Inhibition of noradrenaline release via presynaptic 5-HT$_{1B}$ receptors of the rat vena cava

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Summary. In the rat inferior vena cava preincubated with $^3$H-noradrenaline, the effects of nine serotonin (5-HT) receptor agonists and of eight antagonists (including two $\beta$-adrenoceptor blocking agents) on the electrically evoked $^3$H overflow were determined. 1. 5-HT, 5-carboxamidotryptamine, 5-methoxy-3(1,2,3,6-tetrahydropridine-4-yl)-1H-indole (RU 24969), 5-methoxytryptamine, N,N-dimethyl-5-HT, tryptamine and 5-aminotryptamine inhibited the evoked $^3$H overflow. The potencies of these agonists in inhibiting overflow were significantly correlated with their affinities for 5-HT$_{1B}$ binding sites, but not with their affinities for 5-HT$_{1A}$, 5-HT$_{1C}$ or 5-HT$_{2}$ binding sites. 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a 5-HT$_{1A}$ receptor agonist, and ipsapirone, a partial agonist at these receptors, did not inhibit overflow. 2. Cyanopindolol facilitated the evoked $^3$H overflow, an effect which was abolished by propranolol. The maximum inhibition of overflow obtainable with 5-HT was diminished by cyanopindolol. 3. The concentration-response curve for 5-HT was shifted to the right by metitepine, metergoline, quipazine, 6-chloro-2-(1-piperazinyl)pyrazine (MK 212) and propranolol which, given alone, did not affect $^3$H overflow. The apparent $pA_2$ values of these antagonists tended to be correlated with their affinities for 5-HT$_{1B}$ (but not 5-HT$_{1A}$, 5-HT$_{1C}$ or 5-HT$_{2}$) binding sites. Ketanserin, a 5-HT$_{2}$ receptor antagonist, and spiperone, which blocks 5-HT$_{2}$ and 5-HT$_{1A}$ but not 5-HT$_{1B}$ or 5-HT$_{1C}$ receptors, failed to antagonize the effect of 5-HT. These results suggest that the inhibitory presynaptic 5-HT receptors on the sympathetic nerve terminals of the rat vena cava appear to belong to the 5-HT$_{1B}$ subtype. Cyanopindolol may act as a partial agonist at these receptors, as it does at the facilitatory presynaptic $\beta$-adrenoceptors.

Key words: Noradrenaline release — Serotonin receptors — 5-HT$_{1B}$ receptors — Presynaptic receptors — Inferior vena cava

Introduction

Evidence is accumulating that the sympathetic nerve terminals of blood vessels are endowed with serotonin (5-HT) receptors, activation of which inhibits noradrenaline release. Such receptors have been identified, e.g., in the human and dog saphenous vein (McGrath 1977; Feniuk et al. 1979; Watts et al. 1981; Engel et al. 1983; Göthert et al. 1986b), the rat vena cava and arteria iliaca (Göthert et al. 1986a; Schlicker and Göthert 1987) as well as the rat mesenteric and renal vascular system (Su and Uruno 1985; Charlton et al. 1986).

The aim of the present study was to characterize these presynaptic 5-HT receptors pharmacologically and to determine the 5-HT receptor type and subtype to which they belong. Three main types of 5-HT receptors, termed 5-HT$_{1A}$, 5-HT$_{2}$ and 5-HT$_{3}$, are currently distinguished. The 5-HT$_{1}$ receptors have been further divided into three subtypes denoted as 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ (Bradley et al. 1986; Göthert 1986; Göthert and Schlicker 1987). In rat brain slices and synaptosomes, the release-inhibiting presynaptic 5-HT autoreceptor has been shown to be of the 5-HT$_{1B}$ subtype (Middlemiss 1984; Engel et al. 1986; Raiteri et al. 1986). Similarly, the inhibitory presynaptic 5-HT receptors on the vascular sympathetic nerves of the rat also appear to possess the characteristics of 5-HT$_{1}$ receptors (Charlton et al. 1986; Göthert et al. 1986a), but a clear-cut subclassification was not yet possible. Therefore, in this study, the rat inferior vena cava was used to determine the potencies of a rather large number of 5-HT receptor agonists in inhibiting noradrenaline release and of several antagonists in blocking the inhibitory effect of 5-HT. Then we examined whether these potencies were correlated with the affinities of the drugs to the various 5-HT sites previously determined in radioligand binding studies (Engel et al. 1986; Markstein et al. 1986).

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Methods

The cranial segment of the inferior vena cava of male Wistar rats weighing 250–300 g was dissected and ligated at both ends. Subsequently, the vein was incubated for 30 min in 1.5 ml physiological salt solution (37°C, composition see below) containing (–)[$\text{-ring-2,5,6-3H}1$ noradrenaline 0.1 μmol/l (specific activity 38.7–43.9 Ci/mmol) and then mounted vertically in an organ bath (tension adjusted to 0.5 g) between two parallel platinum electrodes (1.5 cm long), where the adventitial surface of the vein was superfused with $^3$H-noradrenaline-free physiological salt solution of 37°C at a rate of 2 ml/min. The composition of the
maximum effects in the presence and absence of the antagonists, respectively. $B$ is the concentration of the antagonist. 

$$pA_2 = \log (\frac{[E']}{{E}} - 1) - \log [B]$$

$[E']$ and $[E]$ are those concentrations which caused half maximum effects in the presence and absence of the antagonists, respectively. $[B]$ is the concentration of the antagonist.

Linear regression lines and correlation coefficients were calculated in order to detect and quantify correlations between various drug effects.

**Drugs used.** (-)-ring-2,5,6-3H]noradrenaline (New England Nuclear, Dreieich, FRG); desipramine HCl (Ciba-Geigy, Wehr, FRG); corticosterone, 5-methoxytryptamine HCl (5-OCH$_3$-T; Sigma, München, FRG); 5-hydroxytryptamine creatinine sulphate (5-HT), tryptamine HCl (T; Merek, Darmstadt, FRG); 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridyl)-1H indole succinate (RU 24969; Roussel Uclaf, Romainville, France); N,N-dimethyl-5-hydroxytryptamine binoxalate (N,N-(CH$_3$)$_2$-5-HT), 5-aminotryptamine hydroxynoalate (5-NH$_2$-T), 5-carboxamidotryptamine maleinate (N,N-(CH$_3$)$_2$-5-HT), 5-aminotryptamine hydroxynoalate (5-NH$_2$-T), 5-carboxamidotryptamine maleinate (5-COH$_3$-T), 8-hydroxy-2-(di-n-propylamino)tetrahydrobromide (8-OH-DPAT), (+)-cyanoindolol (base; Sandoz, Basel, Switzerland); ipsapirone hydrochloride (TVX 7821; Troponwerke, Köln, FRG); methetine maleate (Hoffmann-La Roche, Basel, Switzerland); quazipine (Miles, Elkhart, IN, USA); mertogline (Farmitalia, Milan, Italy); (+)-propranolol HCl (ICI, Plainstadt, FRG); 6-chloro-2-(1-piperaziny1)pyrazine HCl (MK 212; MSD, München, FRG); spiperone (Janssen, Beerse, Belgium). Drugs were dissolved in saline with the following exceptions: serotonin, T, 5-COH$_3$-T, 5-NH$_2$-T, N,N-(CH$_3$)$_2$-5-HT and 5-OH$_3$-T were dissolved in saline containing ascorbic acid, spiperone and mertogline in saline containing citric acid and methane sulfonic acid, respectively. The vehicles had no effects on basal or evoked tritium efflux.

**Results**

**Effects of 5-HT receptor agonists**

In the concentration range investigated the 5-HT receptor agonists listed in Table 1 (see below) did not affect basal 3H efflux from the inferior vena cava (not shown).

5-HT, tryptamine and 5-NH$_2$-T inhibited the electrically evoked 3H overflow in a concentration-dependent manner; a decrease by about 25–35% represented the maximum effect (Figs. 1, 2). Similar results were obtained with 3H-noradrenaline and superfused with 3H-noradrenaline-free solution containing desipramine (0.6 μmol/l) and corticosterone (40 μmol/l). Five periods of transmural electrical stimulation (240 impulses) at 0.66 Hz were applied (S$_1$ to S$_5$). The ratios of the 3H overflow evoked by S$_3$, S$_4$ or S$_5$ over that evoked by S$_2$ are given, expressed as percentages of the ratios obtained in the respective control experiments (in the latter, these ratios were in the range of unity). The 3H overflow evoked by S$_2$, e.g., in the experiments with 5-NH$_2$-tryptamine was 2.24±0.4 nCi (corresponding to 1.71±0.22% of tissue tritium). The agonist investigated was added to the superfusion fluid at increasing concentrations 9 min before and during S$_3$, S$_4$ and S$_5$ (5-HT 1 μmol/l and tryptamine 10 nmol/l were added in two separate series of experiments 9 min before and during S$_3$). Means±SEM of 7 experiments. **P<0.01 (compared to the corresponding controls)**

**Effects of antagonists**

At the concentrations investigated, the antagonists listed in Table 1 did not alter basal 3H efflux (not shown).