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Characterization of the Subtype of Presynaptic α_2-Adrenoceptors Modulating Noradrenaline Release in Cat and Bovine Cerebral Arteries

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Abstract—The possible existence of a heterogeneous population of α_2-adrenoceptors (α_{2A} and α_{2B}, demonstrated by binding studies) in adrenergic nerve endings of cat and bovine cerebral arteries modulating noradrenaline release was investigated. Electrical field stimulation elicited an increase of tritium secretion from these vessels preincubated with (±)-[^{3}H]noradrenaline, which was reduced by the α_2-agonists, clonidine (1 μM) and B-HT 920 (0·01 and 0·1 μM), in cat cerebral arteries but only by B-HT 920 in bovine cerebral arteries. This reduction was inhibited by the antagonist of the α_{2B}-subtype, prazosin, and the antagonists of α_{2A}- and α_{2B}-subtypes yohimbine and particularly rauwolscine. The effect of B-HT 920 was partially inhibited by clonidine in bovine, but not in cat cerebral arteries. In both types of arteries, prazosin, yohimbine and the α_1-agonist methoxamine (all at 1 μM) failed to modify the stimulated radioactivity liberation, whereas it was increased by 1 μM rauwolscine, and by yohimbine plus prazosin in cat cerebral arteries. The basal tritium release was enhanced by rauwolscine and prazosin in cat cerebral arteries but only by the latter in bovine cerebral arteries. These results suggest: (1) the existence of presynaptic α_2-adrenoceptors, mainly of the α_{2B}-subtype, in these vessels negatively modulating noradrenaline release, their activity being greater in cat than in bovine cerebral arteries, and (2) clonidine has no agonistic but a weak antagonistic action in the latter vessels.

Cerebral vessels are endowed with a dense adrenergic innervation mainly originating in the superior cervical ganglia (Nielsen & Owman 1971; Alborch et al 1977; Marín et al 1980). The stimulation of cerebrovascular adrenergic nerve terminals produces noradrenaline release which increases the tone of these vessels (Alborch et al 1977; Conde et al 1978; Duckles 1979). The noradrenaline liberation in different vessels (Göthert et al 1984; Hentrich et al 1986), including cerebral vessels (Balfagón & Marín 1989; Sánchez-Merino et al 1990), is mediated by presynaptic α_2-adrenoceptors. These receptors appear to constitute a non-homogeneous population (Kawahara & Bylund 1985; Alabaster et al 1986). They have been mainly subclassified into two subtypes, α_{2A} and α_{2B} (Bylund 1988; Bylund et al 1988; Docherty 1989; Young et al 1989). Prazosin is a selective antagonist for α_{2B} (in addition to α_1-adrenoceptors) and yohimbine and particularly rauwolscine are selective for both α_{2A} and α_{2B} (Petrash & Bylund 1986; Bylund 1988; Bylund et al 1988). In spite of these findings, few functional studies have been carried out supporting the binding experiments, and none, to our knowledge, analysing the subtype of α_2-receptors modulating the noradrenaline release from cerebral arteries.

The aim of this paper was to characterize the subtype of presynaptic receptors (α_{2A} or α_{2B}) present in cat and bovine cerebral arteries mediating noradrenaline release, using appropriate agonists and antagonists of α-adrenoceptors. In addition, since in some tissues the activation of presynaptic α_1-adrenoceptors produces an inhibitory noradrenaline release modulation (Kobinger & Pichler 1982; Docherty 1984; Story et al 1985), the existence of these receptors was also investigated.

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Materials and Methods

Bovine and cat cerebral arteries used in the present study were branches of middle cerebral arteries and all arteries of the circle of Willis with their branches, respectively. The bovine brain was obtained from the abbatoir and transported to the laboratory in Krebs-Henseleit solution (KHS) at 4°C. Brains were carefully isolated from animals previously anaesthetized with pentobarbitone (35 mg kg^{-1}, i.p.) and killed by bleeding. The isolated vessels were placed in a Petri dish containing KHS at 4°C, divided into segments of 5 mm in length, pooled and carefully cleaned of traces of blood and adherent tissues. They were then set up in a nylon net and immersed for 30 min in 10 mL of KHS at 37°C continuously gassed with 95% O_2–5% CO_2 (stabilization period). Thereafter, they were incubated for 60 min in 1 mL oxygenated KHS at 37°C containing (±)-[^{3}H]noradrenaline (10 μCi mL^{-1} 5×10^{-7} M, sp. act. 16 Ci mmol^{-1}). Afterwards, the arteries were transferred into a superfusion chamber with two parallel platinum electrodes, 0·5 cm apart, connected to a stimulator (Cibertec model CS9, modified to supply the adequate current strength) for electrical field stimulation (200 mA, 0·3 ms, and 2 or 4 Hz in the case of cat or bovine arteries, respectively, according to Weitzell et al (1979)). The arteries were superfused at a rate of 2 mL min^{-1} with oxygenated KHS at 37°C for 100 min during which time the steady-state level of the basal tritium release was reached. The superfusate was then collected in vials (10 in total) at 30 s intervals, which were distributed in the following manner: 2 before stimulation, to determine the basal level of tritium release, 2 during and 6 after stimulation; the latter were

enough to recover the basal levels of tritium release. Ready Protein solution (Beckman) was added to the vials and the radioactivity measured in a scintillation counter (Beckman LS 2800). Only two electrical stimulation periods of 60 s (S_1 and S_2) were applied to the arteries at 30 min intervals, because a third period did not induce a significant tritium release. The drugs used to modify tritium release were administered 20 min before S_2. When an agonist plus an antagonist were applied, the latter was added 5 min before the former. The concentrations used of α_2-adrenoceptor agonists, clonidine (1 μM) and B-HT 920 (0·01 and 0·1 μM), were those currently used in these type of studies and which produced a significant effect on stimulation-induced tritium release in both types of arteries. Finally, the arteries were blotted, weighed and digested in vials containing 1 mL of H_2O_2 (30% w/v) at 100°C for 3 h, and the radioactivity retained was measured.

The stimulation-induced tritium release was calculated by subtraction of basal tritium release from that evoked by electrical stimulation. Thereafter, the ratios of the net tritium release (S_2/S_1) were calculated in order to eliminate differences among the arteries. For the same reason, the ratio between the basal tritium release before the second period of stimulation (b_2) and the first period (b_1) was also determined. The actions of the drugs on the basal and on the stimulated release were described by their actions on these ratios. The composition of KHS was as follows (mM): NaCl 115, $CaCl_2$ 2·5, KCl 4·6, KH_2PO_4 1·2, $MgSO_4$ $7H_2O$ 1·2, $NaHCO_3$ 25, glucose 11·1, Na_2 EDTA 0·03 (to prevent the oxidation of unstable substances).

Results are given as means ± s.e.m. Statistical analysis was by means of Student's t-test, $P<0.05$ was considered significant. The drugs used were: phentolamine methanesulphonate (Geigy, USA), (±)-[^{3}H]noradrenaline hydrochloride (Amersham, UK), clonidine hydrochloride and B-HT 920 (Boehringer Ingelheim, Germany), methoxamine hydrochloride (Gayoso Wellcome, UK), prazosin hydrochloride (Pfizer, USA), rauwolscine hydrochloride (Carl Roth R.B., Germany), yohimbine hydrochloride and tetrodotoxin (Sigma, USA). Stock solutions (10 mM) of drugs were made in distilled water, except prazosin and noradrenaline, which were made in 50% ethanol solution and saline (0·9% NaCl)-ascorbic acid (0·01% w/v) solution respectively to prevent the oxidation of the catechol group of noradrenaline. These solutions were maintained at −20°C. Appropriate dilutions were made in distilled water on the day of the experiment. Parallel experiments with the same concentration of ethanol as contained in the prazosin solution did not modify tritium release.

Results

Electrical stimulation elicited tritium release from cat cerebral arteries preincubated with [^{3}H]noradrenaline; this release was less in the second stimulation period, S_2 (395 ± 102 counts min^{-1} mg^{-1}), than in S_1 (536 ± 74 counts min^{-1} mg^{-1}); the ratio S_2/S_1 was 0·94 ± 0·10. This ratio was similarly reduced by the α_2-adrenoceptor agonists clonidine (1 μM) and B-HT 920 (0·01 and 0·1 μM) (Fig. 1).

The effect of clonidine (1 μM) and B-HT 920 (0·01 μM) was reversed by 1 μM of the α-adrenoceptor antagonists yohimbine, prazosin and rauwolscine; the response obtained in the presence of rauwolscine was greater than that obtained in the control. However, the inhibition induced by B-HT 920 (0·1 μM) was reversed only by rauwolscine and not affected by the other two α-adrenoceptor antagonists (Fig. 1). The action of B-HT 920 (0·01 μM) was not modified by clonidine (1 μM, results not shown, or 10 μM) (Fig. 1).

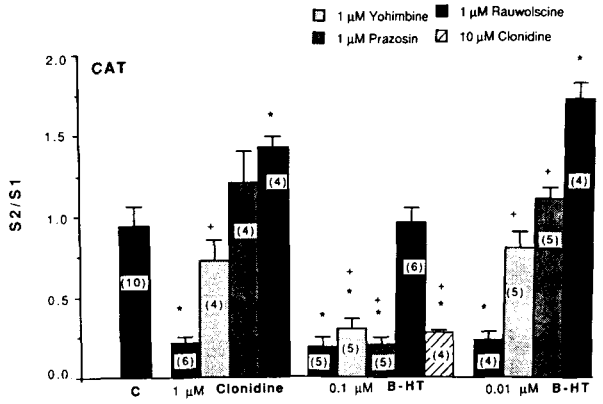

FIG. 1. Effects of the α_2-adrenoceptor agonists clonidine and B-HT 920 and their inhibition by the α-adrenoceptor antagonists yohimbine, prazosin, rauwolscine and clonidine on the electrical (2 Hz, 0·3 ms, 200 mA for 60 s)-induced tritium release from cat cerebral arteries preincubated with [^{3}H]noradrenaline. The ratios of the tritium release induced by the stimulation periods S_2 and S_1 separated by 30 min, are shown on the ordinate. The number of experiments is shown in parentheses. The columns and vertical bars represent means ± s.e.m. $^*P<0.001$, $^+P<0.01$, with respect to control and rauwolscine, respectively. C = Control.

Prazosin, yohimbine and the α_1-agonist methoxamine (all at 1 μM) failed to alter the stimulated tritium release in cat cerebral arteries, whereas the release was increased by 1 μM rauwolscine and yohimbine plus prazosin (Fig. 2).

In bovine cerebral arteries, the tritium release in S_2 (251 ± 25 counts min^{-1} mg^{-1}) was also less than in S_1

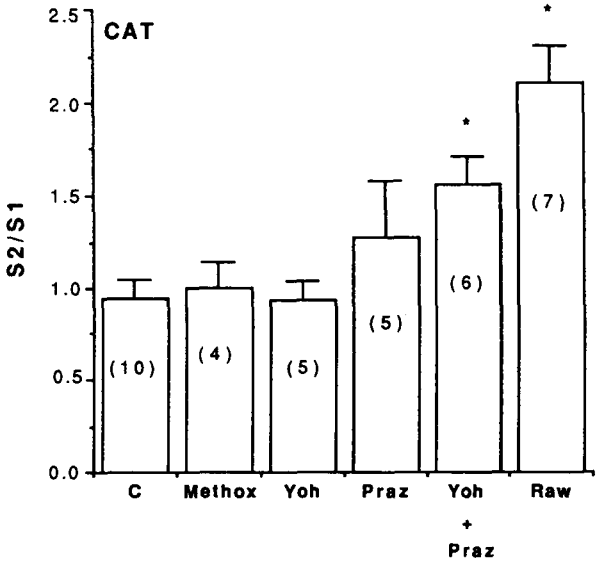

FIG. 2. Effects of methoxamine (Methox), rauwolscine (Raw), prazosin (Praz), yohimbine (Yoh) and yohimbine plus prazosin on the electrically-induced tritium release from cat cerebral arteries preincubated with [^{3}H]noradrenaline. The symbols and experimental procedure are as in Fig. 1. $^*P<0.01$. C = Control.

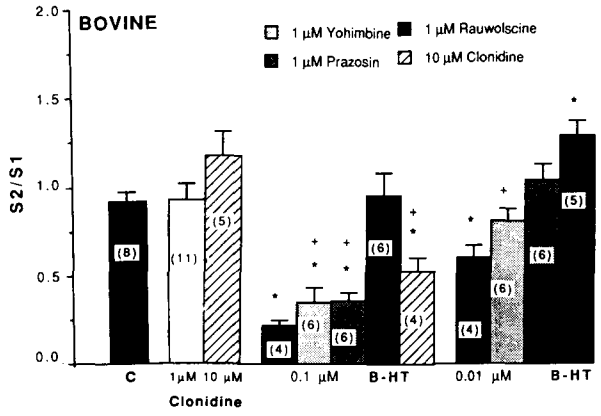

FIG. 3. Effects of the α_2-adrenoceptor agonists clonidine and B-HT 920 and their inhibition by the α-adrenoceptor antagonists yohimbine, prazosin, rauwolscine and clonidine on the electrically (4 Hz, 0·3 ms, 200 mA for 60 s)-induced tritium release from bovine cerebral arteries preincubated with [^{3}H]noradrenaline. The ratios of the tritium release induced by the stimulation periods S_1 and S_2 separated by 30 min are shown on the ordinate. The number of experiments is shown in parentheses. The columns and vertical bars represent means ± s.e.m. $^*P<0.01$, $^+P<0.05$, with respect to control and rauwolscine, respectively. Prazosin produced a weak, but significant ($P<0.05$), inhibition of 0·1 μM B-HT 920 action. C = Control.

(273 ± 23 counts min^{-1} mg^{-1}) and the ratio S_2/S_1 was 0·92 ± 0·05. Clonidine (1 and 10 μM) did not modify this ratio, but it was reduced in a concentration-dependent manner by B-HT 920 (0·01 and 0·1 μM). The effect of 0·1 μM B-HT 920 was partially antagonized by clonidine (10 μM) and prazosin (1 μM), completely inhibited by rauwolscine (1 μM) and not affected by yohimbine (1 μM). Nevertheless, the action of 0·01 μM B-HT 920 was antagonized by prazosin and rauwolscine, and not modified by yohimbine; the tritium release obtained in the presence of rauwolscine was greater than that observed in the control (Fig. 3). The stimulated radioactivity release was not affected by 1 μM methoxamine,

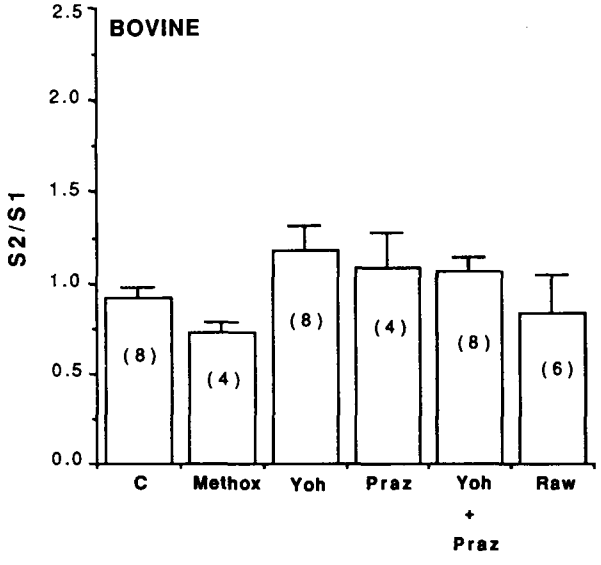

FIG. 4. Effects of methoxamine (Methox), rauwolscine (Raw), prazosin (Praz), yohimbine (Yoh) and yohimbine plus prazosin on the electrically-induced tritium release from bovine cerebral arteries preincubated with [^{3}H]noradrenaline. The experimental procedure is as in Fig. 3.

rauwolscine, prazosin, yohimbine, yohimbine plus prazosin (Fig. 4) or phentolamine (results not shown).

The ratio b_2/b_1 (control $b_1 = 73 \pm 15$ counts min^{-1} mg^{-1}; $b_2/b_1 = 0.75 \pm 0.02$, n = 10) in cat cerebral arteries was increased by 1 μM rauwolscine (0·89 ± 0·01, $P<0.01$, n = 10) and prazosin (1·24 ± 0·1, $P<0.01$, n = 10). In bovine cerebral arteries, this ratio (control $b_1 = 43 \pm 3$ counts min^{-1} mg^{-1}; $b_2/b_1 = 0.85 \pm 0.039$, n = 8) was increased by prazosin ($b_2/b_1 = 1.54 \pm 0.12$, $P<0.01$, n = 4).

Discussion

The present results show that electrical field stimulation induced tritium release in cat cerebral arteries preincubated with [^{3}H]noradrenaline (Endo et al 1977; Duckles & Rapoport 1979; Göthert et al 1984). The stimulated release was reduced by the α_2-adrenoceptor agonists, clonidine and B-HT 920 (Hammer et al 1980; Kobinger & Pichler 1980; Starke & Docherty 1981; Docherty 1989). However, in bovine cerebral arteries, the stimulated noradrenaline secretion was reduced by B-HT 920, but not by clonidine, even at high concentration, showing a lack of intrinsic activity in this tissue. Both the incapacity (Kalsner 1985; Kawasaki et al 1989) and the ability of clonidine (Sakakibora et al 1982; Göthert et al 1984; Kalsner 1985; Ellis et al 1990) and B-HT 920 (Göthert et al 1984; Hentrich et al 1986) to inhibit the stimulated [^{3}H]noradrenaline release in different tissues, including blood vessels, have been reported; the potency of the latter agonist is greater than the former (Göthert et al 1984; Hentrich et al 1986; Balfagón & Marín 1989; Sánchez-Merino et al 1990), as occurs in our vascular preparation. This major effect of B-HT 920 could be related to a greater selectivity and efficacy at the level of α_2-adrenoceptors (Hammer et al 1980; Kobinger & Pichler 1980). Nevertheless, agonistic activity on the presynaptic dopamine receptors in the central nervous system in-vivo has been reported (Anden et al 1982; Pifl et al 1988), which could contribute to the total action of B-HT 920, since the activation of these receptors also decreased the stimulated tritium release (Dubocovich & Langer 1980; Fuder & Muschol 1978). The fact that clonidine partially antagonized the inhibitory effect of B-HT 920 on stimulated radioactivity release in bovine cerebral arteries, indicates an α_2-antagonist component of clonidine and the lack of an agonist component in these vessels. However, this effect was not observed in cat cerebral arteries in which clonidine acts as a full agonist. The partial α-agonist action of clonidine, among other actions, has been reported (Kalsner 1985; Docherty 1989; McGrath et al 1989).

The classical α_2- (yohimbine and rauwolscine) and α_1- (prazosin) adrenoceptor antagonists (Starke & Docherty 1981; Langer & Hicks 1984; Docherty 1989) blocked the inhibitory action of clonidine (in cat cerebral arteries only) and B-HT 920 (mainly at 0·01 μM, because at 0·1 μM only rauwolscine showed a clear effect). The fact that these antagonists reversed the action of B-HT 920 indicates that this agent has agonistic action on α_2-receptors, although other actions, previously mentioned, could contribute to the effect. In human saphenous vein, the inhibitory effect of clonidine and B-HT 920 on [^{3}H]noradrenaline release was antagonized by rauwolscine, but not by prazosin (Göthert et al 1984). In rat cerebral cortex, noradrenaline blocked the

stimulated tritium secretion which was antagonized by yohimbine, but not by prazosin (Raiteri et al 1983), illustrating the involvement of presynaptic α_2-adrenoceptors. Our results also indicate the existence of these latter receptors in cat and bovine cerebral arteries, as we observed in human cerebral arteries (Sánchez-Merino et al 1990). The fact that prazosin antagonizes the action of α_2-agonists suggests that α_1-receptors could be involved in noradrenaline release, as reported for other tissues (Kobinger & Pichler 1982; Docherty 1984; Story et al 1985). Nevertheless, this possibility should be ruled out, as methoxamine, a potent and selective α_1-adrenoceptor agonist (Starke & Docherty 1981; Langer & Hicks 1984; Docherty 1989) failed to modify the stimulated tritium release in both kinds of vessels.

The effect of prazosin in brain vessels can be explained taking into account the results obtained in binding experiments using labelled α_2-antagonists. These experiments show that α_2-receptors constitute a heterogeneous population (Feller & Bylund 1984; Kawahara & Bylund 1985; Alabaster et al 1986), which is formed by, at least, two subclasses α_{2A}- and α_{2B}-adrenoceptors (Petrash & Bylund 1986; Bylund 1988; Bylund et al 1988; Young et al 1989); prazosin specifically blocks α_{2B}-receptors (in addition to α_1-receptors), yohimbine possesses full potency at α_{2A} and little less for α_{2B}, and rauwolscine has similar affinity and potency at both subtypes; the agonists clonidine and noradrenaline are non-selective (Bylund et al 1988). Prazosin antagonized the action of the α_2-agonists, with a potency even higher than yohimbine and less than rauwolscine, which suggests the existence of mainly α_{2B}-adrenoceptors on the perivascular adrenergic nerve terminals of these vessels, which mediate the noradrenaline release. In rat submandibular gland, the stimulated noradrenaline liberation was antagonized by prazosin and also by yohimbine with a 6-fold potency (Turner et al 1984). Also prazosin inhibits the α_2-receptors on noradrenergic nerve endings of rat and mouse vas deferens, but has no action on these receptors on cholinergic nerve endings (Kapocsi et al 1987), which suggests the presence of α_{2B}- and α_{2A}-receptors in these terminals, respectively. In addition, prazosin and yohimbine block the inhibition caused by the exogenous noradrenaline on K^+-evoked [^{3}H]noradrenaline from rat cerebral cortex with an apparent potency (K_B values) of 400 and 91 nM, respectively (Nasseri & Minneman 1987) indicating the existence of α_{2B}-receptors in this preparation. Phentolamine (an α_1- and α_2-blocker), rauwolscine, prazosin, yohimbine and yohimbine plus prazosin did not modify the electrical stimulation-evoked tritium secretion in bovine cerebral arteries. However, in cat cerebral arteries, phentolamine (Balfagón & Marín 1989), rauwolscine, and yohimbine plus prazosin increased the stimulated tritium liberation. These results suggest the existence of a major tonic activity of presynaptic α_2-adrenoceptors in the latter vessels. The association of yohimbine and prazosin produced an effect similar to that found with rauwolscine and phentolamine, which block α_{2A}- and α_{2B}-receptors, indicating the existence of a mutual influence of both receptors on noradrenaline release, especially in cat cerebral arteries. These antagonists also produce an enhancement of [^{3}H]noradrenaline release in different tissues, e.g. rauwolscine in rabbit pulmonary artery (Nedergaard 1988); yohimbine in dog renal arteries (Sakakibora et al 1982), guinea-pig vas deferens (Ellis et al 1990) and rat cerebral cortex (Nasseri & Minneman 1987); prazosin in guinea-pig vas deferens (Ellis et al 1990) and rat cerebral cortex (Nasseri & Minneman 1987) and little effect on dog saphenous vein (Baker et al 1984).

The spontaneous tritium release was not modified by clonidine, nor by B-HT 920, phentolamine or methoxamine, but it was increased by prazosin in both kinds of arteries and by rauwolscine only in cat cerebral arteries. This stimulatory effect of prazosin has been observed in other vessels (Anderson et al 1979; Mishima et al 1984; Skärby 1984) and guinea-pig vas deferens (Ellis et al 1990), and it appears to be due to the already described reserpine-like effect (Anderson et al 1979; Mishima et al 1984). The lack of effect of methoxamine and clonidine on this basal release has also been described in human saphenous veins, whereas B-HT 920 caused a small inhibition (Göthert et al 1984). Phentolamine produces a slight increase in cattle radial artery (Kalsner & Chan 1979) and no change in mesenteric artery (Mishima et al 1984). The effect of rauwolscine or prazosin on basal release of these arteries suggests the existence of a tonic activity of presynaptic α_2-receptors as these antagonists inhibit the feedback inhibitory action increasing the noradrenaline release.

In conclusion, the present results, which support those obtained in binding studies, indicate that cat and bovine cerebral arteries possess functional presynaptic α_2-adrenoceptors, mainly of the subtype α_{2B}, which are involved in noradrenaline release inhibition. The activity of these receptors in cat cerebral arteries appears to be higher than that in bovine cerebral arteries in which clonidine has no agonistic action but does have antagonistic action.

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