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THE EFFECTS OF PIPEROXAN ON UPTAKE OF NORADRENALINE AND OVERFLOW OF TRANSMITTER IN THE ISOLATED BLOOD PERFUSED SPLEEN OF THE CAT

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1 The competitive α-adrenoceptor blocking agent, piperoxan, in concentrations up to 2×10^{-4} M, produced large dose-dependent increases in transmitter overflow from the isolated blood perfused spleen of the cat following nerve stimulation at 10 hertz.

2 At concentrations greater than 2×10^{-4} M, piperoxan produced a rise in perfusion pressure, a contraction of the splenic capsule, and a marked dose-dependent decrease in transmitter overflow.

3 Phenoxybenzamine (10^{-4} M) and desmethylimipramine (3×10^{-5} M) produced further increases in transmitter overflow when added after piperoxan.

4 Piperoxan (5.8 to 6.6×10^{-6} M) had no effect on the recovery of ^3H in the venous blood following the close arterial infusion or injection of [^3H]-(−)-noradrenaline, indicating that the drug does not inhibit uptake of the amine.

5 Piperoxan produced dose-dependent inhibition of responses of the splenic vasculature to close arterial injection of 1 μg of (−)-noradrenaline but was much less effective at inhibiting responses to nerve stimulation. At 2×10^{-6} M piperoxan produced a considerable reduction of the response to injected noradrenaline but potentiated the response to nerve stimulation.

6 In isolated strips of cat splenic capsule, piperoxan produced a shift to the right of the dose-response curve to noradrenaline with no change of the maximum response. There was no evidence of a postsynaptic sensitizing effect of the type observed in the rat vas deferens.

Introduction

Piperoxan (2-piperidino-methyl-1,4-benzodioxane) is a classical competitive α-adrenoceptor blocking agent (Ariens, 1967) at postsynaptic α-receptors. However, in some adrenergically innervated tissues such as the cat nictitating membrane, it has been observed that whilst piperoxan will abolish the effects of adrenaline, it is without effect on responses to adrenergic nerve stimulation (Bacq & Frederique, 1935). In other tissues such as the isolated vas deferens of the rat, piperoxan produces a potentiation of the response following nerve stimulation (Ohlin & Strömblad, 1963; Swedin, 1972). These apparently contradictory findings have been explained in the rat vas deferens in terms of a postsynaptic phenomenon (Jurkiewicz & Jurkiewicz, 1976). The effects of piperoxan on responses to nerve stimulation could be explained in some tissues in terms of differences in the pre- and postsynaptic α-receptors on which α-adrenoceptor blocking agents are known to vary in their effectiveness. For instance, phenoxybenzamine is particularly effective at blocking postsynaptic α-adrenoceptors in rat portal vein and cat spleen (Haggendal, Johansson, Jonason & Ljung, 1972; Dubocovich & Langer, 1974). In the rabbit pulmonary artery piperoxan, tolazoline and yohimbine are preferential blockers of presynpatic α-adrenoceptors whereas clozapine, prazocin and azapetine are preferential inhibitors of postsynaptic α-adrenoceptors (Starke, Borowski & Endo, 1975; Borowski, Ehrl & Starke, 1976; Cambridge, Davey & Massingham, 1977).

Similar differences in effectiveness of α-adrenoceptor blocking drugs at pre- and postsynaptic receptors also exist in the rat vas deferens (Drew, 1977) and in a tissue where the effector response is mediated by β-receptors, the rat heart (Drew, 1976). Phentolamine, piperoxan, yohimbine and tolazoline were effective presynaptic α-adrenoceptor antagonists whereas thymoxamine was without effect.

In this study we have examined the effects of piperoxan on uptake of noradrenaline and on pre- and postsynaptic α-adrenoceptors in the isolated blood perfused spleen of the cat. A preliminary report of

these findings has appeared (Summers & Blakeley, 1975).

Methods

Overflow of transmitter

Cat spleens were perfused with blood *in vitro* (Blakeley, Brown, Dearnaley & Woods, 1969; Blakeley, Powis & Summers, 1973). The splenic nerves were stimulated with shielded bipolar platinum electrodes. Supramaximal stimuli of 20 V and 0.5 ms duration were used throughout. Transmitter overflow was stabilized as previously described (Bacq, Blakeley & Summers, 1976). Transmitter overflow was measured following two trains of 200 stimuli at 10 Hz and two identical trains at 30 hertz. Piperoxan (as hydrochloride) was added to the blood in 0.9% w/v NaCl solution (saline) and allowed 20 min to act. Overflow was then measured following two trains of 200 stimuli at 10 hertz. Only one dose level of piperoxan was studied in each experiment.

Overflowing transmitter in the venous blood was collected for 1 min following stimulation at 30 Hz and for 80 s following stimulation at 10 hertz. The blood was chilled, spun, and the transmitter in the plasma assayed against (−)-noradrenaline on the blood pressure of the pithed rat (Shipley & Tilden, 1947).

Uptake of [^3H]-(−)-noradrenaline

(a) Uptake of [^{3}H]-(−)-noradrenaline was measured from infusions given close arterially at a rate of 361 ng/min to the spleen (perfusion rate 5 to 7 ml/minute). Uptake was taken as the difference between the amount of label given and the amount collected in the venous blood. In order to check that the errors involved in this type of measurement was not large, Evans Blue was added to the infusate to act as an intra-vascular marker (Blakeley & Summers, 1977). The recovery at steady state in the present experiments was 94.3 ± 1.2% (n = 12).

(b) Uptake of [^{3}H]-(−)-noradrenaline was also measured from pulses (Blakeley, Powis & Summers, 1974); 1 μg pulses of [^{3}H]-(−)-noradrenaline were injected close arterially during a 5 s period. Uptake was measured as described previously (Blakeley, *et al.*, 1973).

Isolated strips of cat splenic capsule

Isotonic recordings of contractions of cat splenic capsule in response to noradrenaline were made as described previously (Blakeley & Summers, 1977).

Drugs

[7-^{3}H]-(−)-noradrenaline (10.1 mCi/μmol, Radiochemical Centre, Amersham) was diluted with (−)-noradrenaline (Sigma) to give a specific activity of 248 μCi/μmol; Evans Blue (E. Merck, Darmstadt), piperoxan hydrochloride (2 piperidino-methyl-1,4-benzodioxane hydrochloride; Rhone-Poulenc, Paris); heparin (mucus; Boots Ltd.) and prostaglandin E_1 (Dr John E. Pike, Upjohn, Kalamazoo, U.S.A.) were also used; all doses of amines are expressed as base.

Unless otherwise mentioned results are expressed as means ± s.e. mean. Significance was assessed by Student's t test.

Results

The effects of piperoxan on transmitter overflow following nerve stimulation

Transmitter overflow was measured in each experiment as a fraction of the mean overflow from 200 stimuli at 30 Hz in that experiment. This largely removed the effect of spleen to spleen variation in transmitter overflow from the results. The normal fractional overflow from 200 stimuli at 10 Hz was 0.37 ± 0.03 (n = 26). The addition of piperoxan to the blood perfusing the spleen in concentrations up to 2×10^{-4} M produced a dose-dependent increase in transmitter overflow ($r = 0.67$; $P < 0.001$). The maximum increases in overflow produced by piperoxan in these experiments were greater than 4 fold, and were produced by doses of piperoxan between 5×10^{-6} M and 2×10^{-4} M (Figure 1). At concentrations of piperoxan greater than 2×10^{-4} M, a rise in perfusion pressure and a contraction of the spleen were produced and a marked dose-dependent decrease in transmitter overflow from 200 stimuli at 10 Hz was observed ($r = -0.86$; $P < 0.01$).

In the dose range 5.8×10^{-6} M to 1.1×10^{-5} M piperoxan raised the overflow of transmitter to 1.14 ± 0.09 ($P < 0.001$; n = 14). The addition of phenoxybenzamine (Pbz, 10^{-4} M) after doses of piperoxan (1.3×10^{-6} M to 3.1×10^{-5} M) produced a further increase in transmitter overflow from 200 stimuli at 10 Hz to 1.69 ± 0.11 ($P < 0.001$; n = 8). The addition of desmethylimipramine (DMI) also produced an increase in transmitter overflow from 200 stimuli at 10 Hz to 1.45 ± 0.11 ($P < 0.05$; n = 8). When the overflows after the combined effects of piperoxan with either Pbz or DMI were expressed in terms of the overflow in that experiment after piperoxan alone, DMI produced an increase of 1.44 ± 0.05 fold (n = 8) and Pbz produced an increase of 1.59 ± 0.14 fold (n = 8).

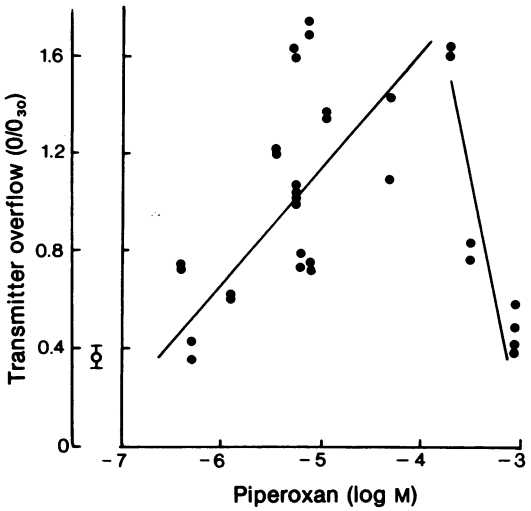

Figure 1 The effect of piperoxan on the overflow of transmitter following nerve stimulation in the isolated blood perfused spleen of the cat. (○) Control overflow of transmitter ($0/\bar{0}_{30}$) (see text) at 10 Hz; (●) overflow of transmitter following 200 stimuli at 10 Hz in the presence of piperoxan.

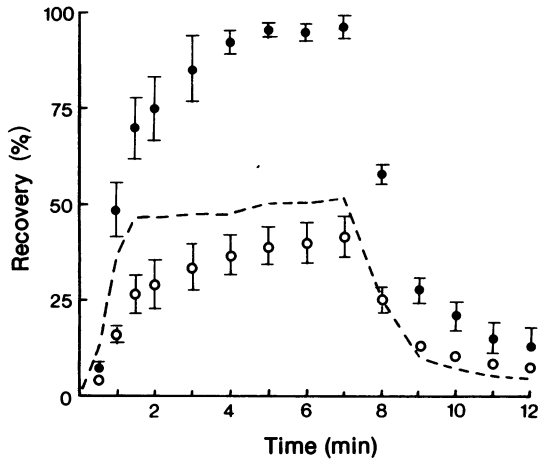

Figure 2 The effect of piperoxan on the recovery of ^3H in the venous blood following close arterial infusion of [^{3}H]-(−)-noradrenaline (361 ng/min) to the isolated blood perfused spleen of the cat. The dotted lines show the recovery of ^3H in the absence of drug and (○) the recovery in the presence of piperoxan (5.8 to 6.6 × 10^{-6} M) $n = 5$; (●) show the recovery of the intravascular marker, Evans Blue, in the venous blood.

The effects of piperoxan on uptake of [^3H]-(−)-noradrenaline infused into the cat spleen

(a) *From infusion (Figure 2)* [^{3}H]-(−)-noradrenaline was infused close arterially into cat spleens at a rate of 361 ng/min over a period of 7 min and the recovery of ^3H monitored in the venous blood. In the absence of piperoxan the recovery of ^3H rose to 48.9 ± 1.9% ($n = 12$) within 3 min of the start of infusion. During the same period the recovery of Evans Blue rose to 94.3 ± 1.2% ($n = 12$) indicating that there was little loss of infusate from the system. In the presence of piperoxan (5.8 to 6.6 × 10^{-6} M) the recovery of ^3H in the venous blood at steady state rose to 38.2 ± 5.4% ($n = 5$). These results indicate that piperoxan in a concentration which produces the maximum increase in overflow had no inhibitory effect on uptake of noradrenaline. In contrast, in the presence of the inhibitors of neuronal and extraneuronal uptake, DMI and 17β oestradiol, the recovery of ^3H rises to near 100% (Blakeley, *et al.*, 1974).

(b) *From 'pulses'* Uptake from 1 μg 'pulses' (see Blakeley, *et al.*, 1974) of [^{3}H]-(−)-noradrenaline injected close arterially was also measured. In 6 experiments the spleen took up 49.8 ± 2.0% from a first 'control pulse' and 51.3 ± 4.1% from a second pulse given 20 min after piperoxan (5.8 × 10^{-6} M to 6.6 × 10^{-6} M). Piperoxan was therefore without significant effect upon the uptake or noradrenaline from pulses.

Effects of piperoxan on responses to nerve stimulation and close arterial injection of noradrenaline in the perfused spleen

Close arterial injection of 1 μg (−)-noradrenaline produced rises in perfusion pressure (vascular responses) and contraction of the splenic capsule (capsular responses) similar to those produced by stimulation of the nerves with 200 stimuli at 10 hertz. Piperoxan produced a dose-dependent blockade of vascular responses to close arterial injection of (−)-noradrenaline. Low doses of piperoxan up to 2 × 10^{-6} M, potentiated the vascular response to nerve stimulation (Figure 3). The potentiation was seen as an increase in the height rather than a prolongation of the response as seen with inhibitors of neuronal uptake. Larger doses of piperoxan (2 × 10^{-6} to 7.8 × 10^{-5} M) which produce an almost complete blockage of responses to (−)-noradrenaline, had only a slight inhibitory effect on responses to nerve stimulation.

The effects of piperoxan on responses of isolated strips of splenic capsule to noradrenaline

In strips of splenic capsule, piperoxan (7.4 × 10^{-6} M) produced a shift to the right of the dose-response curve (Figure 4) with no change in the maximum re-

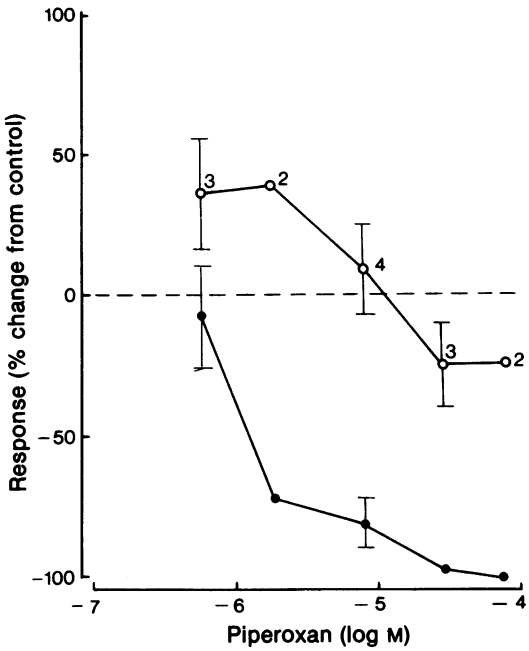

Figure 3 The effect of piperoxan on the response of the splenic vasculature to close arterial injection of 1 μg of (−)-noradrenaline (●) and to nerve stimulation with 200 impulses at 10 Hz (○). The broken line shows the level of no change. Numbers represent the number of observations.

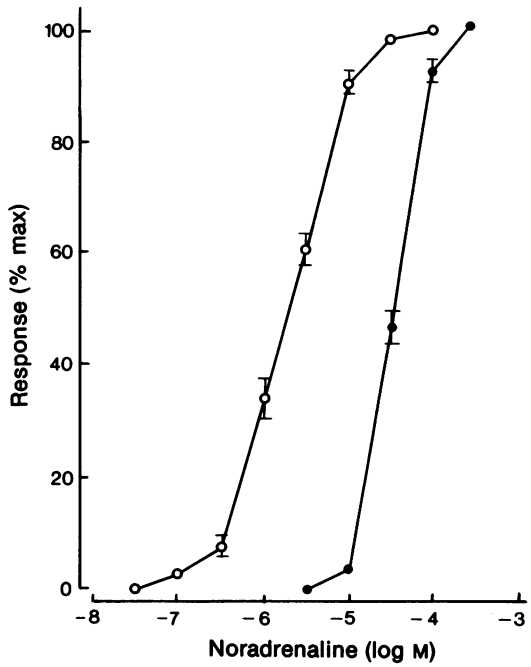

Figure 4 The effect of piperoxan (7.4×10^{-6} M) on responses of isolated strips of cat splenic capsule to noradrenaline. The dose-response curves to noradrenaline were obtained in the same strips in the absence and presence of piperoxan with a 40 min wash between ($n = 4$).

sponse. In the cat splenic capsule piperoxan acts as a competitive α-adrenoceptor blocker. It is not therefore possible to explain the potentiation of the responses to nerve stimulation in terms of an altered postsynaptic response to noradrenaline.

Discussion

Piperoxan acts as a competitive reversible antagonist of (−)-noradrenaline at the α-adrenoceptor in the cat spleen capsule strip. It also antagonizes the vascular effects of noradrenaline injected close arterially into the blood perfused cat spleen. However, as in the cat nictitating membrane (Bacq & Frederique, 1935) the response to adrenergic nerve stimulation is not similarly sensitive to piperoxan. It is not necessary to postulate the existence of an additional postsynaptic or non-adrenergic mechanism to explain the resistance to nerve stimulation; rather it can be explained in terms of an increase in the concentration of noradrenaline at the receptor sites in the presence of the drug. Piperoxan increases transmitter overflow in a dose-dependent manner when present in concentrations below 2×10^{-4} M. Since the drug does not reduce uptake of (−)-noradrenaline by the spleen, this increase in overflow must be due to an increase in transmitter liberation by the nerves. The simplest explanation for this is an inhibition of the presynaptic inhibitory α-adrenoceptors on the noradrenergic nerves (see Endo, Starke, Bangerter & Taube, 1977). The net effect upon the response of the inhibition of pre- and postsynaptic α-adrenoceptors will be determined by the relative potency of the antagonist at the two receptor sites.

A potent presynaptic α-adrenoceptor antagonist without noradrenaline uptake blocking properties, like piperoxan, would make a useful tool in any study of the physiological importance of these receptors.

The only caveat that should be mentioned concerning the use of this drug as a tool for such a study is that in high doses (greater than 2×10^{-4} M) it reduces transmitter overflow from the spleen. In these high doses it constricts the splenic blood vessels and contracts its capsule. The reduction in transmitter overflow may be an artefact due to the altered blood flow in the spleen or could be an indication of an α-agonist or other presynaptic effect. The effect of piperoxan at doses close to 10^{-5} M is variable. Such a large variation in effect is a finding common to other presynaptic α-blockers in this system, for example Pbz (Blakeley, *et al.*, 1969). It is therefore

difficult to be certain of the exact shape of the dose-response curve for piperoxan. However, since the additive effects of piperoxan (in doses close to 10^{-5} M) with either DMI, which inhibits uptake$_1$ (Iversen, 1967) or with the α-blocker Pbz, that blocks all noradrenaline uptake processes (Blakeley, *et al.*, 1974), are not very dissimilar we can conclude that piperoxan almost completely inhibits presynaptic α-receptors, at a dose level of 10^{-5} M. This is more than one order of magnitude less than the dose that reduces transmitter liberation.

In summary, piperoxan is an α-adrenoceptor antagonist that potentiates the overflow of transmitter from the cat spleen in doses that do not inhibit noradrenaline uptake. In high doses piperoxan reduces transmitter overflow by a mechanism that is not yet understood.

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