line (10^{-7} to 10^{-5} M) inhibited contractions evoked by electrical stimulation (0.3 to 4 Hz) of the post-ganglionic adrenergic nerves ($n = 5$) and by exogenous noradrenaline (5×10^{-9} to 5×10^{-6} M, $n = 5$). Amitriptyline (10^{-6} and 10^{-5} M) depressed contractions evoked by noradrenaline (5×10^{-8} M) to a significantly greater extent ($P < 0.05$) than contractions of equivalent amplitude evoked by electrical stimulation (0.6 Hz). In superfusion experiments, amitriptyline (5×10^{-6} M) increased the overflow of radioactivity and of [^{3}H]-noradrenaline from electrically stimulated (1 Hz) strips of vein both in the absence ($n = 5$) and presence ($n = 6$) of cocaine. Thus amitriptyline has a presynaptic effect, unrelated to uptake blockade, which increases the release of noradrenaline from the adrenergic nerve ending. This increase in the stimulated overflow of [^{3}H]-noradrenaline was abolished by phentolamine (10^{-5} M, $n = 6$), indicating that it was due to blockade of presynaptic α receptors by amitriptyline.

Exogenous acetylcholine (2.7×10^{-7} M) depressed the increase in tension and in the overflow of radioactivity and of [^{3}H]-noradrenaline from electrically stimulated (2 Hz) strips of vein ($n = 6$). This presynaptic inhibitory action of acetylcholine was significantly reduced ($P < 0.001$) by amitriptyline (10^{-6} M, $n = 6$). Exogenous histamine (10^{-5} M) also depressed the increase in the overflow of radioactivity from electrically stimulated (2 Hz) veins. This effect was not attenuated by amitriptyline (5×10^{-6} M, $n = 5$).

These results demonstrate that amitriptyline in the above concentrations is an antagonist of presynaptic α and muscarinic receptors. Blockade of these receptors could be important both in the central actions of the drug and in the genesis of the tachycardia and cardiac arrythmias often caused by amitriptyline (Moir, *et al.*, 1972).

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Preliminary characterisation of the presynaptic receptor for 5-hydroxytryptamine in dog isolated saphenous vein

W. FENIUK, P.P.A. HUMPHREY & A.D. WATTS

Pharmacology Department, Glaxo Group Research Limited (Ware Division), Ware, Hertfordshire

We have shown that 5-hydroxytryptamine (5-HT) inhibits contractile responses induced by electrical stimulation in dog saphenous vein and have suggested that this effect is mediated via a specific pre-synaptic 5-HT receptor (Feniuk, Humphrey & Watts, 1978). This receptor like the 5-HT receptor situated post-synaptically in this preparation, is only weakly blocked by the 5-HT antagonist methysergide (Feniuk, Humphrey & Watts, 1979). Indeed methysergide appears to be a partial agonist at the postsynaptic receptor (Apperley, Humphrey & Levy, 1977) and we have therefore investigated whether methysergide has agonistic activity on the presynaptic receptor.

Saphenous veins were removed from barbitone anaesthetised dogs and cut spirally into strips. These were stimulated electrically *in vitro* as described previously (Feniuk, *et al.*, 1978, 1979) and the inhibitory effects of 5-HT and methysergide examined. In other experiments we studied electrically stimulated tritium release following incubation with 1-[7-^{3}H]-noradrenaline (10 m Ci/0.67 $\times 10^{-6}$ mol/l) at 37°C for 2 h in a modified Krebs solution (Apperley, Humphrey & Levy, 1976) containing ascorbic acid (1.1×10^{-4} mol/l) and disodium EDTA (4.0×10^{-6} mol/l). Each strip was then transferred to a bath and washed to remove extracellular tritium using Krebs solution containing ascorbic acid, disodium EDTA, cocaine (3.0×10^{-5} mol/l), indomethacin (2.8×10^{-6} mol/l) and corticosterone (4.0×10^{-5} mol/l). Subsequently the bath was drained and refilled every 2 min and the amount of radioactivity in the Krebs measured by liquid scintillation counting. Each strip was stimulated electrically for 2 min at 2 Hz every 20 minutes. In some experiments [^{3}H]-noradrenaline was separated from the other tritiated metabolites using paper chromatography (Levin, 1973).

5-HT and methysergide inhibited contractions induced by electrical stimulation producing 50% inhi-

bition at $3.2 \pm 0.6 \times 10^{-8}$ mol/l ($n = 20$) and $1.7 \pm 0.4 \times 10^{-6}$ mol/l ($n = 24$) respectively (mean values ± s.e. mean) The inhibitory effects of 5-HT and methysergide were potentiated by cyproheptadine (1.0×10^{-6} mol/l) but were unaffected by haloperidol (1.0×10^{-6} mol/l), propranolol (1.0×10^{-6} mol/l), atropine (1.0×10^{-6} mol/l), mepyramine (1.0×10^{-6} mol/l) or cimetidine (1.0×10^{-5} mol/l). 5-HT (1.0×10^{-7} mol/l) and methysergide (3.0×10^{-6} mol/l) inhibited the electrically stimulated release of tritium by 78 ± 4% ($n = 6$) and 47 ± 7% ($n = 6$) respectively and these inhibitory effects were not antagonised by phentolamine (1.0×10^{-6} mol/l) which itself increased tritium release by about four fold, 5-HT and methysergide also inhibited the electrically stimulated release of [^{3}H]-noradrenaline.

Our results show that methysergide inhibits release of noradrenaline from noradrenergic nerves in dog saphenous vein possibly by activating the presynaptic receptor for 5-HT. This suggests that the presynaptic 5-HT receptor is similar to the postsynaptic 5-HT receptor in this preparation and that both are different from the classical D-receptor (see Apperley, Humphrey & Levy, 1977).

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Evidence for an autoreceptor-mediated presynaptic control of serotonin release in central nerve endings

F. CERRITO & M. RAITERI

Department of Pharmacology, Catholic University, Via Pineta Sacchetti 644, 00168 Rome, Italy

The release of noradrenaline from peripheral and central nerve endings is modulated by presynaptic α-autoreceptors (Langer, 1977; Starke, 1977). We have investigated whether a similar mechanism existed in serotonergic terminals, by analyzing the effect of extra-cellular 5-hydroxytryptamine (5-HT) on the release of [^{3}H]-5-HT previously accumulated by hypothalamic synaptosomes.

Crude synaptosomes, prepared from adult male Wistar rats, were prelabelled with [^{3}H]-5-HT (0.1 μM, 10 min at 37°C) and distributed in parallel superfusion chambers (Raiteri, Angelini & Levi, 1974). The superfusion media contained chlorimipramine (Cl–IMI, 5 μM) to prevent 5-HT uptake. 5-HT was added after 10 min superfusion with Cl–IMI-containing Krebs-Ringer medium and, 8 min later, the synaptosomes were depolarized with KCl (15 mM). Serotonin antagonists were present from the beginning of superfusion. The superfusion rate was 0.5 ml/min. The [^{3}H]-5-HT in each 1-min fraction and in the synaptosomes at the end of superfusion was measured after isolation on Biorex columns.

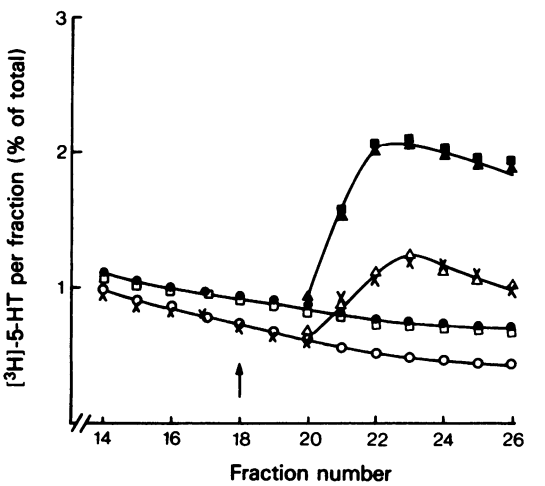

Figure 1 Spontaneous and high K^+-induced release of [^{3}H]-5HT from hypothalamic synaptosomes: inhibition by extracellular 5HT and antagonism between 5HT and methiothepin. (●) control (Cl-IMI present); (○) 0.5 μM 5HT; (□) 0.5 μM 5HT + 0.5 μM methiothepin; (▲) 15 mM KCl; (△) 15 mM KCl + 0.5 μM 5HT; (■) 15 mM KCl + 0.5 μM 5HT + 0.5 μM methiothepin; (×) 15 mM KCl + 0.5 μM 5HT + 1 μM cyproheptadine. Methysergide (1 μM) and mianserin (1 μM) behaved as cyproheptadine and are not shown in the Figure. The [^{3}H]-5HT present in each fraction is expressed as a percentage of the total [^{3}H]-5HT recovered (fractions + synaptosomes). The curves presented are averages of 3-5 quadruplicate experiments.