, 1989.
15. Carey, G. J., Costall, B., Domeney, A. M., Gerrard, P. A., Jones, T. N. C., Naylor, R. J. and Tyers, M. B. Ondansetron and arecoline prevent scopolamine-induced cognitive deficits in the marmoset. *Pharmacol. Biochem. Behav.* 42: 75-83, 1992.
16. Chen, M. F., Chiu, T. H. and Lee, E. H. Y. Noradrenergic mediation of the memory-enhancing effect of corticotropin-releasing factor in the locus coeruleus of rats. *Psychoneuroendocrinology* 17: 113-124, 1992.
17. Chiu, T. H., Chen, M. J., Yang, Y. R., Yang, J. J. and Tang, F. I. Action of dexmedetomidine on rat locus coeruleus neurones: intracellular recording in vitro. *Eur. J. Pharmacol.* 285: 261-268, 1995.
18. Cortes, R., Probst, A. and Palacios, J. M. Quantitative light microscopic autoradiographic localization of cholinergic muscarinic receptors in the human brain: brainstem. *Neuroscience* 12: 1003-1026, 1984.
19. Dorje, F., Wess, J., Lambrecht, G., Tacke, R., Mutschler, E. and Brann, M. R. Antagonist binding profiles of five cloned human muscarinic receptor subtypes. *J. Pharmacol. Exp. Ther.* 256: 727-733, 1991.
20. Egan, T. M. and North, R. A. Acetylcholine acts on $m_2$-muscarinic receptors to excite rat locus coeruleus neurones. *Br. J. Pharmacol.* 85: 733-735, 1985.
21. Egan, T. M. and North, R. A. Actions of acetylcholine and nicotine on rat locus coeruleus neurons in vitro. *Neuroscience* 19: 565-571, 1986.
22. Foote, S. L., Bloom, F. E. and Aston-Jones, G. Nucleus locus coeruleus: new evidence of anatomical and physiological specificity. *Physiol. Rev.* 63: 844-914, 1983.
23. Jaiswal, N., Lambrecht, G., Mutschler, E., Tacke, R. and Malik, K. U. Pharmacological characterization of the vascular muscarinic receptors mediating relaxation and contraction in rabbit aorta. *J. Pharmacol. Exp. Ther.* 258: 842-850, 1991.
24. Kurihara, T., Suzuki, H., Yanagisawa, M. and Yoshioka, K. Muscarinic excitatory and inhibitory mechanisms involved in afferent fiber-evoked depolarization of motoneurones in the neonatal rat spinal cord. *Br. J. Pharmacol.* 110: 61-70, 1993.
25. Maeda, A., Kubo, T., Mishina, M. and Numa, S. Tissue distribution of mRNAs encoding muscarinic acetylcholine receptor subtypes. *FEBS Lett.* 239: 339-342, 1988.
26. McKinney, M., Miller, J. H., Gibson, V. A., Nickelson, L. and Aksoy, S. Interactions of agonists with $M_2$ and $M_4$ muscarinic receptor subtypes mediating cyclic AMP inhibition. *Mol. Pharmacol.* 40: 1014-1022, 1991.
27. Melchiorre, C., Angeli, P., Lambrecht, G., Mutschler, E., Picchio, M. T. and Wess, J. Antimuscarinic action of methoctramine, a new cardioselective $M_2$ muscarinic receptor antagonist, alone and in combination with atropine and gallamine. *Eur. J. Pharmacol.* 144: 117-124, 1987.
28. Nakamura, S., Tani, Y., Maezono, Y., Ishihara, T. and Ohno, T. Learning deficits after unilateral AF64A lesions in the rat basal forebrain: role of cholinergic and noncholinergic systems. *Pharmacol. Biochem. Behav.* 42: 119-130, 1992.
29. Pickwell, S. M., Schimelpfening, S. and Palinkas, L. A. 'Betelmania'. Betel quid chewing by Cambodian women in the United States and its potential health effects. *Western. J. Med.* 160: 326-330, 1994.
30. Raffaele, K. C., Asthana, S., Berardi, A., Haxby, J. V., Morris, P. P., Schapiro, M. B. and Soncrant, T. T. Differential response to the cholinergic agonist arecoline among different cognitive modalities in Alzheimer's disease. *Neuropsychopharmacology* 15: 163-170, 1996.
31. Raffaele, K. C., Berardi, A., Asthana, S., Morris, P. P., Haxby, J. V. and Soncrant, T. T. Effects of long-term continuous infusion of the muscarinic cholinergic agonist arecoline on verbal memory in dementia of the Alzheimer type. *Psychopharmacol. Bull.* 27: 315-319, 1991.
32. Raffaele, K. C., Berardi, A., Morris, P. P., Asthana, S., Haxby, J. V., Schapiro, M. B., Rapoport, S. I. and Soncrant, T. T. Effects of acute infusion of the muscarinic cholinergic agonist arecoline on verbal memory and visuo-spatial function in dementia of the Alzheimer type. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 15: 643-648, 1991.
33. Rapoport, S. I., Ohno, K. and Pettigrew, K. D. Drug entry into the brain. *Brain Res.* 172: 354-359, 1979.
34. Richards, M. H. Relative potencies of agonists and differential sensitivity to N-ethylmaleimide on muscarinic autoreceptors and postsynaptic receptors in rat hippocampus. *J. Pharmacol. Exp. Ther.* 255: 83-89, 1990.
35. Robbins, T. W. and Everitt, B. J. Central norepinephrine neurons and behavior. In: Psychopharmacology: The Fourth Generation of Progress, edited by F. E. Bloom, and D. J. Kupfer. New York, NY: Raven Press, 1995, pp. 363-372.
36. Sitaram, N., Weingartner, H. and Gillin, J. C. Human serial learning: enhancement with arecoline and choline impairment with scopolamine. *Science* 201: 274-276, 1978.
37. Soncrant, T. T., Raffaele, K. C., Asthana, S., Berardi, A., Morris, P. P. and Haxby, J. V. Memory improvement without toxicity during chronic, low dose intravenous arecoline in Alzheimer's disease. *Psychopharmacology* 112: 421-427, 1993.
38. Valentino, R. J., Foote, S. L. and Aston-Jones, G. Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. *Brain Res.* 270: 363-367, 1983.
39. Vanderheyden, P., Gies, J. P., Ebinger, G., De Keyser, J., Landry, Y. and Vauquelin, G. Human $M_1$-, $M_2$- and $M_3$-muscarinic cholinergic receptors: binding characteristics of agonists and antagonists. *J. Neurol. Sci.* 97: 67-80, 1990.
40. Yang, Y. R. and Chiu, T. H. Opioid and antiopioid actions of Tyr-MIF-1, Tyr-W-MIF-1 and hemorphin-4 on rat locus coeruleus neurons: intracellular recording *in vitro*. *Chin. J. Physiol.* 40: 131-135, 1997.
41. Yang, Y. R., Lee, E. H. Y. and Chiu, T. H. Electrophysiological and behavioral effects of Tyr-D-Arg-Phe-Sar on locus coeruleus neurons of the rat. *Eur. J. Pharmacol.* 351: 23-30, 1998.