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Determination of the somatodendritic α_2-adrenoceptor subtype located in rat locus coeruleus that modulates cortical noradrenaline release in vivo

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Abstract

The regulation of extracellular noradrenaline levels in the cingulate cortex by somatodendritic α_2-adrenoceptors located in the locus coeruleus was evaluated in the rat by using dual-probe microdialysis. The concentration of noradrenaline in the cingulate cortex was decreased (37%–40%) by administration into the locus coeruleus (1 μM) of the agonists clonidine and UK14304 (bromoxidine), whereas it was increased by similar administration of the nonselective antagonist RX821002 (2-methoxyidazoxan) (+103%) and the selective α_{2A}-adrenoceptor antagonist BRL44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazole) (+148%). The selective $\alpha_{2B/C}$-adrenoceptor antagonist ARC239 (2-[2[4-(*o*-methoxyphenyl)piperazin-1-yl]ethyl]-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolinedione) did not induce changes. In the presence of BRL44408, the effects of clonidine and UK14304 were abolished, but they were not modified in the presence of ARC239. The data demonstrate that noradrenaline release in terminal areas is tonically modulated by somatodendritic α_{2A}-adrenoceptors.

Keywords: α_2-Adrenoceptor subtype; Noradrenaline; Locus coeruleus; Microdialysis; Cortex, cingulate; Brain, rat

1. Introduction

The locus coeruleus is the largest group of noradrenergic neurones in the central nervous system. Noradrenergic processes, arising in the locus coeruleus, project extensively throughout the brain and the spinal cord (Moore and Bloom, 1979). Presynaptic α_2-adrenoceptors are located on noradrenergic nerve terminals as well as on the cell bodies and/or dendrites of the locus coeruleus neurones. Systemic administration of α_2-adrenoceptor agonists induces in vivo an inhibitory effect on the firing activity of locus coeruleus cells (Cedarbaum and Aghajanian, 1976) and reduces the release of noradrenaline from axonal terminals (L'Heureux et al., 1986; Van Veldhuizen et al., 1993; Meana et al., 1997).

Three different α_2-adrenoceptors, termed as α_{2A}, α_{2B} and α_{2C} subtypes, have been identified by molecular and pharmacological criteria (Bylund et al., 1994). In the central nervous system, the α_{2A}-adrenoceptor subtype is expressed, among others, by the noradrenergic cells of the locus coeruleus (Rosin et al., 1993; Aoki et al., 1994) and in noradrenergic terminal fields such as the cingulate cortex (Aoki et al., 1994; Talley et al., 1996). The α_{2C}-adrenoceptor subtype is also expressed by catecholaminergic neurones in the locus coeruleus (Lee et al., 1998) and other brain areas with noradrenergic innervation (Rosin et al., 1996).

In the rat, the modulation of noradrenaline release by α_{2A}-autoreceptors located on cortical noradrenergic terminals has been extensively evaluated (Maura et al., 1992; Trendelenburg et al., 1993; Van Veldhuizen et al., 1993; Dalley and Stanford, 1995; Mateo et al., 1998). Additionally, the α_{2A}-adrenoceptor subtype contributes to the regulation of somatodendritic release of noradrenaline in the locus coeruleus (Callado and Stamford, 1999). However, clear discrepancies related to the α_2-adrenoceptor subtype that controls the neuronal firing activity of locus coeruleus noradrenergic cells have been reported (Nörenberg et al., 1997; Arima et al., 1998). Since the release of noradrenaline in nerve terminals is determined by the pattern of discharge of locus coeruleus cells (Florin-Lechner et al.,

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1996), somatodendritic α_2-adrenoceptors located at this level may modulate the efficacy of noradrenergic transmission in terminal areas. In fact, local administration within the locus coeruleus of nonselective agonists or antagonists of the α_2-adrenoceptor subtypes modulates in vivo the extracellular levels of noradrenaline in the rat brain cortex (Van Gaalen et al., 1997; Mateo et al., 1998).

The aim of the present study was to clarify which of the different α_2-adrenoceptor subtypes located in the locus coeruleus nucleus controls in vivo noradrenaline release in projection areas such as the cingulate cortex. Subtype-selective α_2-adrenoceptor drugs were applied into the locus coeruleus by reverse dialysis and simultaneously extracellular noradrenaline levels were measured in the cingulate cortex. This dual-probe microdialysis approach has been previously applied to the study of the central noradrenergic system (Van Gaalen et al., 1997; Mateo et al., 1998).

2. Materials and methods

2.1. Animals and surgery

Male Sprague–Dawley rats (250–300 g) were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and placed for stereotaxic surgery with the incisor bar lowered to a 15° angle (nose down). One microdialysis probe (2.0 × 0.25 mm) was implanted in the vicinity of the right locus coeruleus (AP − 3.7; L + 1.3; V − 8.2, taken in millimeters from lambda suture point) and the other probe (4.0 × 0.25 mm^2) was implanted in the ipsilateral cingulate cortex (AP + 2.8; L + 1.0; V − 5.0, taken in millimeters from bregma), as previously reported (Mateo et al., 1998).

2.2. Microdialysis procedures and drug administration

Microdialysis experiments were carried out approximately 20 h after surgery. Animals were placed in a CMA/120 system for freely moving animals and the probes were perfused with a modified cerebrospinal fluid (CSF) solution (148 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$; pH 7.4) at a flow rate of 1 μl/min. Following 1 h for stabilization, dialysate samples were collected every 35 min. The three samples that were collected before drug administration were considered as basal levels. The local administration by reverse dialysis was performed by dilution of the drugs in the artificial CSF. When the effects of agonist drugs were evaluated in the presence of antagonists, these antagonist drugs were included in the perfusion medium from the beginning of the experiment. Under these conditions, basal levels were considered as those in the presence of the antagonist before agonist administration. The correct implantation of the probes was verified after the experiments and the in vitro recovery for noradrenaline was calculated to be in the range 10%–15%.

2.3. Chromatographic analysis

Noradrenaline levels from dialysate samples (35 μl of the dialysate medium plus 5 μl of 0.1 M $HClO_4$) were quantified by high-performance liquid chromatography (HPLC) with electrochemical detection as previously described (Mateo et al., 1998). The limit of detection of the assay was 20–25 fmol per sample.

2.4. Statistical analyses

The mean value of the three basal samples before drug administration was considered to be 100%. Other measures are expressed as a percentage of these baseline values. The concentration–response effects of clonidine were assessed by one-way analysis of variance (ANOVA). When a single concentration of drug was used, the differences among basal and maximal effect values were evaluated by Student's *t*-test. Results are expressed as mean ± S.E.M. values and the level of significance was set at $P = 0.05$.

2.5. Drugs and chemicals

ARC239 (2-[2[4-(*o*-methoxyphenyl)piperazin-1-yl]ethyl]-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolinedione) and BRL44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazole) were generously provided by Thomae (Biberach, Germany) and Smith Kline Beecham (Essex, UK), respectively. Clonidine HCl was obtained from Sigma (St. Louis, MO, USA) and RX821002 (2-methoxyidazoxan) was synthesized at LASA Laboratorios (Barcelona, Spain). UK14304 (bromoxidine) was supplied by Tocris Cookson (Bristol, UK).

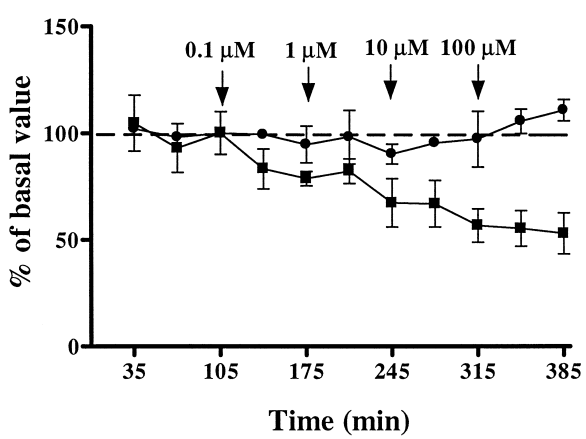

Fig. 1. Effect of local administration of clonidine into the locus coeruleus area on extracellular noradrenaline levels in the cingulate cortex in the absence (■) or in the presence (●) of the α_2-adrenoceptor antagonist RX821002 (1 μM). Concentrations of clonidine were progressively increased every two fractions (70 min) in tenfold increments (arrows). Data are mean ± S.E.M. (bars) values from four separate experiments and are expressed as percentages of the corresponding baseline values.

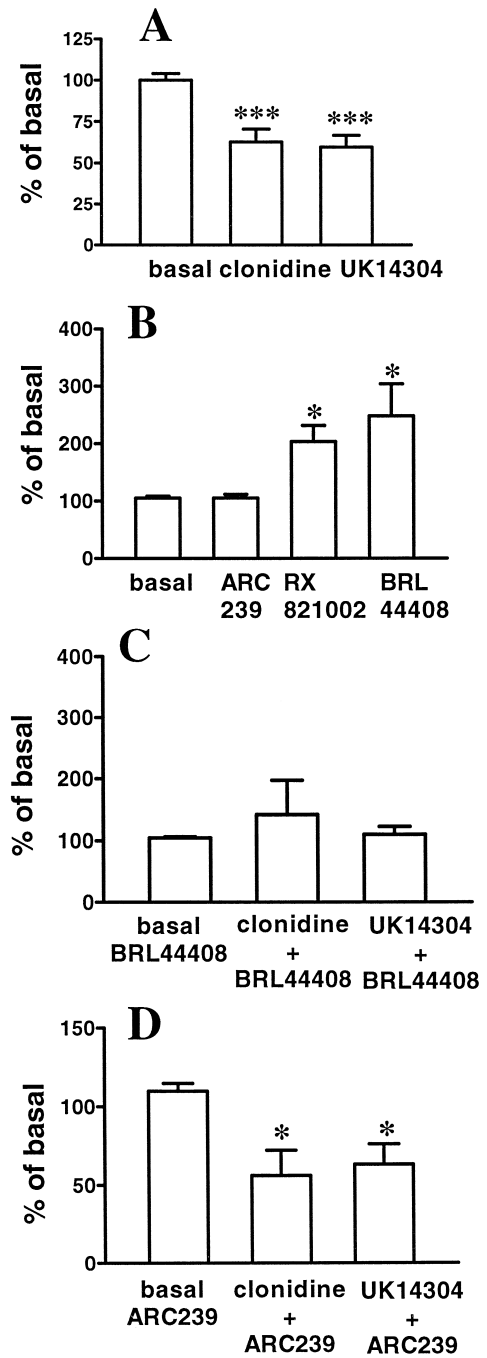

Fig. 2. Effect on noradrenaline levels in the cingulate cortex of local administration into the locus coeruleus area of (A) the α_2-adrenoceptor agonists clonidine or UK14304; (B) the α_2-adrenoceptor antagonists ARC239 ($\alpha_{2B/C}$-adrenoceptor selective), RX821002 (α_2-adrenoceptor nonselective) and BRL44408 (α_{2A}-adrenoceptor selective); (C,D) the agonists in the presence of antagonists. Drugs were administered at 1 μM concentration by reverse dialysis through the probe and the maximal effects were measured after 70 min of continuous administration. In C and D, basal levels represent noradrenaline levels in the presence of BRL44408 and ARC239, respectively. The columns represent the mean ± S.E.M. (bars) values from four to seven separate experiments and are expressed as percentages of the corresponding baseline values. $^{*}P < 0.05$, $^{***}P < 0.001$ (Student's *t*-test vs. basal values).

3. Results

The administration into the locus coeruleus of the α_2-adrenoceptor agonist clonidine (0.1–100 μM) induced a concentration-dependent decrease of noradrenaline levels (basal level 5.68 ± 0.75 nM) in the cingulate cortex ($F[10,42] = 3.82$; $P < 0.005$), with a maximal inhibitory effect of 47 ± 9% being reached with 100 μM clonidine (Fig. 1). When the nonselective α_2-adrenoceptor antagonist RX821002 (1 μM) was added to the artificial CSF and perfused into the locus coeruleus area for at least 60 min before the collection of baseline samples and for all the experiment, the noradrenaline level was 8.74 ± 0.67 nM and no effect of clonidine could be observed ($F[9,19] = 1.92$; $P = 0.16$) (Fig. 1).

On the basis of these data, the next experiments were carried out with a single drug concentration (1 μM). The basal level of noradrenaline in the cingulate cortex was 5.29 ± 0.74 nM and significantly decreased following local perfusion into the locus coeruleus (1 μM) of the agonists clonidine (−37 ± 8%; $t = 4.91$; $P < 0.001$) or UK14304 (−40 ± 7%; $t = 5.95$; $P < 0.001$) (Fig. 2A). Conversely, the basal noradrenaline level in the cingulate cortex was increased by the perfusion, at a concentration of 1 μM, into the locus coeruleus of the nonselective α_2-adrenoceptor antagonist RX821002 (+103 ± 28%; $t = 3.68$; $P < 0.05$) or the selective α_{2A}-adrenoceptor antagonist BRL44408 (+148 ± 59%; $t = 2.39$; $P < 0.05$) (Fig. 2B). In contrast, the selective $\alpha_{2B/C}$-adrenoceptor antagonist ARC239 administered also into the locus coeruleus did not have effect on noradrenaline levels in the cingulate cortex (+5 ± 7%; $t = 0.07$; $P = 0.94$) (Fig. 2B).

In the presence of BRL44408 (1 μM), administered from the beginning into the locus coeruleus area, the basal level of noradrenaline in the cingulate cortex was 12.61 ± 3.15 nM. Under such conditions, local administration into the locus coeruleus area of 1 μM clonidine (+48 ± 55%; $t = 1.14$; $P = 0.28$) or 1 μM UK14304 (+10 ± 13%; $t = 1.04$; $P = 0.32$) did not affect noradrenaline levels in the cingulate cortex (Fig. 2C). However, in the presence of ARC239 (1 μM) in the locus coeruleus, the noradrenaline level in the cingulate cortex (4.55 ± 0.70 nM) was significantly reduced by local administration of 1 μM clonidine (−44 ± 16%; $t = 2.85$; $P < 0.05$) or UK14304 (−37 ± 13%; $t = 3.32$; $P < 0.05$) into the locus coeruleus (Fig. 2D).

4. Discussion

The present results confirm that in vivo noradrenaline release in the cingulate cortex is modulated by α_2-adrenoceptors located in the locus coeruleus and demonstrate for the first time that these somatodendritic receptors corre-

spond to the α_{2A}-adrenoceptor subtype. The increase of noradrenaline levels in the cingulate cortex following the administration of α_2-adrenoceptor antagonists into the locus coeruleus suggests the presence of a tonic autoinhibitory regulation of noradrenergic neurones (Cedarbaum and Aghajanian, 1976), which is probably mediated by the presence of noradrenaline at the somatodendritic level (Mateo et al., 1998).

The agonists clonidine and UK14304 display comparable affinity for the α_{2A}- and the $\alpha_{2B/C}$-adrenoceptor subtypes (Renouard et al., 1994) and the antagonist RX821002 shows a small selectivity for the α_{2A}- vs. the $\alpha_{2B/C}$-adrenoceptor subtype (Marjamäki et al., 1993; Renouard et al., 1994). This fact implies that the effects of these drugs could be mediated via the α_{2A}- or the α_{2C}-adrenoceptor subtype because α_{2B}-adrenoceptor expression is restricted in the rat central nervous system to the diencephalon (Zeng and Lynch, 1991; Nicholas et al., 1993; Scheinin et al., 1994). Currently, agonist drugs with important selectivity for α_{2A}-, α_{2B}-, or α_{2C}-adrenoceptor subtypes are not available. The compound BRL44408 is considered the most selective antagonist for the α_{2A}-adrenoceptor subtype, whereas ARC239 is thought to have selectivity for $\alpha_{2B/C}$-adrenoceptor subtypes (Devedjian et al., 1994). Therefore, based on the ability of RX821002 and BRL44408, but not ARC239 to antagonize the effects of clonidine and UK14304, it could be suggested that the α_{2A}-adrenoceptor subtype expressed in the locus coeruleus area is involved in the regulation of noradrenaline release by noradrenergic terminals. The agonists probably inhibit the firing activity of locus coeruleus cells (Cedarbaum and Aghajanian, 1976) leading to a subsequent attenuation of noradrenaline release in a projection area, the cingulate cortex.

Electrophysiological studies performed with slices of rat locus coeruleus have characterized the somatodendritic α_2-adrenoceptors that control the firing activity as being the α_{2A}-adrenoceptor subtype (Nörenberg et al., 1997). The present data confirm in vivo the functional role of somatodendritic α_{2A}-adrenoceptors in central noradrenergic activity. In contrast, the activity of dissociated locus coeruleus noradrenergic neurones seems to be modulated by the $\alpha_{2B/C}$-adrenoceptor subtype (Arima et al., 1998). Immunohistochemical studies also provide evidence for the presence of both α_{2A}- and $\alpha_{2B/C}$-adrenoceptor subtypes in the locus coeruleus (Aoki et al., 1994; Rosin et al., 1996; Talley et al., 1996; Lee et al., 1998). These apparent discrepancies could reflect a segregated localization of each subtype on different neurones or the selective targeting within the same cell of different functions. In this regard, it is interesting to note that in the rat brain cortex, the modulation of noradrenaline synthesis by the enzyme tyrosine hydroxylase and the release of noradrenaline are controlled by different α_2-adrenoceptors, the α_{2C}- and the α_{2A}-adrenoceptor subtypes, respectively (Trendelenburg et al., 1993; Esteban et al., 1996).

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