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Short communication

Activation of 5-HT_{1A} receptors potentiates the clonidine inhibitory effect in the locus coeruleus

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Abstract

Using in vivo extracellular recordings, we have examined the effect of the application of the prototypical 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), on the firing rate of locus coeruleus neurons. 8-OH-DPAT (1 μg/kg, i.v.) did not modify the basal activity of the locus coeruleus but shifted to the left the dose–response curve for the clonidine induced inhibition of firing rate and reduced the corresponding ED_{50} by 77%. 2-[2-[4-(*o*-methoxyphenyl)piperazin-1-yl]ethyl]-4,4-dimethyl-1,3(2H,4H)-isoquinolinedione (ARC 239; 75 μg/kg, i.v.), and chlorpromazine (75 μg/kg, i.v.) also shifted to the left the dose–response curve for clonidine and reduced by 38 and 46%, respectively, the ED_{50}, while slightly increasing the basal firing rate. The results indicated that 5-HT_{1A} receptors may modulate the responses mediated by α_{2A}-adrenoceptors in the locus coeruleus. © 1997 Elsevier Science B.V.

Keywords: Locus coeruleus; α_{2A}-Adrenoceptor; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)-tetralin); ARC 239 (2-[2-[4-(*o*-methoxyphenyl)piperazin-1-yl]ethyl]-4,4-dimethyl-1,3(2H,4H)-isoquinolinedione); Chlorpromazine

1. Introduction

The locus coeruleus, the principal noradrenergic cell group in the brain, contains tyrosine and tryptophane hydroxylases (Pickel et al., 1977) and a high density of 5-HT_{1A} binding and immunoreactive sites (Weissmann-Nanopoulos et al., 1985; Azmitia et al., 1996). In addition, it is densely innervated by serotonergic fibers and terminals (Imai et al., 1986), most probably coming from pericoerulear 5-HT neurons (Aston-Jones et al., 1991b). Administration of 5-HT to the locus coeruleus produces varied effects on its spontaneous discharge. Thus, a long lasting suppression of cell activity (Segal, 1979) and the absence of any clear effects (Aston-Jones et al., 1991a; Haddjeri et al., 1997; Koyama and Kayama, 1993) have been observed. However, 5-HT receptor agonists inhibit the depolarizing synaptic potentials of locus coeruleus cells in vitro with 5-HT_{1A} receptors mediating in part this effect (Bobker and Williams, 1989). 5-HT plays a role in modulating the electrophysiological responses of the locus coeruleus. For example, locus coeruleus neuron activation by noxious stimulation (Segal, 1979; Aston-Jones et al., 1991a) and by excitatory amino acids is attenuated by 5-HT (Shiekhattar and Aston-Jones, 1993), the 5-HT_{1A} receptor being involved in these modulatory effects (Aston-Jones et al., 1991a).

Binding studies on the rat brain have revealed that the $\alpha_{2B/C}$-adrenoceptor antagonist 2-[2-[4-(*o*-methoxyphenyl)-piperazin-1-yl]ethyl]-4,4-dimethyl-1,3(2H,4H)-isoquinolinedione (ARC 239), which is considered a good tool to study $\alpha_{2B/C}$-adrenoceptors (Uhlen and Wikberg, 1991), binds to 5-HT_{1A} receptors (Meana et al., 1996). The antipsychotic and α_2-adrenoceptor agonist agent, chlorpromazine, used to characterize the $\alpha_{2B/C}$-adrenoceptor subtype (Bylund et al., 1988), also binds to 5-HT receptors (Roth and Meltzer, 1995). These abilities to bind 5-HT sites should be taken into account when α_2-adrenoceptor subtypes are studied.

The aims of this study were to elucidate if activation of the 5-HT system modulates the basal activity and the α_2-adrenoceptor-mediated effects in the locus coeruleus and to determine which receptor mediated the effects of ARC 239 and chlorpromazine in the locus coeruleus.

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2. Materials and methods

Male albino Sprague–Dawley rats weighing 200–300 g were anesthetized with choral hydrate (400 mg/kg i.p.). Subsequently, a cannula was inserted into the trachea and the right jugular vein was cannulated for additional injections of anesthetic and drugs. The rat was placed in a stereotaxic frame with the head oriented at a 15° angle to the horizontal plane (nose down). Body temperature was maintained at 37°C with a heating pad.

Procedures for single-extracellular recording from locus coeruleus cells were carried out as described previously (Pineda et al., 1993). Individual neuronal spikes were fed into a computer to generate interspike time interval histograms. The computer was programmed to recognize a burst onset when there occurred an interspike interval shorter than 80 ms, and a burst termination at the next interval exceeding 160 ms. Burst firing was quantified by the burst percentage, i.e. the ratio between spikes in bursts and total number of spikes of an interspike time interval histogram. The variation coefficient of the interspike time interval histograms was the ratio between the standard deviation and the mean interval value of an interspike time interval histogram, expressed as a percentage and was employed to study the regularity of the cell firing pattern.

Changes in firing rate are expressed as percentages of the baseline firing rate and were measured after administration of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), ARC 239 or chlorpromazine. In some experiments 5-HT was depleted by administration of the 5-HT synthesis inhibitor, *p*-chlorophenylalanine (400 mg/kg, 24 h before the experiment, i.v.). Clonidine (0.08–20 μg/kg) was injected at 1 min intervals, in doubling doses. Dose–effect curves for the inhibition of locus coeruleus cells were analyzed using the non-linear least squares fitting program, GraFit. Experimental data in each group were pooled and analyzed for the best, simple non-linear fit to a standard three-parameter equation (Parker and Waud, 1971): $E = E_{max}[A]^n/(ED_{50}^n + [A]^n)$, where $[A]$ is the i.v. dose of the drug and E is the effect; E_{max} is the maximal percentage change at 'infinite' dose (100%); ED_{50} is the effective dose for eliciting 50% of E_{max}; n is the slope factor. The S.E. values obtained by non-linear regression were not used in further formal statistical calculations. Dose–effect curve parameters obtained under different experimental conditions were compared by determining the goodness of fit to a model, with and without a set of constraints, by means of an *F*-test (Ratkowsky, 1983).

Basal firing rates are given as means ± S.E.M. Statistical evaluation was made by means of the Student's paired *t*-test or the Wilcoxon test. The level of significance was chosen as $P < 0.05$.

3. Results

All locus coeruleus cells recorded had a discharge rate of 0.5–5 Hz and a firing pattern consisting mainly of single spikes and less frequently of bursts. 8-OH-DPAT (1 μg/kg, i.v.) did not modify this basal activity (Table 1). ARC 239 (75 μg/kg, i.v.) and chlorpromazine (75 μg/kg, i.v.) induced a slight increase of 11 ± 5% ($n = 20$, $P < 0.05$) and of 16 ± 6% ($n = 11$, $P < 0.05$) respectively in the firing rate, while they did not induce any change in the degree of burst or pattern of discharge (Table 1).

Dose–response curves for the clonidine-induced inhibition of the firing rate were obtained, for control rats ($ED_{50} = 2.79$ μg/kg, $n = 9$) and for experimental rats 3–5 min after administration of 8-OH-DPAT, ARC 239 and chlorpromazine, when the firing discharge of the cell had stabilized. 8-OH-DPAT (1 μg/kg, i.v.) caused potentiation of the clonidine-induced inhibition of locus coeruleus neurons ($ED_{50} = 0.64$ μg/kg, $n = 5$; $P < 0.05$) and shifted the dose–effect curve to the left (Fig. 1A). This effect had not been observed after depletion of 5-HT by *p*-chlorophenylalanine administration (400 mg/kg, 24 h before the experiment, i.v.) ($ED_{50} = 3.14$ μg/kg, $n = 4$). Similarly, ARC 239 (75 μg/kg, i.v.) and chlorpromazine (75 μg/kg, i.v.) potentiated the clonidine-induced inhibition of locus coeruleus neurons ($ED_{50} = 1.74$ μg/kg, $n = 6$, $P < 0.05$ and $ED_{50} = 1.55$ μg/kg, $n = 5$, $P < 0.05$,

Table 1
Parameters of locus coeruleus cell activity before and after a single intravenous dose of 8-OH-DPAT, ARC 239 or chlorpromazine

Drug	Basal			Effect			*n*
	Firing rate (Hz)	Variation coefficient (%)	Bursts (%)	Firing rate (hz)	Variation coefficient (%)	Bursts (%)	
8-OH-DPAT (1 μg/kg)	1.91 ± 0.56	41.91 ± 0.56	1.28 ± 0.57	2.06 ± 0.60	39.08 ± 5.5	1.91 ± 0.56	5
ARC 239 (75 μg/kg)	2.28 ± 1.11	43.61 ± 1.72	2.16 ± 0.72	2.54 ± 1.11 *	44.33 ± 2.08	2.62 ± 0.77	20
Chlorpromazine (75 μg/kg)	1.55 ± 0.15	39.08 ± 1.7	1.11 ± 0.57	1.80 ± 0.20 *	39.00 ± 5.58	0.53 ± 0.33	11

Parameters of locus coeruleus neurons were obtained from 3–5 interspike time interval histograms samples before and after drug administration. Each value represents the mean ± S.E.M. of *n* cells per group. Student's paired *t*-test, or the Wilcoxon test in the case of burst values, were employed to evaluate statistical significance. The level of significance was chosen as $P < 0.05$.

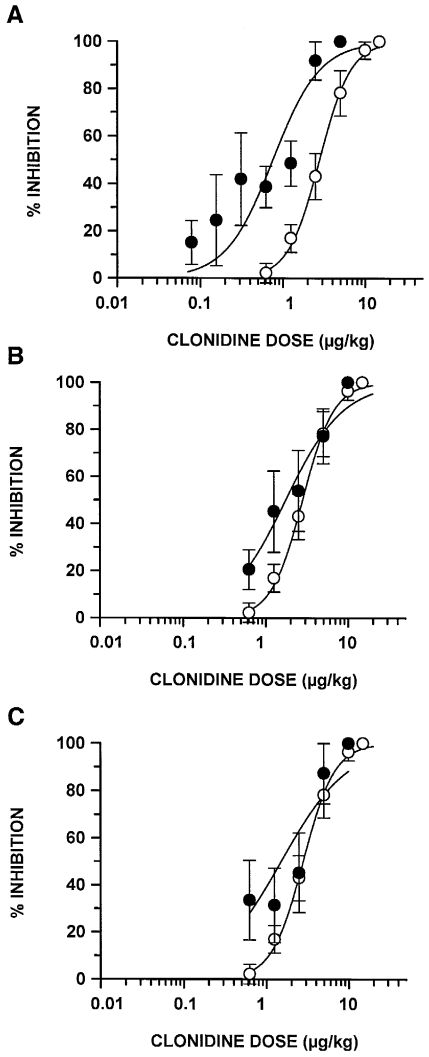

Fig. 1. Dose–effect curves for the inhibitory effect of clonidine on locus coeruleus neuron firing rate. (○) Control group and (●) experimental groups 3–5 min after administration of: (A) 8-OH-DPAT (1 μg/kg), (B) ARC 239 (75 μg/kg) and (C) chlorpromazine (75 μg/kg). Clonidine was injected in doubling doses (0.08–20 μg/kg, i.v.) at 1 min intervals.

respectively) and shifted to the left the clonidine dose–effect curves (Fig. 1B, C). In all cases the maximal inhibition (i.e. $E_{max} = 100\%$) of locus coeruleus neuron activity was reached.

4. Discussion

The results of this study indicated that 5-HT_{1A} receptors may act in synergy with α_2-adrenoceptors in the locus coeruleus; the clonidine-induced inhibition of the locus coeruleus firing rate, mediated through α_2-adrenoceptors is potentiated by the concurrent administration of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, which when administered alone has no effect on the firing rate. After 5-HT depletion, no effect of potentiation was observed, which suggests that this effect might be due to presynaptic receptor activation on 5-HT neurons. Consistent with these results, it has been shown that the central 5-HT system influences responses mediated through α_2-adrenoceptors. Thus, lesion of 5-HT neurons antagonizes the clonidine induced suppression of an avoidance behavior and of locomotion activity in rats (Kostowski et al., 1981). Destruction of 5-HT neurons or administration of 5-HT receptor antagonists potentiates the clonidine-induced hypoactivity response in mice (Heal and Philpot, 1987) and 5-HT depletion reduces the release of growth hormone induced by clonidine in rats (Söderpalm et al., 1987). On the other hand, it has been suggested that 5-HT_{1A} receptors do not tonically influence locus coeruleus activity. Here we show that 8-OH-DPAT did not modify the basal activity of the locus coeruleus as did 5-HT_{1A} receptor drugs in previous studies (Gorea and Adrien, 1988; Haddjeri et al., 1997).

Locus coeruleus neurons are tonically regulated by α_2-adrenoceptors (Svensson et al., 1975; Cedarbaum and Aghajanian, 1977) and these receptors are of the α_{2A}-adrenoceptor subtype (Scheinin et al., 1994; Ruiz-Ortega and Ugedo, 1996). 8-OH-DPAT binds to α_2-adrenoceptors (Crist and Surprenant, 1987) but its lack of effect on the basal activity of the locus coeruleus at the dose employed in this study eliminates the possibility of an α_2-adrenoceptor interaction. ARC 239 and chlorpromazine increased the firing of locus coeruleus neurons. This effect could be interpreted as being due to their activity as α_2-adrenoceptors antagonists. However, the fact that these compounds potentiated the clonidine inhibitory effect is not consistent with their behaving as α_2-adrenoceptor antagonists. It is more likely that these compounds are acting as 5-HT receptor agonists. Thus, ARC 239, has been reported to bind to 5-HT_{1A} receptors with an affinity similar to that for α_2-adrenoceptors ($K_{i\,5\text{-}HT1A} = 63$ to 163 nM; K_i $\alpha_2 = 14$ to 219 nM) (Meana et al., 1996). Thus, ARC 239 also modulates the synthesis of 5-HT in the hippocampus of the rat through 5-HT_{1A} receptors (Esteban et al., 1996). Chlorpromazine also binds 5-HT receptors but mainly the 5-HT_2 ($K_i = 2.3$ nM), 5-HT_6 ($K_i = 4.1$ nM), and 5-HT_7 ($K_i = 21$ nM) receptor subtypes (Roth and Meltzer, 1995). Therefore the potentiation of the locus coeruleus inhibition by clonidine could be due to an interaction with these receptor subtypes. In this context, it has been shown that 5-HT_2 receptor agonists increase evoked locus coeruleus activity (Rasmussen et al., 1986).

In conclusion, 5HT_{1A} receptor activation potentiates the inhibition of locus coeruleus neurons mediated by α_2-adrenoceptors. The finding that the clonidine-induced inhibition of the locus coeruleus was potentiated following administration of ARC 239 or chlorpromazine suggests that the effects of these drugs might be mediated not only by α_2-adrenoceptors but also by 5HT receptors, a fact which should be taken into account when functions associated with each α_2-adrenoceptor subtype are studied.

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