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PRESYNAPTIC INHIBITORY ACTION OF 5-HYDROXYTRYPTAMINE IN DOG ISOLATED SAPHENOUS VEIN

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1 The effect of 5-hydroxytryptamine on contractile responses to sympathetic nerve stimulation has been studied in the dog isolated saphenous vein.

2 Electrical stimulation (0.1 to 10 Hz) of dog saphenous vein strips produced frequency-dependent contractions. Contractions produced by stimulation at 2 Hz were almost completely blocked by tetrodotoxin (3.1×10^{-8} mol/l) or phentolamine (5.0×10^{-6} mol/l) but mecamylamine (5.0×10^{-6} mol/l) had little effect. This suggests that the contractions were mediated predominantly through noradrenaline release from postganglionic noradrenergic nerves.

3 Contractions produced by intermittent electrical stimulation at 2 Hz were inhibited by 5-hydroxytryptamine (1.0×10^{-9} to 1.0×10^{-7} mol/l) in a concentration-dependent manner whilst contractions induced by exogenous noradrenaline were not affected.

4 The inhibitory action of 5-hydroxytryptamine was most marked at low frequencies of stimulation and with low pulse numbers.

5 High external calcium concentrations (3.9 and 5.2×10^{-3} mol/l) reduced the inhibitory action of 5-hydroxytryptamine.

6 Cyproheptadine (1.0×10^{-8} mol/l to 1.0×10^{-6} mol/l) or morphine (1.0×10^{-7} mol/l to 1.0×10^{-5} mol/l) did not antagonize the inhibitory action of 5-hydroxytryptamine. Methysergide (1.0×10^{-7} mol/l) slightly reduced the contractions produced by electrical stimulation and only weakly antagonized the action of 5-hydroxytryptamine.

7 It is suggested that a 5-hydroxytryptamine receptor exists presynaptically in the dog isolated saphenous vein strip and that stimulation of this receptor by low concentrations of 5-hydroxytryptamine inhibits the release of noradrenaline from noradrenergic nerves. This receptor type is resistant to blockade by 'classical' 5-hydroxytryptamine antagonists.

Introduction

Several different types of pharmacologically distinct receptors appear to exist on the presynaptic terminals of sympathetic nerves in vascular smooth muscle. For example, noradrenaline and other α-adrenoceptor agonists (Starke, Endo & Taube, 1975), acetylcholine (Vanhoutte & Shepherd, 1973), prostaglandins (de la Lande, Hall, Kennedy & Higgins, 1975), histamine (McGrath & Shepherd, 1976) and adenosine (Verhaeghe, Vanhoutte & Shepherd, 1977) will all stimulate presynaptic receptors and inhibit noradrenaline release during periods of sympathetic nerve stimulation. However, until recently there was little evidence in the literature to demonstrate the existence of similar receptors for 5-hydroxytryptamine (see Westfall, 1977).

Nevertheless, it has been known for many years that the cardiovascular actions of 5-hydroxytryptamine in anaesthetized animals can depend upon the degree of activity of the sympathetic nervous system (McCubbin, Kaneko & Page, 1962). Furthermore, recent studies have demonstrated an inhibitory action of 5-hydroxytryptamine on contractions produced by electrical stimulation of dog isolated saphenous vein (McGrath, 1977, Feniuk, Humphrey & Watts, 1978). In this study we have analysed the pharmacological characteristics of this inhibitory action in the saphenous vein in more detail.

Methods

Preparation

Lateral saphenous veins were removed from dogs anaesthetized with barbitone and cut spirally into strips. Four preparations were obtained from each

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vein and each was suspended in Krebs solution between platinum electrodes approximately 0.5 cm apart. Isometric contractions were recorded as described previously (Apperley, Humphrey & Levy, 1976). The length of each preparation was adjusted initially to produce a tension of 0.5 g. The composition ($\times 10^{-3}$ mol/l) of the modified Krebs solution was: Na^+ 143.4, K^+ 5.9, Mg^{2+} 0.6, Ca^{2+} 1.3, Cl^- 124.5, $H_2PO_4^-$ 1.2, SO_4^{2-} 0.6, HCO_3^- 25.0 and glucose 11.1. The solution was continuously gassed with 95% O_2 and 5% CO_2 at 37°C. Indomethacin (2.8×10^{-6} mol/l) and cocaine (3.0×10^{-5} mol/l) were continually present in the Krebs solution in order to inhibit endogenous prostaglandin biosynthesis and the uptake$_1$ process for noradrenaline respectively. When the effects of varying the frequency of electrical stimulation, or varying the calcium concentration, were studied, cyproheptadine (1.0×10^{-6} mol/l) was also continually present in the Krebs to inhibit the contractile responses produced by 5-hydroxytryptamine.

The effect of 5-hydroxytryptamine on responses to electrical stimulation

The preparations were stimulated with square wave pulses of 0.1 ms pulse width using a Farnell physiological stimulator in conjunction with an Amcron (D150A) amplifier. In all experiments supramaximal voltages of about 30 V (measured between the electrodes) were used. In the majority of experiments (and unless otherwise stated in the text) each strip was stimulated intermittently at a frequency of 2 Hz for a period of 10 s every 180 s. This produced submaximal contractions. When constant responses were obtained to electrical stimulation, 5-hydroxytryptamine was added cumulatively to each of the baths and inhibitory concentration-effect curves determined.

The effect of 5-hydroxytryptamine on contractions produced by noradrenaline

In some experiments 5-hydroxytryptamine was administered approx. 60 s before a concentration of noradrenaline which produced a submaximal contraction. The contraction produced was compared with the contraction produced by the same concentration of noradrenaline administered in the absence of 5-hydroxytryptamine.

In a more detailed study, cumulative concentration-effect curves to noradrenaline (1.0×10^{-9} mol/l to 2.0×10^{-4} mol/l) were obtained in each of the preparations and then repeated in the presence of a single concentration of 5-hydroxytryptamine (1.0×10^{-8}, 1.0×10^{-7} or 1.0×10^{-6} mol/l). One preparation was used as a control and the noradrenaline concentration-effect curve was repeated in the absence of 5-hydroxytryptamine.

The effects of frequency and pulse number

Each preparation was stimulated electrically with either 10 or 100 pulses delivered at varying frequencies of 0.5, 2 and 5 Hz at 3 to 6 min intervals. When constant contractile responses were obtained at each frequency the inhibitory effect of a single concentration of 5-hydroxytryptamine (1.0×10^{-8} mol/l) was examined. Each frequency was studied in each preparation in a randomised order using a 3×3 Latin square design.

The effects of calcium

Inhibitory concentration-effect curves for 5-hydroxytryptamine were obtained in 4 preparations as described above. In three preparations the curves were re-determined after 30 min in Krebs containing three different concentrations of calcium. The fourth preparation was exposed to Krebs with normal calcium and therefore acted as a control to monitor any spontaneous changes in sensitivity of the preparation to 5-hydroxytryptamine. However the changes in sensitivity were always less than two fold in the control preparations.

The effects of antagonists

When the effects of antagonists were studied a similar experimental protocol to that described for the calcium studies (above) was employed. Inhibitory concentration-effect curves for 5-hydroxytryptamine were obtained in 4 preparations. Three were then exposed to Krebs containing three different concentrations of antagonist. The fourth was exposed to Krebs containing no antagonist and acted as a control.

Drugs used

The following drugs were used: atropine sulphate, mol. wt. 694.8 (BDH); cimetidine, mol. wt. 252.3 (Smith, Kline and French); cocaine hydrochloride, mol. wt. 339.8 (May and Baker); cyproheptadine hydrochloride, mol. wt. 350.9 (Merck, Sharp and Dohme); 5-hydroxytryptamine creatinine sulphate, mol. wt. 405.4. (Koch-Light); indomethacin, mol. wt. 375.5 (Sigma); mecamylamine hydrochloride, mol. wt. 203.8 (Merck, Sharp and Dohme); methysergide bimaleate, mol. wt. 469.5 (Sandoz); mepyramine maleate, mol. wt. 401.5 (May and Baker); (−)-noradrenaline bitartrate, mol. wt. 337.3 (Koch-Light); phentolamine mesylate, mol. wt. 337.5 (Ciba); propranolol hydrochloride, mol. wt. 295.8 (ICI); tetrodotoxin, mol. wt. 319.2 (Calbiochem).

All drugs were initially dissolved in distilled water with the exception of indomethacin (10% w/v sodium

bicarbonate solution) and (−)-noradrenaline bitartrate (1.14×10^{-3} mol/l ascorbic acid in isotonic saline). All subsequent dilutions were made with isotonic saline, except the noradrenaline solutions, which always contained ascorbic acid (1.14×10^{-3} mol/l).

Results

Electrical stimulation

Electrical stimulation of dog isolated saphenous veins produced frequency-dependent contractions. At 2 Hz the mean increase in tension (± s.e. mean) from 20 experiments was 0.75 ± 0.10 g. The α-adrenoceptor blocking agent, phentolamine (5.0×10^{-6} mol/l), inhibited contractions to intermittent stimulation at 2 Hz by 91 ± 2% (mean ± s.e. mean, $n = 15$), whilst the neuronal blocking agent, tetrodotoxin (3.1×10^{-8} mol/l) produced an 87 ± 4% inhibition ($n = 12$). The ganglion blocking agent, mecamylamine (5.0×10^{-6} mol/l), had little effect on these responses producing a 4 ± 3% potentiation ($n = 5$).

The effect of 5-hydroxytryptamine on responses to electrical stimulation

Contractile responses of saphenous vein strips caused by electrical stimulation at a frequency of 2 Hz were inhibited by 5-hydroxytryptamine in a concentration-dependent manner (Figure 1). The threshold concentration for this inhibitory action was approximately 1.0×10^{-9} mol/l. 5-Hydroxytryptamine also contracted the preparations, although the threshold concentration for contraction was about five times greater than the threshold concentration for inhibition of the electrically-induced contractions. The inhibitory action of 5-hydroxytryptamine was rapid in onset and lasted for as long as the 5-hydroxytryptamine remained in the bath, but on washing was readily reversed (Figure 1).

The effect of 5-hydroxytryptamine on contractile responses to exogenous noradrenaline

5-Hydroxytryptamine had no inhibitory effect on the submaximal contractions produced by a single dose of noradrenaline (Figure 1). The effect of 5-hydroxytryptamine on contractile responses to a range of nor-

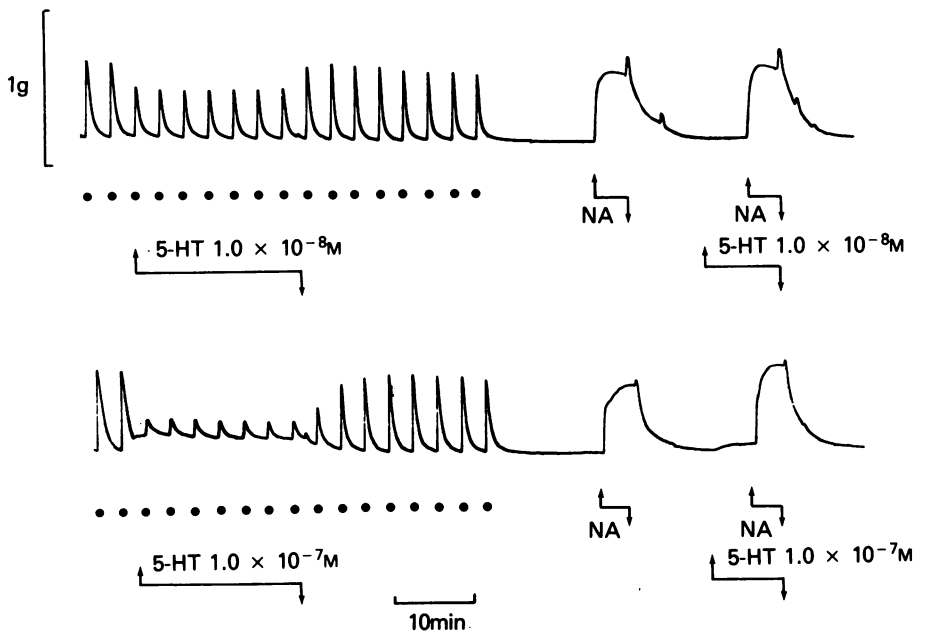

Figure 1 Dog isolated saphenous vein strip. The effects of 5-hydroxytryptamine (5-HT 1.0×10^{-8} mol/l and 1.0×10^{-7} mol/l) on contractile responses to electrical stimulation (●, 2 Hz for 10 s) or exogenous noradrenaline (NA, 1.0×10^{-7} mol/l). Upward arrows indicate addition of drug to the bath. Downward arrows indicate washing from the bath.

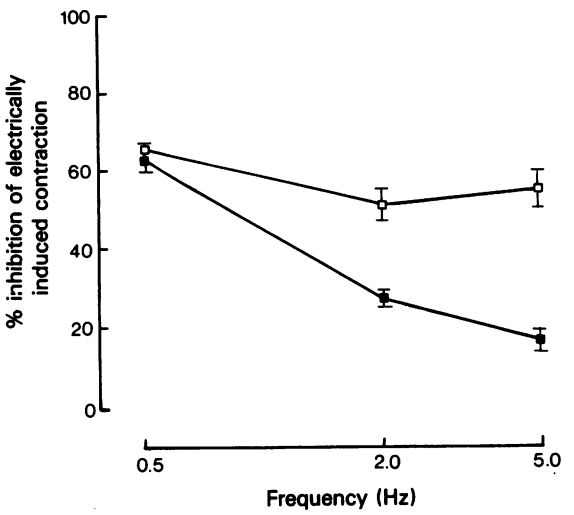

Figure 2 Dog isolated saphenous vein strip. The effect of frequency on the degree of inhibition produced by 5-hydroxytryptamine (1.0×10^{-8} mol/l) on contractile responses to electrical stimulation. Each frequency was studied in preparations with either trains of 10 pulses (□) or 100 pulses (■). The Krebs contained cyproheptadine (1.0×10^{-6} mol/l) to antagonize the contractile effects of 5-hydroxytryptamine (see text). Each point is the mean of 4 observations; vertical lines show s.e. mean.

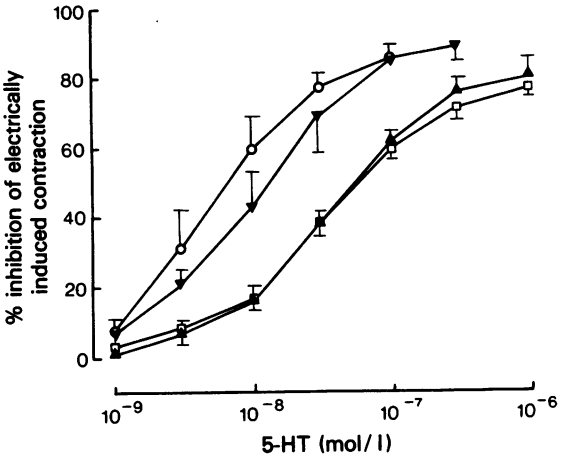

Figure 3 Dog isolated saphenous vein strip. Electrically stimulated at 2 Hz for 10 s every 180 s and inhibitory concentration-effect curves for 5-hydroxytryptamine obtained in the presence of different concentrations of calcium (○ 0.65×10^{-3} mol/l, ▼ 1.3×10^{-3} mol/l i.e. second control curve, □ 3.9×10^{-3} mol/l and ▲ 5.2×10^{-3} mol/l). The Krebs contained cyproheptadine (1.0×10^{-6} mol/l) to antagonize the contractile effect of 5-hydroxytrypamine (see text). Each point is the mean of 4 or 5 observations; vertical lines show s.e. mean.

adrenaline concentrations (1.0×10^{-8} to 2.0×10^{-4} mol/l) was also studied. 5-Hydroxytryptamine (1.0×10^{-8}, 1.0×10^{-7}, or 1.0×10^{-6} mol/l) had little or no effect on noradrenaline cumulative concentration-effect curves.

Effects of frequency and number of pulses on the inhibitory action of 5-hydroxytryptamine

We have examined the effect of varying the frequency and the number of pulses on the inhibitory action of a single concentration of 5-hydroxytryptamine (1.0×10^{-8} mol/l) in the presence of cyproheptadine (1.0×10^{-6} mol/l, Figure 2). With trains of 100 pulses the inhibitory action of 5-hydroxytryptamine was dependent upon the frequency of stimulation, greater inhibition being achieved with the lower frequencies. However, when trains of 10 pulses were used, this frequency-dependence was only evident with the two lowest frequencies. The inhibitory action of 5-hydroxytryptamine was also dependent upon the train length, since there was a significantly greater inhibition when trains of 10 pulses rather than 100 pulses were used at both 2 Hz and 5 Hz. Essentially similar results were obtained in the absence of cyproheptadine, although in these experiments the inhibitory action of 5-hydroxytryptamine was less marked and more difficult to quantitate because of its direct contractile effect.

Effects of external calcium concentration on the inhibitory action of 5-hydroxytryptamine

Decreasing the calcium concentration in the Krebs from 1.3 to 0.65×10^{-3} mol/l caused the response produced by electrical stimulation (2 Hz for 10s) to be reduced by $73 \pm 5\%$ ($n = 4$), whilst increasing the calcium concentration to 3.9 and 5.2×10^{-3} mol/l caused the response to increase by $42 \pm 7\%$ ($n = 4$) and $91 \pm 21\%$ ($n = 5$) respectively.

The 5-hydroxytryptamine-induced inhibition of these electrically-induced contractions was also calcium-dependent. Increases of the calcium concentration above 0.65×10^{-3} mol/l produced parallel displacements of the 5-hydroxytryptamine concentration-effect curve to the right (Figure 3). However, the reduction in sensitivity to the inhibitory effect of 5-hydroxytryptamine was not significantly different whether 3.9 or 5.2×10^{-3} mol/l calcium was used. As in the frequency-dependence studies similar results were obtained in the absence of cyproheptadine.

Effects of antagonists on the inhibitory action of 5-hydroxytryptamine

Cyproheptadine Cyproheptadine (1.0×10^{-7} and 1.0×10^{-6} mol/l) reduced the magnitude of the contractile responses to electrical stimulation by $13 \pm 5\%$ and $37 \pm 6\%$ respectively ($n_1 = n_2 = 10$). The higher concentration of cyproheptadine almost completely abolished the contractile effect of 5-hydroxytryptamine. However, cyproheptadine (1.0×10^{-8} to 1.0×10^{-6} mol/l) did not prevent the inhibitory action of 5-hydroxytryptamine on contractions produced by electrical stimulation. Indeed, concentration-effect curves to 5-hydroxytryptamine were displaced to the left in a concentration-dependent manner (Figure 4).

Morphine Morphine (1.0×10^{-7} mol/l to 1.0×10^{-5} mol/l) neither affected contractile responses of dog isolated saphenous vein strips to electrical stimulation, nor did it affect the inhibitory action of 5-hydroxytryptamine.

Methysergide The effect of methysergide on the inhibitory action of 5-hydroxytryptamine was examined at concentrations of 1.0×10^{-8} mol/l and 1.0×10^{-7} mol/l. At the higher concentration, methysergide itself slightly reduced the contractions produced by electrical stimulation ($17 \pm 4\%$ inhibition, $n = 5$) and was only a weak antagonist of the inhibitory action of 5-hydroxytryptamine, producing a three-fold displacement to the right of the 5-hydroxytryptamine concentration-effect curve.

Other antagonists Propranolol (1.0×10^{-6} mol/l), atropine (1.0×10^{-6} mol/l), mepyramine (1.0×10^{-6} mol/l) and cimetidine (1.0×10^{-5} mol/l) had no effect on the inhibitory action of 5-hydroxytryptamine on contractile responses caused by electrical stimulation of dog saphenous vein strips at 2 Hz. Phentolamine (5.0×10^{-8} mol/l) and yohimbine (1.0×10^{-7} mol/l) also had little or no effect on the inhibitory action of 5-hydroxytryptamine at concentrations which themselves produced about 20% reduction of the contractile response to electrical stimulation (mean 5-hydroxytryptamine concentration ratios of 1.0 and 2.3 respectively). Each compound was examined on four strips obtained from different dogs.

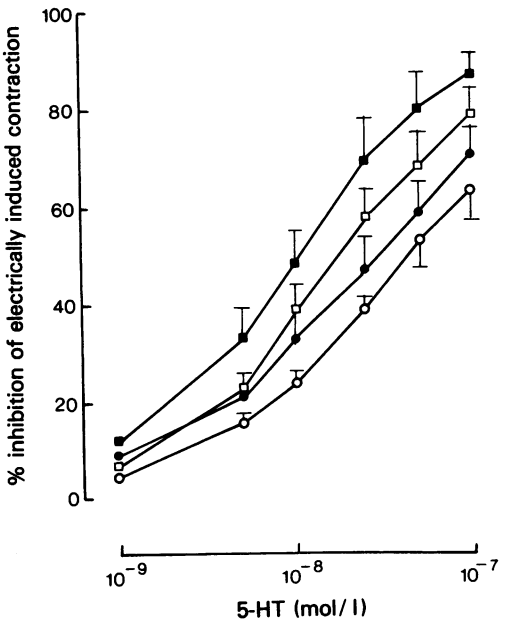

Figure 4 Dog isolated saphenous vein strip. Electrically stimulated at 2 Hz for 10 s every 180 s and inhibitory concentration-effect curves for 5-hydroxytryptamine obtained in the absence i.e. second control curve (○) and presence of different concentrations of cyproheptadine (● 1.0×10^{-8} mol/l, □ 1.0×10^{-7} mol/l and ■ 1.0×10^{-6} mol/l). Each point is the mean of 6 observations; vertical lines show s.e. mean. Note the potentiation produced by cyproheptadine.

Discussion

Intermittent electrical stimulation of spirally cut strips of dog isolated saphenous veins caused contractions which could be inhibited by phentolamine or tetrodotoxin, but not by mecamylamine. This suggests that the contractile responses were mediated predominantly by activation of postganglionic noradrenergic nerves. These contractile responses to electrical stimulation were also inhibited by 5-hydroxytryptamine in a concentration-dependent manner and since contractions caused by noradrenaline were not affected, or were even slightly potentiated, the inhibitory action of 5-hydroxytryptamine appears to involve a presynaptic mechanism.

Although prostaglandins can inhibit the release of noradrenaline from sympathetic nerves in vascular smooth muscle (de la Lande *et al.*, 1975) and 5-hydroxytryptamine can release prostaglandin-like substances from rat perfused lung (Alabaster & Bakhle, 1976), an indirect action involving prostaglandin release can be ruled out in the present experiments since the tissues were continually exposed to the prostaglandin synthetase inhibitor, indomethacin. An action involving an enhanced re-uptake of liberated noradrenaline by uptake$_1$ can also be excluded, since cocaine was continually present in the bathing medium. From our own data we can only sepeculate that the mechanism of the inhibitory action of 5-hydroxytryptamine is presynaptic. However, the recent

finding of McGrath (1977), that 5-hydroxytryptamine can inhibit the electrically-induced overflow of noradrenaline from dog saphenous vein, confirms a presynaptic inhibitory action on the transmitter release process. Our experiments with a variety of blocking agents have excluded the possibility of stimulation of β-adrenoceptors, muscarinic, histamine H_1- and H_2-receptors. Furthermore, our results with the selective antagonist, yohimbine, have ruled out a presynaptic α-adrenoceptor mechanism (Doxey, Smith & Walker, 1977). McGrath (1977) too has shown that 5-hydroxytryptamine will inhibit release of tritiated noradrenaline even in the presence of high concentrations of phentolamine (3.6×10^{-6} mol/l). In view of the high potency of 5-hydroxytryptamine it seems likely therefore that this effect of 5-hydroxytryptamine is via a specific 5-hydroxytryptamine receptor.

The inhibitory action of 5-hydroxytrypamine on contractions produced by electrical stimulation, was most evident at low frequencies. Since in this study the number of pulses was kept constant whilst varying the frequency, we have demonstrated a true frequency-dependence. Other experimental protocols for examining presynaptic mechanisms, where workers have used different frequencies for a fixed time, have been criticized (Hadhazy, Vizi, Magyar & Knoll, 1976). With this in mind we have also examined the effect of varying the number of electrical pulses at fixed frequencies. Our results indicate that the inhibitory action of 5-hydroxytryptamine is most marked with low numbers of pulses and is, therefore, dependent not only on frequency, but also on the number of pulses. The same has been found to be the case for the presynaptic α-adrenoceptor, the presynaptic prostaglandin receptor and the presynaptic muscarinic receptor (Hume, de la Lande & Waterson, 1972; Steinsland, Furchgott & Kirpekar, 1973; Vizi, Somogyi, Hadhazy, & Knoll, 1973; Hadhazy *et al.*, 1976). However, in our experiments we have only examined the effect of a single concentration of 5-hydroxytryptamine and the exact nature of the inter-relationship between the effects of frequency and pulse number awaits further analysis.

Another similarity between the inhibitory action of 5-hydroxytryptamine and other presynaptic mechanisms, is the dependence upon external calcium concentration. The inhibitory action of 5-hydroxytryptamine was antagonized by raising the external calcium concentration, which has also been found to be the case for α-adrenoceptor agonist-, prostaglandin-, and acetylcholine-induced inhibition (Stjarne, 1973; Hadhazy, Todorov & Nador, 1975; Leighton & Westfall, 1976). Since the characteristics of the 5-hydroxytryptamine-induced inhibition so closely resemble those of three other known presynaptic inhibitory mechanisms, it is tempting to conclude that they all act via a common pathway. It has been suggested that the other presynaptic inhibitory mechanisms limit the availability of calcium ions which are essential for neurotransmitter release. This is consistent with the dependence on the parameters of electrical stimulation since high frequency and large numbers of pulses would prolong the periods of membrane depolarization at the presynaptic terminals. This in turn would be expected to increase the influx of extracellular calcium, thereby antagonizing the inhibitory mechanism (see Westfall, 1977).

In the present study an attempt has been made to classify the 5-hydroxytryptamine receptor which is apparently involved in this inhibitory action. Gaddum & Picarelli (1957) described two types of 5-hydroxytryptamine receptor in mammalian smooth muscle. The D-receptor is believed to occur in the smooth muscle itself and is blocked by dibenamine, methysergide and cyproheptadine (Gaddum & Picarelli, 1957; Drakontides & Gershon, 1968; Apperley *et al.*, 1976). The M-receptor is pharmacologically distinct from the D-receptor and is believed to be situated in neuronal tissue (Day & Vane, 1963). The response to M-receptor activation is antagonized by morphine (Gaddum & Picarelli, 1957) although there is considerable doubt about whether morphine is a specific 5-hydroxytryptamine receptor blocking agent (Lewis, 1960). Nevertheless, the inhibitory action of 5-hydroxytryptamine described in this study did not appear to involve stimulation of D-receptors, since cyproheptadine potentiated the inhibitory effect of 5-hydroxytryptamine and methysergide was only weakly effective as an antagonist; neither did the inhibitory effect of 5-hydroxytryptamine involve a morphine-sensitive mechanism. We are continuing attempts to characterize this effect of 5-hydroxytryptamine by looking for a specific antagonist and by determining agonist potencies in a series of tryptamine analogues for comparison with those obtained in other preparations containing 5-hydroxytryptamine receptors.

The mechanism by which cyproheptadine potentiates the inhibitory action of 5-hydroxytryptamine in the dog saphenous vein is not clear. A similar finding has been described in the nasal vasculature of the dog, where cyproheptadine did not block but potentiated vasoconstrictor responses to 5-hydroxytryptamine (Vargaftig & Lefort, 1974). It is tempting to speculate that, in the former case at least, cyproheptadine produced potentiation by reducing the availability of calcium for secretion coupling. It is known that relatively low concentrations of cyproheptadine can antagonize a variety of agonists that produce contraction of vascular smooth muscle (Apperley *et al.*, 1976; Fozard, 1976). It has been suggested that this non-specific blocking action is mediated by a mechanism involving reduction of available calcium (Jogi, Roy & Sarkar, 1974) and such a mechanism would

be expected to potentiate, not block, a presynaptic inhibitory effect.

At present one can only speculate about the possible physiological significance of a presynaptic inhibitory 5-hydroxytryptamine receptor. It could explain the vasodilator action of 5-hydroxytryptamine *in vivo* (McCubbin *et al.*, 1962) and why arterioles with a dense sympathetic innervation are dilated by 5-hydroxytryptamine, whilst large arteries, which are sparsely innervated, are constricted (Harper & MacKenzie, 1977). Another possibility is that 5-hydroxytryptamine could modify intestinal tone and motility by an inhibitory neuronal action. These findings could also have important consequences for the pharmacological control of brain function in view of a recent study which shows that there are presynaptic inhibitory receptors for 5-hydroxytryptamine in the dorsal raphé nucleus (Haigler & Aghajanian, 1977). In summary, the inhibitory action of 5-hydroxytryptamine demonstrated in this study represents one of the most potent actions of 5-hydroxytryptamine yet described. The physiological characteristics of the mechanism closely resemble those of other presynaptic mechanisms and suggest a final common pathway. This 5-hydroxytryptamine receptor type has yet to be characterized fully and the potential clinical significance of these findings remains to be determined.

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