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Short communication

α_{2A}- But not $\alpha_{2B/C}$-adrenoceptors modulate noradrenaline release in rat locus coeruleus: voltammetric data

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Abstract

In this study, we used subtype-selective antagonists to determine the subtype of α_2-adrenoceptor controlling noradrenaline release in rat locus coeruleus. Noradrenaline release was measured in locus coeruleus slices using fast cyclic voltammetry at carbon fibre microelectrodes. On long stimulation trains (40 pulses, 20 Hz), the α_{2A}-adrenoceptor selective antagonist BRL 44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole) methyl]-4,5-dihydroimidazole) at 100 nM and 1 μM significantly increased stimulated noradrenaline release, whereas the $\alpha_{2B/C}$-selective antagonist ARC 239 (2-[2[4-(*o*-methoxyphenyl)piperazin-1-yl] ethyl]-4,4dimethyl-1,3-(2*H*,4*H*)-isoquinolinedione) at 50 and 500 nM had no effect. On short stimuli (20 pulses, 200 Hz), the non-specific α_2-adrenoceptor agonist dexmedetomidine (10 nM) significantly decreased noradrenaline release, an effect reversed by BRL 44408 (1μM) but not by ARC 239 (500 nM). These data demonstrate that autoreceptor control of noradrenaline release in the locus coeruleus is mediated by α_{2A} but not $\alpha_{2B/C}$-adrenoceptors.

Keywords: Locus coeruleus; Noradrenaline; Voltammetry, in vivo; α_2-Adrenoceptor subtype

1. Introduction

Noradrenergic cells in the locus coeruleus fire in a slow repetitive pacemaker-like fashion and their activity is determined by α_2 autoreceptors (Saunier et al., 1993). Previous studies in our laboratory have shown that these cells release noradrenaline in the rat locus coeruleus which may be detected by voltammetry (Palij and Stamford, 1994). Moreover, this noradrenaline release is under α_2-adrenoceptor control (Jorm and Stamford, 1993).

Based on molecular and pharmacological evidence, the existence of at least three different α_2-adrenoceptor subtypes (α_{2A}, α_{2B} and α_{2C}) is now widely accepted (Bylund et al., 1994). Each subtype has been reported to exist in the brain and messenger RNA for all three different α_2-adrenoceptor subtypes has been reported in the locus coeruleus (Tavares et al., 1996; Winzer-Serhan et al., 1997).

However, the lack of highly selective ligands to discriminate between the different α_2-adrenoceptor subtypes has made it difficult to ascribe a role for each subtype in the central functions of the α_2-adrenoceptors. More recently, a number of drugs have been demonstrated to possess subtype selectivity for α_2-adrenoceptors. The aim of the present study was therefore to determine, using these new ligands, the subtype of α_2-adrenoceptor involved in the control of noradrenaline release in the rat locus coeruleus.

2. Methods

2.1. Brain slice preparation.

Male Wistar rats (150–200 g) were stunned and then killed by rapid cervical dislocation. The brain was rapidly excised while being irrigated with ice cold artificial cerebrospinal fluid (ACSF). A vibrotome was used to obtain 350 μm thick slices containing the locus coeruleus that were transferred to the incubation chamber. The locus coeruleus slice was placed on a stainless steel grid and immobilised with a nylon meshed frame. The internal temperature of the chamber was maintained at 32°C and

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the slice was superfused with oxygenated ACSF at 1 ml/min for 1 h before the first stimulation and throughout the experiment.

2.2. Measurement of noradrenaline release

A carbon fibre (7 μm diameter) recording electrode was inserted into the slice, 200 μm from a bipolar tungsten stimulating electrode. Quantitative real-time noradrenaline release was measured using fast cyclic voltammetry, as previously described (Palij and Stamford, 1994). An input voltage consisting of 1.5 cycles of a triangular waveform (−1.0 to +1.4 V vs. Ag/AgCl) at a scan rate of 480 V/s was applied to the potentiostat every 500 ms. A sample and hold circuit monitored the current at the oxidation potential for noradrenaline (+0.6 V vs. Ag/AgCl). Fast cyclic voltammetry is able to distinguish between signals for noradrenaline and adrenaline (Pihel et al., 1994) and we saw no evidence of adrenaline contaminating the noradrenaline signals in the locus coeruleus.

2.3. Electrical stimulation

In experiments involving α_2-adrenoceptor antagonists, noradrenaline release was evoked with trains of 40 pulses (0.1 ms duration, 10 mA constant current) at 20 Hz, applied every 10 min. These long stimulation trains allow time for autoreceptor activation by released transmitter to occur and thus enabled detection of antagonist effects (Palij and Stamford, 1994). Conversely, experiments using agonist drugs were performed with shorter trains: 20 pulses at 200 Hz every 5 min. These short trains minimise autoreceptor activation by released noradrenaline (Thienprasert and Singer, 1993) and allow exogenous α_2-adrenoceptor agonist effects to be seen.

2.4. Drugs

Dexmedetomidine was a gift from Farmos (Finland), and BRL 44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole) methyl]-4,5-dihydroimidazole) from SmithKline Beecham (UK). ARC 239 (2-[2[4-(*o*-methoxyphenyl)piperazin-1-yl] ethyl]-4,4dimethyl-1,3-(2*H*,4*H*)-isoquinolinedione) was obtained from Tocris (UK). All salts used to prepare the ACSF were obtained from BDH.

2.5. Statistical analysis

All noradrenaline release data were calculated as percentages of the pre-drug period, six stimulations in the short trains experiments and three stimulations in the long train experiments. Data were expressed as mean ± S.E.M. and were analysed by One-way Analysis of Variance (ANOVA) with post hoc application of the Tukey–Kramer multiple comparisons test.

3. Results

Electrical stimulation in the locus coeruleus evoked release of noradrenaline that was detected at a carbon fibre microelectrode adjacent to the stimulating electrode.

On long stimulus trains (40 pulses at 20 Hz) the selective α_{2A}-adrenoceptor antagonist BRL 44408 significantly increased stimulated noradrenaline release compared with matched controls (Fig. 1A). The effect of BRL 44408 was higher at 1 μM (+60 ± 3%, $P < 0.001$, $n = 5$) than at 100 nM (+21 ± 8%, $P < 0.01$, $n = 4$). In the case of the higher concentration of BRL 44408, the onset of effect was extremely rapid, being already manifest only 10 min after addition. The selective $\alpha_{2B/C}$-adrenoceptor antagonist ARC 239 had no effect on stimulated noradrenaline release at 50 or at 500 nM (Fig. 1B).

On short stimulus trains (20 pulses at 200 Hz), the subtype non-selective α_2-adrenoceptor agonist dexmedetomidine (10 nM) significantly decreased noradrenaline release (Fig. 2) by 47 ± 12% ($P < 0.001$, $n = 5$). This effect was blocked in the presence of 1 μM BRL 44408 ($P < 0.001$), but not by ARC 239 (500 nM). Each antagonist

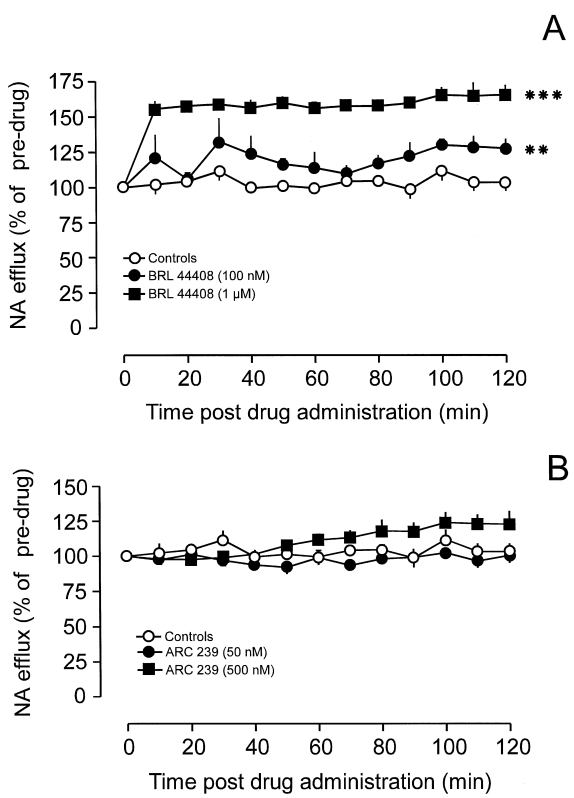

Fig. 1. Effects of α_2 adrenoceptor subtype-selective antagonists upon noradrenaline release on long stimulation trains. (A) Effects of BRL 44408 (100 nM ●, and 1 μM ■) compared to controls (○). All values are mean ± S.E.M. ($n = 4$–7) percentages of pre-drug values. $**P < 0.01$ and $***P < 0.001$ vs. control (One-way ANOVA). (B) Effects of ARC 239 (50 nM ●, and 500 nM ■) compared to controls (○). All values are mean ± S.E.M. ($n = 4$–7) percentages of pre-drug values. Stimulation parameters: trains of 40 pulses, 20 Hz, 0.1 ms, 10 mA, every 10 min.

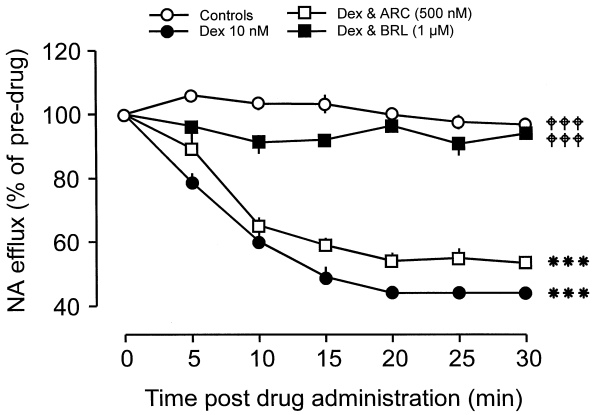

Fig. 2. Effects of α_2 adrenoceptor subtype-selective antagonists upon inhibition of noradrenaline release by dexmedetomidine on short stimulation trains. Effects of the α_2-adrenoceptor agonist dexmedetomidine (10 nM) on stimulated noradrenaline release alone (●) and in the presence of the α_{2A}-selective antagonist BRL 44408 (1 μM: ■) or the $\alpha_{2B/C}$-selective antagonist ARC 239 (500 nM: □) each added 30 min prior to dexmedetomidine. Controls (○) are shown for comparison. All values are mean ± S.E.M. ($n = 4–5$) percentages of pre-drug values. $***P < 0.001$ vs. control (One-way ANOVA). ⧺⧺⧺ $P < 0.001$ vs. dexmedetomidine. Stimulation parameters: trains of 20 pulses, 200 Hz, 0.1 ms, 10 mA, every 5 min.

was added to the chamber 30 min before the dexmedetomidine. Neither ARC 239 nor BRL 44408 had any effect on stimulated noradrenaline release (94.5 ± 7.2% and 99.1 ± 1.7% of pre-drug values, respectively, 30 min post-administration).

4. Discussion

It has been demonstrated that noradrenergic cell *firing* appears to be under α_{2A}-autoreceptor control (Nörenberg et al., 1997) although recent work in dissociated rat locus coeruleus neurones has suggested that α_{2B} or α_{2C}-adrenoceptors may also modulate noradrenergic cell activity (Arima et al., 1998). Furthermore, one should be cautious of assuming that the same receptors control noradrenaline *release*. For instance, serotonergic cell *firing* in the dorsal raphe nucleus is under 5-HT_{1A} receptor control while 5-HT *release* in the nucleus is modulated also by 5-HT_{1B} and 5-HT_{1D} receptors (Davidson and Stamford, 1995).

The purpose of the present study was to ascertain, using selective antagonists, whether the same phenomenon prevailed in locus coeruleus. BRL 44408 and ARC 239 are now considered as highly selective compounds for the discrimination of α_{2A} and $\alpha_{2B/C}$-adrenoceptors, being currently used for such a purpose in functional and biochemical studies in several tissues (Millan et al., 1994; Sastre and García-Sevilla, 1994; Callado et al., 1996). BRL 44408 has a K_i of 7.17 and 194 nM at rat α_{2A} and α_{2B} receptors, respectively, compared with values of 337 (α_{2A}) and 7.7 (α_{2B}) for ARC 239 (Renouard et al., 1994).

The evidence of Figs. 1 and 2 suggests that noradrenaline release is controlled not by a multiplicity of α_2-adrenoceptors but solely by the α_{2A}-adrenoceptor subtype. Only BRL 44408 was able to increase noradrenaline efflux on long stimulations and block the effect of dexmedetomidine on short trains. ARC 239 had no effect on either measure, even at concentrations considerably above its affinity constant for $\alpha_{2B/C}$-adrenoceptors. The present results confirm the involvement of the α_2-adrenoceptors in the control of the noradrenaline release in the rat locus coeruleus as previously reported (Jorm and Stamford, 1993; Mateo et al., 1998) and are, to our knowledge, the first demonstration that this control is mediated exclusively by the α_{2A}-adrenoceptor subtype. It has however been reported that the rat cerebrocortical α_2 autoreceptor is of the $\alpha_{2A/D}$ subtype (Trendelenberg et al., 1993) and suggested that this subtype may generally comprise the main mammalian α_2-autoreceptor.

It is worth emphasising at this point that the source of the released noradrenaline is unclear. For instance, catecholamine storage vesicles have been demonstrated in the dendrites of noradrenergic neurones in the locus coeruleus (Fillenz, 1990). Furthermore, the locus coeruleus also receives noradrenergic inputs from the A1, A2, A5 catecholaminergic cell groups as well as the contralateral locus coeruleus (Cederbaum and Aghajanian, 1978) and there are extensive recurrent axon collaterals within the nucleus (Aghajanian et al., 1977). Any or all of these structures may be capable of releasing noradrenaline.

Both BRL 44408 and ARC 239 also recognise 5-HT_{1A} receptors in the rat brain (Meana et al., 1996). Although 5-HT_{1A} receptors have been implicated in the regulation of noradrenaline release in rat brain (Rollema et al., 1996), the 5-HT_{1A} selective agonist 8-hydroxy-DPAT, at a concentration known to inhibit 5-HT release (Davidson and Stamford, 1995), had no significant effect on evoked release of noradrenaline in locus coeruleus (data not shown) excluding any role of these receptors in the present results.

The high α_{2A}-adrenoceptor immunoreactivity in the locus coeruleus (Talley et al., 1996) and the presynaptic presence of this subtype (Lee et al., 1998a), as well as the mainly postsynaptic localisation of α_{2C}-adrenoceptors (Lee et al., 1998b) support our findings. These results are also in agreement with previous work reporting that the α_{2A}-adrenoceptor subtype mediates noradrenaline release in other rat brain regions (Gobbi et al., 1993; Kiss et al., 1995; Gobert et al., 1998) as well as in other tissues (Schwartz and Malik, 1992; Molderings and Gothert, 1995). This predominant role of α_{2A}-adrenoceptors is supported by studies in transgenic mice, indicating that α_{2A} rather than α_{2B} or α_{2C}-adrenoceptor subtypes control the activity of the noradrenergic neurons in the central nervous system (Limbird, 1996; Sallinen et al., 1997).

As previously mentioned, the locus coeruleus firing activity is regulated by the α_{2A}-adrenoceptor subtype (Nörenberg et al., 1997). This fact, in conjunction with the present results, would imply that the apparent dissociation of dexmedetomidine's actions on cell *firing* and nor-

adrenaline *release* (Jorm and Stamford, 1993) is indicative of differing receptor reserves for these processes rather than the involvement of different α_2-adrenoceptor subtypes, as originally speculated. In this context, it has been reported that α_2-adrenoceptors regulating the firing rate of locus coeruleus cells are characterised by a high receptor reserve (Pineda et al., 1997). Nevertheless, in the light of the recent paper by Arima et al. (1998) on dissociated rat locus coeruleus neurones, the involvement of α_{2B} or α_{2C}-adrenoceptors in control of noradrenergic cell *firing*, although not release, must be considered.

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