J. Pharm. Pharmacol. 1981, 33: 515–520
Received December 12, 1980

0022–3573/81/080515–06 $02.50

A pre-junctional action of 5-hydroxytryptamine and methysergide on noradrenergic nerves in dog isolated saphenous vein

A. D. WATTS, W. FENIUK* AND P. P. A. HUMPHREY

Department of Pharmacology, Glaxo Group Research Limited, Ware Division, Hertfordshire, SG12 0DJ, U.K.

Electrical stimulation (2 Hz for 2 min) of dog isolated saphenous vein strips pre-incubated with tritiated noradrenaline increased the overflow of tritium of which about 80% was noradrenaline. 5-Hydroxytryptamine (5-HT; $1{\cdot}0 \times 10^{-9}$–$1{\cdot}0 \times 10^{-7}$ mol litre^{-1}) and methysergide ($3{\cdot}0 \times 10^{-8}$–$3{\cdot}0 \times 10^{-6}$ mol litre^{-1}) inhibited the induced overflow of total tritium by a maximum of 78 ± 4% and 47 ± 7% respectively (mean ± s.e. mean, n = 6 for each). Methysergide was about 30 times less potent than 5-HT and the maximum inhibition obtained was less than with 5-HT. Both compounds inhibited electrically-induced contractions and overflow of tritiated noradrenaline. Their inhibitory actions on tritium overflow were little affected by phentolamine ($1{\cdot}0 \times 10^{-6}$ mol litre^{-1}) or cyproheptadine ($1{\cdot}0 \times 10^{-6}$ mol litre^{-1}), nor was the inhibitory effect of methysergide on electrically induced contractions antagonized by atropine, mepyramine, cimetidine or propranolol. The findings suggest that the prejunctional inhibitory effect of methysergide may be mediated via stimulation of a 5-HT receptor which, unlike the D-receptor, is not blocked by cyproheptadine. The possibility that the pre-junctional 5-HT receptor in the dog saphenous vein is the same as the post-junctional receptor in this preparation is discussed.

Low concentrations of 5-hydroxytryptamine (5-HT) will inhibit neuronally mediated contractions of dog isolated saphenous vein (McGrath 1977; Feniuk et al 1979a). It has been suggested that this effect is produced by inhibition of release of neuronal noradrenaline via stimulation of a specific pre-junctional receptor for 5-HT (McGrath & Shepherd 1978). This receptor is not a D-receptor since it is only weakly blocked by the classical 5-HT receptor blocking drug methysergide (Feniuk et al 1979a).

The 5-HT receptor located post junctionally and mediating contraction in this vein is also only weakly blocked by methysergide, which appears to be a partial agonist at the post-junctional 5-HT receptor (Apperley et al 1980). We therefore wondered if methysergide also had agonistic activity at the *pre*-junctional receptor in this preparation. To determine this we have examined the effects of methysergide on sympathetic neuronal transmission by measurement of its effects on electrically induced contractions and transmitter overflow in dog saphenous vein strips. 5-HT was also examined in an attempt to further characterize the nature of the pre-junctional 5-HT-receptor. A preliminary account was presented to the British Pharmacological Society (Feniuk et al 1979b).

* Correspondence.

MATERIALS AND METHODS

Lateral saphenous veins were removed from beagle dogs, cut spirally into 4 strips and mounted in individual organ baths containing physiological solution as described by Humphrey (1978).

Electrical stimulation

The strips were situated between parallel platinum electrodes to allow electrical field stimulation (Feniuk et al 1979a).

Effect of antagonists on the inhibitory action of 5-HT or methysergide on contractile responses to electrical stimulation

Each strip was stimulated intermittently using pulses of supra-maximal voltage and 0·1 ms duration, at 2 Hz for 10 s every 180 s. When constant responses were obtained, 5-HT or methysergide was added cumulatively to each of 4 baths and inhibitory concentration-effect curves determined. When the effect of antagonists was examined, three strips were then exposed to three different concentrations of antagonist for 30 min in the absence of electrical stimulation before re-determining inhibitory concentration-effect curves to 5-HT or methysergide. The fourth strip was not incubated with antagonist and therefore

acted as a control to monitor any spontaneous changes in sensitivity of the preparation to the agonist. The changes in sensitivity were always less than 2-fold in controls. In the absence of drug, responses to electrical stimulation did not vary by more than about 10% during the period required to obtain a concentration effect curve.

Measurement of tritium overflow

Each strip was incubated in 3 ml physiological solution containing 1-[7-^{3}H] noradrenaline (Radiochemical Centre, Amersham; 10 m Ci/0.67 $\times$ 10^{-6} mol litre^{-1}) or 1[7,8-^{3}H]noradrenaline (Radio-chemical Centre, Amersham; 10 m Ci/0·29 $\times$ 10^{-6} mol litre), ascorbic acid (1·1 $\times$ 10^{-4} mol litre^{-1}) and disodium EDTA (4·0 $\times$ 10^{-6} mol litre^{-1}) for 2 h at 37 °C and gassed with 95% O_2, 5% CO_2. Each strip was then mounted isometrically under an initial tension of 0·5 g in a 2 ml organ bath and incubated in physiological solution containing ascorbic acid (1·1 $\times$ 10^{-4} mol litre^{-1}), disodium EDTA (4·0 $\times$ 10^{-6} mol litre^{-1}), cocaine (3·0 $\times$ 10^{-5} mol litre^{-1}), corticosterone (4·0 $\times$ 10^{-5} mol litre^{-1}) and indomethacin (2·8 $\times$ 10^{-6} mol litre^{-1}) throughout the remainder of the experiment. Before the start of the experiment proper, the organ bath was drained and then refilled every 10 min for 100 min to wash away extracellular tritium. Subsequently the bath was drained every 2 min. The 2 ml samples of physiological solution were collected for measurement of tritium content by liquid scintillation counting.

Experimental design

After washing, each vascular strip was electrically stimulated using a maximal voltage for 2 min at 2 Hz (0·5 ms pulse width) every 20 min. In each experiment there were nine periods of stimulation. The results from the first two (S_1, S_2) were discarded because the basal overflow of radioactivity was waning markedly. Subsequent samples provided a more constant overflow and were designated S_3 (control) S_4 (lowest concentration of drug), S_5 (control), S_6 (middle concentration of drug), S_7 (control), S_8 (highest concentration of drug), S_9 (final control). In some experiments 0·9% NaCl (saline) was added instead of drug. The volume of drug solution or saline added was always 0·02 ml and was present 6 min before, and during electrical stimulation.

Scintillation counting

The samples were collected in plastic scintillation vials and mixed with scintillation fluid (6 ml Picofluor 30, Packard Instrument Co.) and the total amount of tritium measured using a Packard scintillation counter. Vials were counted for 5 min or until 20000 counts had been obtained. Correction for quenching was made using an external standard and calculated by computer. Counting efficiency was about 30%.

Measurement of tritiated noradrenaline overflow

In some experiments the amount of tritiated noradrenaline in the physiological solution was measured. The method was similar to that of Drew et al (1979) itself a modification of those of Muldoon et al (1978) and Levin (1973). From each 2 ml sample of physiological solution, 1·5 ml was taken and acidified with 0·2 ml 0·5 mol litre^{-1} HCl containing 100 μg disodium EDTA. After addition of 6 ml methanol and 50 μg each of unlabelled noradrenaline and the five major metabolites of noradrenaline as carriers, the samples were evaporated to near dryness under a partial vacuum. The residues were mixed with 1 ml ethanol–acetone (1 : 1) which dissolved about 98% of the radioactivity from the residue. Finally the tritiated noradrenaline and total tritium content of the redissolved samples was measured after separation of noradrenaline from its metabolites by descending paper chromatography in butanol–ethanol (95%)–distilled water (1 : 1 : 1) (see Levin 1973). In these experiments there were only 4 periods of electrical stimulation. Samples S_1 and S_2 were discarded as previously, while sample S_3 was obtained in the absence of drug and sample S_4 was obtained in the presence of the drug being tested.

Calculation of results

Herein, basal tritium overflow is the mean tritium overflow per 2 ml sample of physiological solution in the 3 samples preceding electrical stimulation. Electrically induced tritium overflow was calculated as the overflow of tritium during stimulation minus basal tritium overflow. Changes in electrically-induced tritium overflow produced by drugs or saline were calculated by comparing the overflow in the presence of drug or saline as a percentage of the overflow produced by the preceding control period of electrical stimulation. Changes in tritiated noradrenaline overflow were calculated in a similar way.

Statistical analysis

Where appropriate, mean results have been shown $\pm$ s.e. of the mean of n obse. vations (dogs).

Drugs Used

Ascorbic acid (BDH); $(atropine)_2$ sulphate, (BDH); cimetidine (SKF); cocaine hydrochloride (May &

Baker); corticosterone (Sigma); cyproheptadine hydrochloride (Merck, Sharp & Dohme); disodium ethylenediaminetetraacetic acid (disodium EDTA) (BDH); haloperidol (Searle); 5-hydroxytryptamine creatinine sulphate (Koch-Light); indomethacin (Sigma); mepyramine maleate, (May & Baker); methysergide bimaleate (Sandoz); morphine hydrochloride (Macfarlan Smith); phentolamine mesylate (Ciba); propranolol hydrochloride (ICI).

Ascorbic acid, cocaine and EDTA were dissolved in physiological solution. Stock solutions of 5-hydroxytryptamine and methysergide were made in distilled water, phentolamine in saline, corticosterone in 99·8% ethanol and indomethacin in 10% sodium bicarbonate. All dilutions were made in saline.

Drug concentrations are given in mol litre^{-1}.

RESULTS

Effect of 5-HT and methysergide on electrically induced contractions

Contractile responses of saphenous vein strips at 2 Hz for 10 s were inhibited by 5-HT ($1{\cdot}0 \times 10^{-9}$–$1{\cdot}0 \times 10^{-7}$) and methysergide ($5{\cdot}0 \times 10^{-8}$–$1{\cdot}0 \times 10^{-5}$) in a concentration dependent manner (Fig. 1).

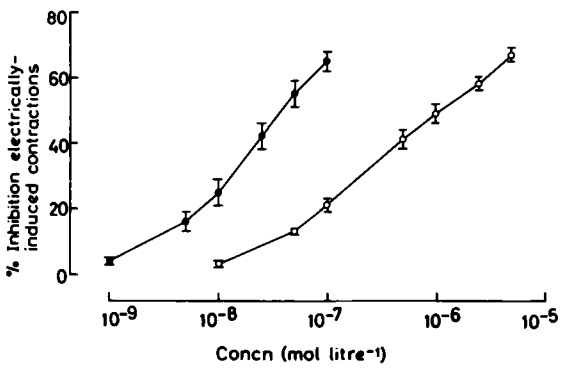

FIG. 1. Dog isolated saphenous vein strip. Inhibition of electrically induced contractions by 5-HT (●) and methysergide (○). Each point is the mean of 6 determinations. Vertical bars represent the s.e. mean.

Comparison of equiactive concentrations (at absolute 50% inhibition) shows that methysergide is about 30 times weaker than 5-HT in this respect.

Effect of antagonists on the inhibitory action of 5-HT and methysergide on electrically induced contractions

As with 5-HT (Feniuk et al 1979a) the inhibitory action of methysergide on the contractions of the saphenous vein was not modified by morphine ($1{\cdot}0 \times 10^{-5}$), propranolol ($1{\cdot}0 \times 10^{-6}$), atropine base ($1{\cdot}0 \times 10^{-6}$), mepyramine ($1{\cdot}0 \times 10^{-6}$) or cimetidine ($1{\cdot}0 \times 10^{-5}$). Neither was the inhibitory action of 5-HT or methysergide antagonized by haloperidol ($1{\cdot}0 \times 10^{-6}$). Each compound was examined on at least 3 strips from different dogs. However, as with 5-HT (Feniuk et al 1979a) cyproheptadine potentiated the inhibitory action of methysergide (Fig. 2.)

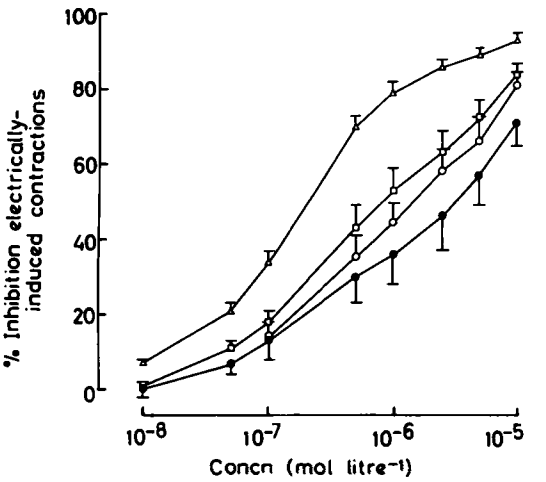

FIG. 2. Dog isolated saphenous vein strip. Inhibition of electrically induced contractions by methysergide in the absence (●) and presence of $1{\cdot}0 \times 10^{-9}$ (○), $1{\cdot}0 \times 10^{-7}$ (□) and $1{\cdot}0 \times 10^{-6}$ (△) mol litre^{-1} cyproheptadine. Each point is the mean of 6 determinations. Vertical bars represent the s.e. mean. Note the potentiation of the inhibitory effect of methysergide which was only statistically significant with 10^{-6} mol litre^{-1} cyproheptadine (at $P = 0{\cdot}05$).

Effects of electrical stimulation on total tritium and tritiated noradenaline overflow

Stimulation at 2 Hz for 2 min (period S_3) increased total tritium overflow by 102 ± 14% (n = 17) and was associated with an increase in strip tension of 0·96 ± 0·11 g (n = 17). After saline (0·02 ml), electrically induced tritium overflow changed by +5 ± 4%, −4 ± 4% and +1 ± 8% (n = 5), corresponding to the administration of low (S_4), middle (S_6) and high (S_8) concentrations of drug (see Fig. 3a). The tritium overflow during the final period of electrical stimulation (S_9) was 16 ± 6% (n = 5) less than that during the first control period (S_3).

Stimulation at 2 Hz for 2 min (period S_3) increased tritiated noradrenaline overflow by 417 ± 57% (n = 6). Basal overflow of tritium consisted of 17 ± 0·4% tritiated noradrenaline (n = 6) and the electrically induced overflow of tritium consisted of 80 ± 3% tritiated noradrenaline (n = 6).

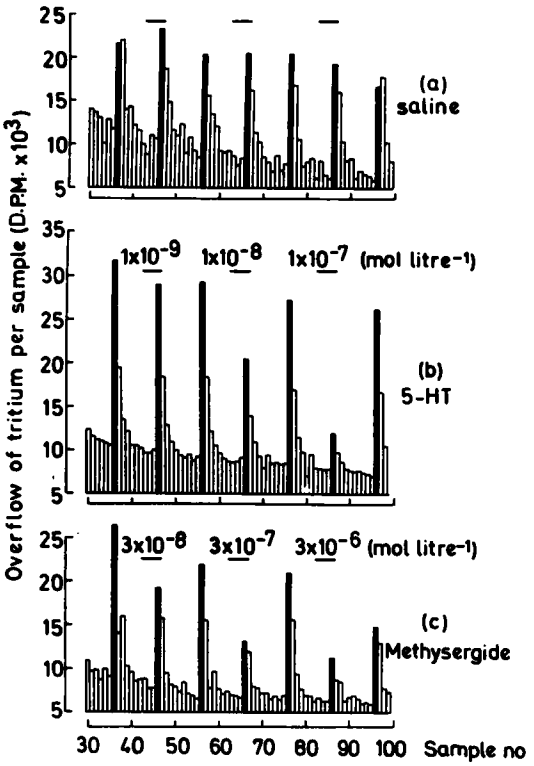

FIG. 3. Three dog isolated saphenous vein strips pre-incubated with [³H]noradrenaline. Effects of (a) saline (0·02 ml), (b) 5-HT and (c) methysergide on electrically induced tritium overflow. Vertical column represent the tritium overflow in samples of physiological solution collected at 2 min intervals. Filled vertical columns represent the tritium overflow during a 2 min period of electrical stimulation.

Effects of 5-HT and methysergide on electrically induced total tritium and tritiated noradrenaline overflow

Neither 5-HT ($1{\cdot}0 \times 10^{-9}$–$1{\cdot}0 \times 10^{-7}$) nor methysergide ($3{\cdot}0 \times 10^{-8}$–$3{\cdot}0 \times 10^{-6}$) produced any change in basal tritium overflow.

5-HT ($1{\cdot}0 \times 10^{-9}$–$1{\cdot}0 \times 10^{-7}$) inhibited electrically induced tritium overflow in a concentration dependent manner (see Fig. 3b). This effect could be reversed by washing. Methysergide ($3{\cdot}0 \times 10^{-8}$–$3{\cdot}0 \times 10^{-6}$) also inhibited the overflow in a concentration dependent manner (see Fig. 3c). After the highest concentration used ($3{\cdot}0 \times 10^{-6}$) the effect could not be fully reversed by washing for 20 min. At the highest concentration of 5-HT the mean inhibition was 78 ± 4% whilst the maximum mean inhibition produced by methysergide was 47 ± 7% (Fig. 4). Methysergide was about 30 times less potent than 5-HT.

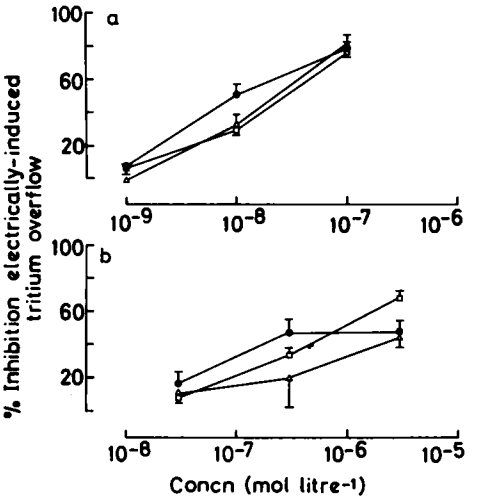

FIG. 4. Dog isolated saphenous vein strip pre-incubated with [³H]noradrenaline. Inhibition of electrically induced tritium overflow by (a) 5-HT and (b) methysergide in the absence of (●) and presence of phentolamine ($1{\cdot}0 \times 10^{-6}$ mol litre^{-1} □) or cyproheptadine ($1{\cdot}0 \times 10^{-6}$ mol litre^{-1} △). Each point is the mean of 2–6 determinations. Vertical bars represent the s.e. mean and are shown only for 3 or more determinations.

Methysergide ($3{\cdot}0 \times 10^{-6}$) inhibited induced tritiated noradrenaline overflow by 58 ± 14% (n = 5) whilst 5-HT reduced the overflow by 86% (1 exp.).

Effects of cyproheptadine on the inhibition of electrically induced tritium overflow

Cyproheptadine ($1{\cdot}0 \times 10^{-6}$) increased the induced overflow of tritium by about 50% but caused no marked displacement of the inhibitory dose-effect curves to either 5-HT or methysergide. (Fig. 4).

Effects of phentolamine on the inhibition of electrically induced tritium overflow

Phentolamine ($1{\cdot}0 \times 10^{-6}$) increased the induced overflow of tritium by about 300% but had little effect on the inhibition of the overflow produced by 5-HT or methysergide (Fig. 4).

DISCUSSION

It has been shown that contractions produced by electrical stimulation of dog isolated saphenous vein are mediated via release of noradrenaline from post-ganglionic noradrenergic nerves (Brandao & Guimaraes 1974; Feniuk et al 1979a). We have found that electrical stimulation of vein strips, pre-incubated with tritiated noradrenaline, increased the overflow of tritium and tritiated noradrenaline. It seems reasonable to assume that tritium overflow reflects the release of neuronal transmitter in response to nerve activation since the proportion of tritiated

noradrenaline in that overflow was both high and consistent, and presumably the result of the presence of cocaine and corticosterone in the physiological solution, as inhibitors of uptake$_1$ and uptake$_2$ respectively (see Vanhoutte 1978; Curro et al 1978; Muldoon et al 1978; Brandao et al 1980).

Low concentrations of 5-HT inhibited the electrically induced contractions of dog saphenous vein strips and the electrically induced overflow of transmitter, which confirms the work of McGrath (1977). We have also confirmed McGrath's finding that the latter effect was little affected by a high concentration of phentolamine which rules out involvement of a pre-junctional α-adrenoceptor. In addition, the inhibitory action of 5-HT on tritium overflow was little affected by cyproheptadine which suggests that a classical 5-HT D-receptor is not involved (see Apperley et al 1980). However, it is surprising that cyproheptadine did not potentiate this effect of 5-HT since it did potentiate the inhibitory effect of 5-HT on the electrically induced contractions of this vein (Feniuk et al 1979a). Perhaps this potentiating effect relates in some way to the ability of cyproheptadine to inhibit the contractile action of agonists post-synaptically and not as previously suggested to the ability to interfere with calcium availability at a pre-junctional site (see Feniuk et al 1979a).

Methysergide, a D-receptor-blocking drug like cyproheptadine, also failed to antagonize the pre-junctional inhibitory actions of 5-HT in dog saphenous vein, except at concentrations which themselves caused inhibition (Feniuk et al 1979a). In the present experiments, methysergide mimicked the actions of 5-HT causing inhibition of contractile responses produced by electrical stimulation but was about 30 times less potent than 5-HT. Our results on tritium overflow, a measure of the effect of methysergide on transmitter release, show that methysergide mimics 5-HT and is about 30 times less potent.

High concentrations of methysergide are known to stimulate α-adrenoceptors (Apperley et al 1976). However, its inhibitory action on transmitter release was not mediated via a pre-junctional α-adrenoceptor since its action was little modified by a high concentration of phentolamine that would be expected to produce about a 100 fold shift to the right in the concentration effect curve to a pre-junctional α-adrenoceptor agonist (Doxey et al 1977; Drew 1977). In the present experiments phentolamine increased the electrically induced overflow of tritium by about 4 fold which suggests that it had produced a high degree of pre-junctional α-adrenoceptor blockade.

As was found with 5-HT (Feniuk et al 1979a), the experiments with a variety of other blocking agents suggest that methysergide does not inhibit electrically-induced contractions of the preparation via stimulation of β-adrenoceptors or of muscarinic, histamine or dopamine receptors. In addition the inhibitory effect was not blocked by morphine. Since indomethacin was continually present throughout all the experiments, an indirect mechanism involving prostaglandin release can also be excluded.

The remarkable finding in this study is that methysergide, a well known 5-HT antagonist, mimicked the pre-junctional effects of 5-HT. Similar findings have been reported in the central nervous system where the excitation of cat cortical neurons induced by glutamate was inhibited by iontophoretic administration of 5-HT and methysergide (Curtis & Davis 1962; Krnjevic & Phillis 1963). Both 5-HT and methysergide also depress the firing of raphe neurons in the rat (Haigler & Aghajanian 1974) and depress sympathetic outflow by a central mechanism in the cat (Tadepalli et al 1979). More recently it has been shown that methysergide does not potently antagonize, and indeed will mimic, the contractile actions of 5-HT in the dog isolated saphenous vein (Apperley et al 1980). From such observations it has been postulated that there is a specific receptor for 5-HT which is distinct from the 'D'-receptor or 'M'-receptor described by Gaddum & Picarelli (1957) and at which methysergide is a partial agonist (Apperley et al 1980). It may be that all these agonistic effects of 5-HT and methysergide are mediated via a common receptor but a specific receptor blocking drug will be necessary to substantiate the hypothesis.

Our findings add support to the suggestion that the pre-junctional 5-HT receptor which mediates inhibition of neuronal noradrenaline release in dog saphenous vein is pharmacologically similar to the 5-HT receptor type situated post-junctionally in this preparation. This belief is based on the finding that at both sites methysergide is a partial agonist. At both pre- and post-junctional sites it is about 30 times weaker than 5-HT and in both cases the maximum response to methysergide is some 40–60% of that to 5-HT (this study; Apperley et al 1980). Furthermore we have now shown that the rank orders of agonistic potency for certain 5-carboxamide analogues of tryptamine are similar for both pre- and post-junctional actions in the dog saphenous vein but different for their contractile actions in the rabbit aorta, a 'D-receptor' containing preparation (Feniuk et al 1981a). That the pre- and post-junctional 5-HT receptors in the dog saphenous vein are similar is

interesting in view of the fact that α_2-adrenoceptors (Hamilton & Reid 1980; Drew 1980) and muscarinic receptors (Steinsland et al 1973) can also be pharmacologically similar whether or not they occur pre- or post-junctionally.

In the present experiments both 5-HT and methysergide inhibited tritium overflow at similar concentrations to those which were needed to inhibit electrically-induced contractions. Indeed, with 5-HT there was a close correlation between the degree of inhibition of the two parameters (cf. Figs 1, 4). However, this was not so with high concentrations of methysergide (about $1{\cdot}0 \times 10^{-6}$ or more) since inhibition of electrically induced tritium overflow was maximal and yet electrically induced contractions were inhibited further. This probably relates to the known, albeit weak, α-adrenoceptor blocking activity of methysergide (Apperley et al 1976, 1980).

Nevertheless, the important point is that at low concentrations, methysergide does have a pre-junctional inhibitory action on noradrenaline overflow. This in turn probably explains the vasodilator action of methysergide in the anaesthetized dog which has been shown to depend on sympathetic neurogenic tone (Feniuk et al 1981b). Other experiments in vivo have demonstrated a similar action for 5-HT which suggests that the pre-junctional 5-HT receptor in the saphenous vein also exists on the sympathetic nerves which supply the resistance vessels of various arterial beds of the dog (Mena & Vidrio 1979; Feniuk et al 1981b).

If, as now seems apparent, pre-junctional 5-HT receptors can be demonstrated in the vasculature in vivo it is interesting to speculate about their presence in other locations. There is now some evidence to suggest they may occur on parasympathetic nerves to the intestine (Gintzler & Musacchio 1974) and also on cardiac sympathetic nerves (Martinez & Lokhandwala 1980). Whether or not the pre-junctional action of 5-HT in the central nervous system also involves a similar receptor remains to be determined (Haigler & Aghajanian 1977). Indeed lysergic acid diethylamide has pre-junctional inhibitory actions which may in some instances be mediated via 5-HT receptors (see McGrath 1979).

In conclusion, the results indicate that 5-HT and methysergide inhibit transmitter release by a pre-junctional action on noradrenergic nerves in the dog isolated saphenous vein. Furthermore, they suggest that these effects are mediated via a specific 5-HT receptor which is unlike the classical D-receptor and similar to the vein's post-junctional 5-HT receptor.

REFERENCES

Apperley, E., Humphrey, P. P. A. Levy, G. P. (1976) Br. J. Pharmacol. 58: 211–221

Apperley, E., Feniuk, W., Humphrey, P. P. A., Levy, G. P. (1980) Ibid. 68: 215–224

Brandao, F., Guimaraes, S. (1974) Blood Vessels 11: 45–54

Brandao, F., Paiva, M. Q., Guimaraes, S. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 311: 1–7

Curro, F. A., Greenberg, S., Verbeuren, T. J., Vanhoutte, P. M. (1978) J. Pharmacol. Exp. Ther. 207: 936–949

Curtis, D. R., Davis, R. (1962) Br. J. Pharmacol. 18: 217–246

Doxey, J. C., Smith, C. F. C., Walker, J. M. (1977) Ibid. 60: 91–96

Drew, G. M. (1977) Eur. J. Pharmacol. 42: 123–130

Drew, G. M. (1980) Ibid. 65: 85–87

Drew, G. M., Levy, G. P., Sullivan, A. T. (1979) Br. J. Pharmacol. 66: 151P

Feniuk, W., Humphrey, P. P. A., Watts, A. D. (1979a) Br. J. Pharmacol. 67: 247–254

Feniuk, W., Humphrey, P. P. A., Watts, A. D. (1979b) Ibid. 67: 423P–424P

Feniuk, W., Humphrey, P. P. A., Watts, A. D. (1981a) Ibid. 73: 191P–192P

Feniuk, W., Humphrey, P. P. A. Watts, A. D. (1981b) J. Autonom. Pharmacol. 1: 127–132

Gaddum, J. H., Picarelli, Z. P. (1957) Br. J. Pharmacol. 12: 323–328

Gintzler, A. R., Musacchio, J. M. (1974) J. Pharmacol. Exp. Ther. 189: 484–489

Haigler, H. J., Aghajanian, G. K. (1974) J. Neural. Transm. 35: 257–273

Haigler, H. J., Aghajanian, G. K. (1977) Fed. Proc. 36: 2159–2164

Hamilton, C. A., Reid, J. L. (1980) Br. J. Pharmacol. 70: 63P

Humphrey, P. P. A. (1978) Ibid. 63: 671–675

Krnjevic, K., Phillis, J. W. (1963) Br. J. Pharmacol. 20: 471–490

Levin, J. A. (1973) Analytical Biochem. 51: 42–60

Martinez, A. A., Lokhandwala, M. F. (1980) Eur. J. Pharmacol. 63: 303–311

McGrath, J. C. (1979), Paton, D. M. in: (ed.) The release of Catecholamines from Adrenergic Neurones, Pergamon Press, Oxford, pp 229–239

McGrath, M. A. (1977) Circ. Res. 41: 428–435

McGrath, M. A., Shepherd, J. T. (1978) Fed. Proc. 37: 195–197

Mena, M. A., Vidrio, H. V. (1979) J. Cardiovasc. Pharmacol. 1: 149–154

Muldoon, S. M., Vanhoutte, P. M., Tyce, G. M. (1978) Am. J. Physiol. 234: H235–H243

Steinsland, O. S., Furchgott, R. F., Kirpekar, S. M. (1973) J. Pharmacol. Exp. Ther. 184: 346–356

Tadepalli, A. S., Ho, K. W., Buckley, J. P. (1979) Eur. J. Pharmacol. 59: 85–93

Vanhoutte. P. M. (1978) Fed. Proc. 37: 181–186