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Different α-adrenoceptors modulate the release of 5-hydroxytryptamine and noradrenaline in rat cortex

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1 The potassium-evoked release of [^{3}H]-noradrenaline from slices of rat occipital cortex and the potassium-evoked release of [^{3}H]-5-hydroxytryptamine from slices of rat frontal cortex were measured using a superfusion system.

2 The rank order of potency for a number of α-adrenoceptor agonists was different for the two neuronal systems, clonidine and azepexole being the most potent inhibitors of noradrenaline release and methoxamine and phenylephrine being the most potent against 5-hydroxytryptamine release.

3 The rank order of potency for a series of α-adrenoceptor antagonists in reversing the inhibition of noradrenaline release produced by clonidine was: phentolamine > rauwolscine = yohimbine = corynanthine >> WB4101, whereas against methoxamine-inhibition of 5-hydroxytryptamine release the rank order of potency was: WB4101 > phentolamine > corynanthine > yohimbine > rauwolscine.

4 The results suggest that the α-adrenoceptors which modulate potassium-evoked 5-hydroxytryptamine release are not identical with the α_2-adrenoceptors located on noradrenergic nerve terminals and may more closely resemble α_1-than α_2-adrenoceptors.

Introduction

α-Adrenoceptors were originally subdivided into two classes on the basis of their anatomical location. Thus the 'post-synaptic' α-adrenoceptors which mediate smooth muscle contraction were termed α_1 and those which were 'pre-synaptic' and reduced the output of neurotransmitter from nerve terminals were termed α_2 (Berthelsen & Pettinger, 1977). The development of selective agonists and antagonists for the two subclasses of receptor, however, has revealed that this anatomical classification is simplistic and in particular that α_2-adrenoceptors may be located post-junctionally in some peripheral tissues (McGrath, 1982).

The terms 'pre and post-synaptic' are even less relevant in the central nervous system but by the use of specific agonists and antagonists, α_2-adrenoceptors have been shown to be present on noradrenergic nerve terminals in the rat cortex and hippocampus (Taube, Starke & Borowski, 1977; Frankhuyzen & Mulder, 1982). These α_2-adrenoceptors appear to resemble closely the α_2-adrenoceptors that have been characterized in the periphery. Frankhuyzen & Mulder (1982) have also shown that the 5-hydroxytryptamine (5-HT) nerve terminals in the hippocampus are endowed with α_2-adrenoceptors. These receptors, whilst superficially resembling the α_2-adrenoceptors on noradrenergic nerve terminals, were not identical to them in that the rank order of intrinsic activities of agonists was different for the two receptors.

Gothert, Huth & Schlicker (1981) have also identified α-adrenoceptors on 5-HT nerve terminals in the occipitoparietal cortex and have classified them as being of the α_2-subtype. The present study was undertaken in order to investigate whether the 5-HT nerve terminals in the frontal cortex, previously shown to possess presynaptic 5-HT receptors (Mounsey, Brady, Carroll, Fisher & Middlemiss, 1982), were also endowed with α-adrenoceptors.

The characteristics of the α_2-adrenoceptors on the noradrenergic terminals in the occipital cortex were used for comparison.

Methods

In each experiment two male Sprague-Dawley rats (160–200 g) were killed by decapitation, the frontal or occipital cortex dissected out and chopped into 250 × 250 μm slices with a McIlwain tissue chopper.

The brain slices were incubated at 37°C in Krebs-Henseleit solution containing ascorbic acid (2×10^{-4}M), pargyline (10^{-6}M) and ^{3}H-neurotransmitter (10^{-7}M), either [^{3}-H]-5-HT (frontal cortex) or [^{3}H]-noradrenaline (NA) (occipital cortex). After 20 min the slices were washed and equal aliquots placed in each of 10 superfusion chambers, approximately 25 mg tissue per chamber. The volume of the superfusion chamber was 300 μl. The slices were superfused with previously oxygenated Krebs-Henseleit solution at 37°C at a rate of 0.4 ml min^{-1}. Superfusate fractions were collected every 4 min and the radioactivity in each fraction together with that remaining in the tissue at the end of the experiment was determined by liquid scintillation counting.

Two pulses of Krebs-Henseleit solution containing 25 mM KCl obtained by iso-osmotic replacement of NaCl with KCl, were administered for 4 min, 42 (S_1) and 66 (S_2) min after the start of the superfusion. Drugs were added to the superfusing medium immediately after S_1. The percentage efflux of tritium was calculated as the fractional release, i.e. the radioactivity in each superfusate fraction was divided by the amount of radioactivity present in the slices at the start of that collection period. Basal or spontaneous release was taken as the fractional release occurring immediately prior to S_1 and immediately prior to S_2. The total percentage of radioactivity released above the basal values by the two pulses of potassium is expressed as the ratio S_2/S_1 for both control and drug-treated slices. The S_2/S_1 ratio for drug-treated slices is expressed as a percentage of the control S_2/S_1 ratio. In each experiment designed to determine agonist potency, individual concentration-effect curves were obtained from which the maximum response, slope and pD_2 value were calculated. In each experiment designed to determine antagonist potency, three concentrations of agonist from the linear portion of the concentration-effect curve were tested alone and in the presence of increasing concentrations of antagonist. The shift in the agonist concentration-effect curve was measured at the EC_{50} level. The pA_2 value was calculated using 4 concentrations of antagonist according to the method of Arunlakshana & Schild (1959). Statistical analysis of the results was performed by the Mann Whitney U test, 2 tailed.

The drugs used were: ascorbic acid (B.D.H.), azepexole HCl (Boehringer Ingleheim), chlorimipramine HCl (Ciba-Geigy, Ltd), clonidine HCl (Boehringer Ingleheim), corynanthine HCl (Roth, FDR), [^{3}H]-5-hydroxytryptamine creatinine sulphate (sp. act., 20 Ci mmol^{-1}, New England Nuclear), methoxamine HCl (Wellcome Foundation), [^{3}H]-noradrenaline bitartrate (sp. act., 32 Ci mmol^{-1}, Amersham International), pargyline HCl (Sigma), phentolamine mesylate (Ciba Laboratories), phenylephrine HCl (Sigma), rauwolscine HCl (Roth, FDR) WB 4101 HCl (N-[2-(2,6 dimethoxyphenoxy) ethyl]-1, 4 benzodioxane-2-methylamine) (Ward-Blenkinsop & Co.), yohimbine HCl (Sigma).

Results

[^{3}H]-noradrenaline release

The spontaneous release of tritium from slices of occipital cortex preloaded with [^{3}H]-NA was initially rapid but slowed and became constant after 30 min of superfusion with Krebs-Henseleit solution at $0.8 \pm 0.06\%$ min^{-1}. Superfusion with Krebs-Henseleit solution containing 25 mM K^+ increased the rate of release of tritium to a maximum of $1.54 \pm 0.09\%$ min^{-1} during S_1 and to $1.08 \pm 0.07\%$ min^{-1} for S_2. The S_2/S_1 ratio was 0.83 ± 0.03 ($n = 40$). The K^+-evoked release of tritium but not the spontaneous release was calcium-dependent; the S_2/S_1 ratio in the absence of calcium was 0.14 ± 0.07 ($n = 4$).

Effect of α-adrenoceptor agonists Clonidine (3×10^{-9} to 10^{-6}M) produced a concentration-related inhibition of K^+-evoked tritium release by a maximum of 70%. The pD_2 value was 7.12 ± 0.05 ($n = 12$).

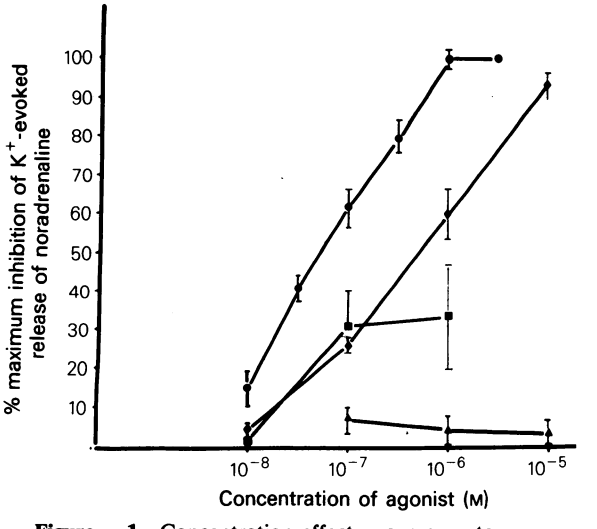

Figure 1 Concentration-effect curves to α-adrenoceptor agonists for the inhibition of potassium-evoked tritium release from slices of rat occipitoparietal cortex preloaded with [^{3}H]-noradrenaline. Each point represents the mean of at least 4 determinations: clonidine (●), azepexole (◆), methoxamine (■) and phenylephrine (▲). Vertical bars indicate s.e.mean.

Table 1 pA_2 values for the antagonism of clonidine-induced inhibition of K^+-evoked noradrenaline release and methoxamine-induced inhibition of K^+-evoked 5-hydroxytryptamine release

	Clonidine		*Methoxamine*	
Antagonist	*pA_2*	*Slope*	*pA_2*	*Slope*
Yohimbine	7.70±0.02	1.1±0.3	7.05±0.36	1.0±0.2
Rauwolscine	7.61±0.30	1.3±0.1	6.07±0.56	0.7±0.4
Corynanthine	7.52±0.12	0.8±0.1	7.54±0.21	1.2±0.1
Phentolamine	8.50±0.60	0.8±0.2	7.96±0.16	0.9±0.3
WB 4101	<6.5	—	8.29±0.09	0.9±0.2

Results are expressed as mean ±s.e.mean $n = 4$.

Azepexole produced a similar inhibition of K^+-evoked tritium release with a pD_2 value of 6.28±0.08 ($n = 4$), whereas phenylephrine and methoxamine were inactive in concentrations up to 10^{-5}M. These results are shown in Figure 1. Phenylephrine (10^{-5}M) significantly increased the spontaneous release of tritium, but none of the remaining compounds tested affected this parameter.

Effect of α-adrenoceptor antagonists on the inhibition of K^+-evoked tritium release produced by clonidine

Rauwolscine, yohimbine, corynanthine and phentolamine all produced parallel rightward shifts in the concentration-effect curve to clonidine over the concentration range 3×10^{-9} to 10^{-7}M. The pA_2 values are shown in Table 1 and an example of the antagonism of the response to clonidine by yohimbine is shown in Figure 2. The antagonist WB 4101 was without effect in concentrations up to 3×10^{-7}M. Similarly the 5-HT receptor antagonist methiothepin was inactive in concentrations up to 10^{-6}M.

[^{3}H]-5-hydroxytryptamine release

The spontaneous release of tritium from slices of frontal cortex preloaded with [^{3}H]-5-HT was initially rapid but became constant after 30 min of superfusion with Krebs-Henseleit solution, containing chlorimipramine (5×10^{-6}M), at 0.85±0.05% min^{-1} ($n = 30$). Superfusion with 25 mM K^+ increased the release of tritium to a maximum of 1.38±0.09% min^{-1} during S_1 and to 1.14±0.07% min^{-1} during S_2. The S_2/S_1 ratio was 0.87±0.03 ($n = 30$). The K^+-evoked but not the spontaneous release of tritium was Ca^{2+}-dependent. The S_2/S_1 ratio in the absence of calcium was 0.12±0.08 ($n = 4$).

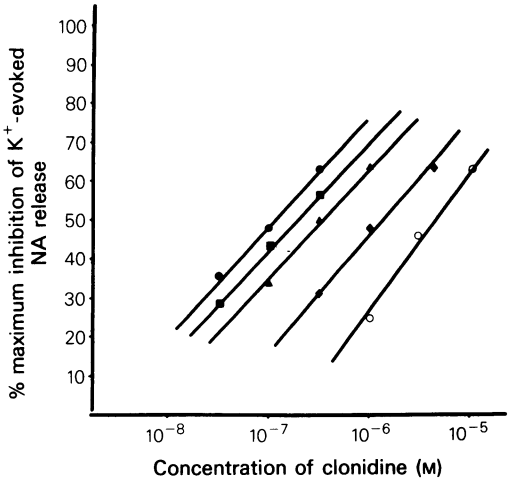

Figure 2 Calculated concentration-effect regression lines for clonidine alone (●) and in the presence of yohimbine 10^{-8}M (■), 3×10^{-8}M (▲), 10^{-7}M (◆) and 3×10^{-7}M (○) for the inhibition of the potassium-evoked release of tritium from slices of rat occipitoparietal cortex, preloaded with [^{3}H]-noradrenaline.

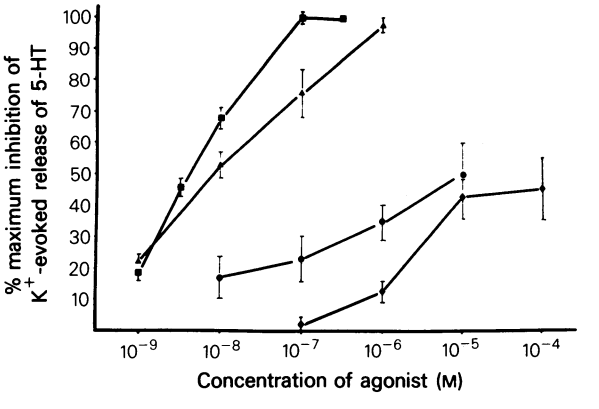

Figure 3 Concentration-effect curves to α-adrenoceptor agonists for the inhibition of potassium-evoked tritium release from slices of rat frontal cortex preloaded with [^{3}H]-5-hydroxytryptamine. Each point represents the mean of at least 4 determinations: clonidine (●), azepexole (◆), methoxamine (■) and phenylephrine (▲). Vertical bars indicate s.e.mean.

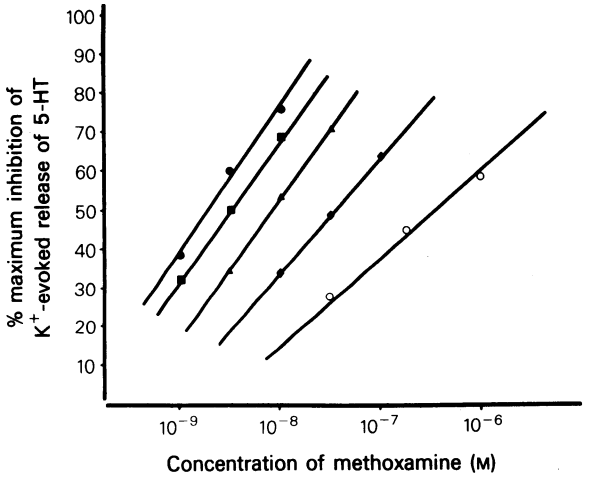

Figure 4 Calculated concentration-effect regression lines for methoxamine alone (●) and in the presence of WB 4101 3×10^{-9}M (■), 10^{-8}M (▲), 3×10^{-8}M (◆) and 10^{-7}M (○) for the inhibition of the potassium-evoked release of tritium from slices of rat frontal cortex preloaded with [^{3}H]-5-hydroxytryptamine.

Effect of α-adrenoceptor agonists Methoxamine (10^{-9} to 10^{-7}M) produced a dose-related inhibition of K^+-evoked tritium release by a maximum of 70%. The pD_2 value was 8.37 ± 0.07 ($n = 16$). These results are shown in Figure 3. Phenylephrine produced a similar concentration-related inhibition of release whilst clonidine and azepexole were only active at high concentrations and never achieved the same maximum effect as methoxamine. The pD_2 values for clonidine and azepexole were 5.22 ± 0.40 ($n = 4$) and 4.21 ± 0.30 ($n = 4$) respectively. None of the compounds affected the spontaneous release of tritium.

Effect of α-adrenoceptor antagonists on the inhibition of K^+-evoked tritium release produced by methoxamine WB 4101, phentolamine, corynanthine, yohimbine and rauwolscine all produced parallel rightward shifts in the concentration-effect curve to methoxamine over the concentration range 3×10^{-9}M to 10^{-6}M. The pA_2 values obtained are shown in Table 1 and an example of the antagonism of methoxamine by WB 4101 in Figure 4. The 5-HT receptor antagonist methiothepin had no effect in concentrations up to 10^{-6}M.

Discussion

The K^+-evoked release of tritium from slices of rat cortex preloaded with either [^{3}H]-NA or [^{3}H]-5-HT was inhibited by a number of α-adrenoceptor agonists. However, the relative order of potency for the agonists, was different for the two neuronal systems. Thus the order of potency for the inhibition of noradrenaline release was clonidine > azepexole >> methoxamine = phenylephrine, and for 5-HT was methoxamine > phenylephrine > clonidine > azepexole. Clonidine and azepexole, which show preferential activity at the α_2-subtype of adrenoceptor (Pichler, Placheta & Kobinger, 1980; Starke, 1981), were 100 times more potent as inhibitors of K^+-evoked NA than 5-HT release. Conversely, methoxamine and phenylephrine, which are selective for the α_1-subtype of adrenoceptor, were ineffective in concentrations up to 10^{-5}M as inhibitors of K^+-evoked NA release but were potent inhibitors of 5-HT release.

The finding that methoxamine was more potent than clonidine in inhibiting K^+-evoked 5-HT release is in contrast to the result obtained by Gothert *et al.* (1981) who showed clonidine and BHT 920 to be more potent than methoxamine and phenylephrine. It is difficult to explain these discrepancies other than by postulating differences in the subtypes of α-adrenoceptor located on 5-HT neurones in the frontal and occipitoparietal cortex or in the mechanism of transmitter release evoked by electrical and potassium stimulation.

The α-adrenoceptor antagonists also proved to have differing orders of potency in reversing the clonidine-induced inhibition of K^+-evoked NA release and the methoxamine-induced inhibition of K^+-evoked 5-HT release. Thus, against clonidine the order of antagonist potency was phentolamine > rauwolscine = yohimbine = corynanthine >> WB 4101 and against methoxamine was WB 4101 > phentolamine > corynanthine > yohimbine > rauwolscine. However, there appear to be some discrepencies in the relative orders of potency obtained in the present study with those reported for α_1 and α_2-adrenoceptors in the periphery. Thus, corynanthine is usually considered to be less potent than rauwolscine and yohimbine at α_2-adrenoceptors (Starke, 1981) whilst in the present study the two agents were equipotent. Furthermore, phentolamine was more potent than yohimbine as an antagonist of clonidine. This result confirms the findings of Delini-Stula, Baumann & Büch, (1979) who found phentolamine to be more potent than yohimbine in increasing tritium overflow from slices of rat cortex preloaded with [^{3}H]-NA. In contrast, yohimbine was more potent than phentolamine in increasing tritium overflow in the rabbit pulmonary artery (Borowski, Starke, Ehrl & Endo, 1977). Thus there appear to be some differences between the α_2-adrenoceptors in the CNS and those in the periphery. It may be more circumspect, therefore, to characterize the receptors with which clonidine interacts in the CNS as more

closely resembling peripheral α_2 than α_1-adrenoceptors. It is also possible that there may be a number of different receptor types on the same neuroterminal so the order of antagonist potency would then reflect the mean effect of the antagonists at these receptors.

It has been reported that the stereoisomers of yohimbine are potent antagonists at 5-HT receptors (Lambert, Lang, Friedman, Meller & Gershon, 1978). Since presynaptic 5-HT receptors have been shown to be present on the 5-hydroxytryptaminergic nerve terminals in the frontal cortex of the rat (Mounsey *et al.*, 1982) and on noradrenergic nerve terminals in the cortex (Taube *et al.*, 1977) the effect of the 5-HT antagonist, methiothepin, was investigated. Methiothepin has been shown to be active at either postsynaptic (S_1) or autoreceptors (S_2) in rat brain (Ennis & Cox, 1982). In the present study, methiothepin in concentrations up to 10^{-6}M was ineffective as an antagonist of either clonidine or methoxamine indicating that these agonists were not acting at a 5-HT receptor to inhibit ^{3}H-neurotransmitter release. In conclusion, the results of the present study demonstrate that the release of 5-HT in the frontal cortex of the rat is modulated by α-adrenoceptors. However, the adrenoceptor mediating this effect is not identical with the α-adrenoceptor located on noradrenergic nerve terminals in the rat cortex and appears to resemble more closely the α_1-adrenoceptor subtype. Since Gothert *et al.* (1981) demonstrated the presence of α_2-adrenoceptors on the 5-HT nerve terminals in the occipitoparietal cortex, the discrepency with the results of the present study may reflect differences in the noradrenergic innervation in various regions of the rat cortex.

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