J. Pharm. Pharmacol. 1983, 35: 750–751
Communicated June 8, 1983

The release of 5-hydroxytryptamine in the occipital and frontal cortex is modulated by different subtypes of α-adrenoceptor

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It has previously been reported that the potassium evoked release of 5-hydroxytryptamine (5-HT) from slices of rat frontal cortex was modulated by α-adrenoceptors which closely resemble the α_1-subtype (Ennis 1983). This finding however did not agree with those of Gothert et al (1981) who reported that the electrically evoked release of 5-HT from slices of rat occipital cortex was modulated by α_2-adrenoceptors. There are therefore two variables which could account for the discrepency between the results of the two studies, i.e. (a) the region of the cortex that was used and (b) the method employed to depolarize the nerve terminals. The present study was designed to identify the type of α-adrenoceptor that modulates the release of 5-HT from slices of rat occipital cortex evoked by high external potassium concentrations, in an attempt to distinguish between these two variables.

Method

The method used has been extensively described (Ennis 1983). Briefly, slices (250 × 250 μm) of rat occipital or frontal cortex were preloaded with [^{3}H]5-HT, washed and superfused with oxygenated Krebs-Henseleit solution at 37 °C at a rate of 0·4 ml min^{-1}. Two pulses of Krebs-Henseleit solution containing 25 mM K^+, obtained by iso-osmotic replacement of NaCl with KCl, were administered for 4 min, at 42 (S_1) and 66 (S_2) min after the start of the superfusion. 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. Modifying drugs were added to the superfusion medium immediately after S_1. The fractional release of tritium was calculated. Spontaneous release was taken as the fractional release occurring immediately before S_1 and S_2. The change in percentage of tissue radioactivity released above the spontaneous level by the two pulses of potassium was expressed as the ratio S_2/S_1 and the S_2/S_1 ratio for drug treated slices was expressed as a percentage of the control S_2/S_1 ratio. Concentration-effect curves to the agonists were constructed to allow comparison of maximum effect and potency. Determinations of pA_2 values for antagonists were performed according to the method of Arunlakshana & Schild (1959). Statistical significance of results was assessed using the Mann-Whitney U test, 2 tailed. The compounds used were: ascorbic acid (BDH), [^{3}H]5-hydroxytryptamine creatinine sulphate (spec. act. 20 Ci mmol^{-1}, New England Nuclear), methoxamine HCl (Wellcome Foundation), pargyline HCl (Sigma), tetrodotoxin (Sankyo), WB 4101 (Ward Blenkinsop), yohimbine HCl (Sigma).

Results

The release of tritium from slices of rat occipital cortex was initially rapid but became constant at 0·47 ± 0·03% total tissue radioactivity released per minute after 30 min of superfusion. Addition of 25 mM potassium to the superfusing medium increased the release of tritium to a maximum of 0·82 ± 0·046% min^{-1} for S_2. The increase in release persisted for 12 min and then returned to spontaneous values. The S_2/S_1 ratio was 0·82 ± 0·05 (n = 9). The potassium evoked release, but not the spontaneous release, was calcium-dependent.

Clonidine produced a concentration-related inhibition of potassium evoked tritium release with a maximum effect of 60% at 10^{-6} M. The concentration which produced a 25% inhibition of release was 10^{-8} M. Methoxamine also produced an inhibition of release but the maximum effect obtained was only 30% at 10^{-5} M. From a linear regression analysis of the linear portion of

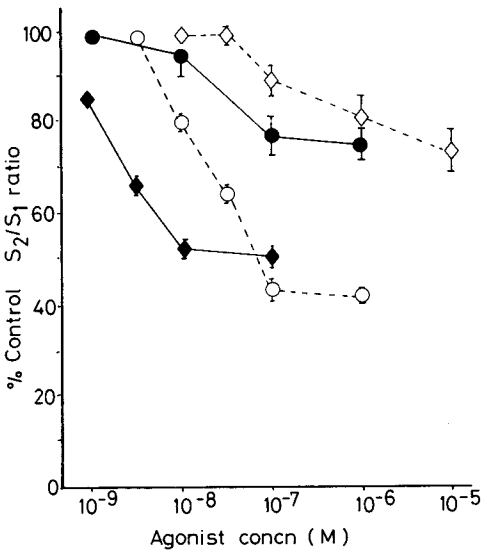

FIG. 1. Concentration-effect curves for methoxamine (◆) and clonidine (●) for the inhibition of potassium-evoked [^{3}H]5-HT release from slices of rat occipital cortex (open symbols) and frontal cortex (closed symbols). Each point represents the mean of at least 4 determinations and the vertical bars the standard error of the mean.

Table 1. Comparison of the potency of clonidine and methoxamine to inhibit the release of 5-HT from slices of rat occipital and frontal cortex.

	Frontal cortex			
Agonist	IC25 (nM)	Max. effect	Slope	s.e.m.
Clonidine	280 (70–500)	30%	9·8	1·9
Methoxamine	2·0 (0·9–3·0)	50%	30·5	0·8
	Occipital cortex			
	IC25	Max. effect	slope	s.e.m.
Clonidine	10 (3–20)	60%	36·1	4·9
Methoxamine	3000 (700–40 000)	30%	9·8	0·6

Figures in brackets refer to 95% confidence limits (n = 4).

the concentration-effect curve the concentration producing a 25% inhibition of release was 3×10^{-6} M. These results are shown in Table 1 and are compared with those previously obtained using slices of rat frontal cortex. The concentration-effect curves are shown in Fig. 1.

The concentration-effect curve to clonidine for the inhibition of 5-HT release from slices of rat occipital cortex was shifted to the right in the presence of yohimbine. The pA_2 value for yohimbine was 7·80 ± 0·09 and the slope of the Arunlakshana-Schild plot was 0·9 ± 0·1 (n = 4). WB 4101 had no significant effect on the response to clonidine in concentrations up to 10^{-6} M. The effect of antagonists on the response to methoxamine was not investigated in view of the very shallow concentration-effect curve making it difficult to analyse any shift.

Tetrodotoxin (10^{-7} M) had no significant effect on the inhibition of tritium release from slices of rat occipital cortex produced by clonidine. The inhibition of tritium release produced by clonidine (3×10^{-8} M) was 30 ± 4·8% and in the presence of tetrodotoxin (10^{-7} M) was 31 ±4·2% (n = 4). Tetrodotoxin had no significant effect on the inhibition of tritium release from slices of rat frontal cortex produced by methoxamine. Thus, methoxamine alone produced 41 ± 7·4% inhibition of release and in the presence of tetrodotoxin 33 ± 7·0% (n = 4).

Discussion

The potassium evoked release of 5-HT from slices of rat occipital cortex was inhibited by clonidine and to a much lesser extent by methoxamine. The inhibition of release produced by clonidine was antagonized by the selective α-adrenoceptor antagonist yohimbine but not by the selective α-adrenoceptor antagonist WB 4101. These results appear to confirm the findings of Gothert et al (1981) who reported that the electrically evoked release of 5-HT from slices of rat occipital cortex was modulated by α_2-adrenoceptors. Under conditions that were virtually identical to those of the present study the release of 5-HT from slices of rat frontal cortex has been shown to be modulated by α_1-adrenoceptors (Ennis 1983). It would appear therefore that there are regional differences in the type of adrenoceptor involved in the modulation of 5-HT release in the rat cortex and that it is this difference rather than the method used to depolarize the cortex slices that is responsible for the discrepancies between the results of Gothert et al (1981) and those of Ennis (1983).

In order to investigate the location of the α-adrenoceptors the effect of tetrodotoxin was examined on the inhibition of 5-HT release produced by clonidine in the occipital cortex and methoxamine in the frontal cortex. The response to neither compound was significantly affected by the presence of tetrodotoxin suggesting that both types of α-adrenoceptor are located on the 5-HT nerve terminals.

Maura et al (1982) have recently reported that the potassium evoked release of 5-HT from synaptosomes prepared from whole cortex was modulated by α_2-adrenoceptors. This finding suggests that the α_1-subpopulation of adrenoceptors found in the frontal cortex represents only a small proportion of the total α-adrenoceptor population on 5-HT neurones in the cortex of the rat. Thus when whole cortex is used the effect of compounds on the α_1-adrenoceptors is probably masked by their effects on the more numerous α_2-adrenoceptors.

There is now evidence that the modulation of 5-HT release by both α_1- and α_2-adrenoceptors may occur in-vivo. Handley & Brown (1982) have reported that both α_1- and α_2-adrenoceptors can modify head-twitches induced by 5-hydroxytryptophan in mice.

REFERENCES

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. 14: 48–58

Ennis, C. (1983) Ibid. 79: 279–283

Gothert, M., Huth, H. Schlicker, E. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 317: 199–203

Handley, S. L., Brown, J. (1982) Neuropharmacol. 21: 507–510

Maura, G., Gemigrani, A., Raiteri, M. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 320: 272–275