Presynaptic agonist effect of phentolamine in the rabbit vas deferens and rat cerebral cortex

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Clonidine inhibited the electrically-induced twitch response of the rabbit and rat isolated vas deferens preparations and also the K^+-evoked release of [^{3}H]noradrenaline from rat cortical slices. This effect of clonidine was antagonized competitively by yohimbine. Phentolamine inhibited the electrically-induced twitch response of the rabbit, but not the rat, vas deferens and in low concentrations (<0.1 μM) also inhibited the K^+-evoked release of [^{3}H]NA from rat cortical slices. These inhibitory effects of phentolamine were antagonized by yohimbine in a competitive manner but were not antagonized by indoramin, an α_1-adrenoceptor antagonist. In the rabbit vas deferens, the effects of phentolamine were shown not to be due to the stimulation of β-, H_1-, H_2-, 5-HT- or muscarinic receptors. These results are consistent with the view that phentolamine can act as an agonist at presynaptic α_2-adrenoceptors in the rabbit vas deferens and rat cortex but not in the rat vas deferens.

Adebanjo & Ambache (1978) reported that the electrically-induced twitch response of the rabbit isolated vas deferens preparation was completely inhibited by the presence of phentolamine in the bathing fluid, but not by another α-adrenoceptor antagonist, phenoxybenzamine. The inhibitory effect of phentolamine therefore does not appear to be related to α-adrenoceptor blockade. The electrically-induced twitch responses of the isolated vas deferens from the rat (Drew 1977), mouse (Marshall et al 1978) and guinea-pig (Stjarne 1975) are inhibited by α_2-adrenoceptor agonists via a negative feedback mechanism controlling transmitter release (Langer 1977; Starke 1977). This negative feedback system is also known to operate in the central nervous system and can be shown to control the K^+-induced release of [^{3}H]noradrenaline ([^{3}H]NA) from brain slices (Taube et al 1977). In the present study, the effects of phentolamine and clonidine on the electrically-induced twitch response of the rabbit isolated vas deferens have been compared with their effects on the electrically-induced twitch response of the rat isolated vas deferens and on the K^+-induced release of [^{3}H]NA from rat occipitoparietal cortex.

MATERIALS AND METHODS

Rat and rabbit isolated vasa deferentia

New Zealand white rabbits (2–3 kg) and Wistar rats (250–300 g) were killed by a blow to the neck and the vasa deferentia removed. The prostatic ends of the

vasa deferentia (2–3 cm lengths) were mounted between platinum ring electrodes in 5 ml organ baths and bathed in Krebs bicarbonate solution of the following composition (mM), NaCl 118·0; KCl 4·7; $CaCl_2$ 2·5; $MgSO_4$ 1·2; KH_2PO_4 1·2; Glucose 11·0; $NaHCO_3$ 25·0; maintained at 35 °C and bubbled with 95% O_2 and 5% CO_2. The initial tension on the preparation was 0·5 g and they were left to equilibrate for 30 min before stimulation was started. Then they were continuously field-stimulated using a square wave stimulator (Scientific Research Instruments Ltd) with a constant current output device at a frequency of 0·1 Hz, pulse width 1 ms and current of 90 mA. The contractions of the tissues were recorded isometrically using Grass force-displacement transducers and displayed on a Grass model 7D polygraph. Cumulative concentration response curves were obtained to clonidine HCl or phentolamine mesylate with a 4 min contact time at each concentration. The reduction in response height was measured and expressed as a percentage of the height of the response before addition of clonidine or phentolamine. The antagonist yohimbine or indoramin was then added to the Krebs solution and allowed to equilibrate with tissues for 30 min after which the concentration-response curves to clonidine HCl or to phentolamine were repeated. Increasing concentrations of the antagonists were tested against clonidine or phentolamine. The effect of desmethylimipramine (DMI) on the twitch responses of the vasa deferentia was determined by adding increasing concentrations to the organ bath

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and leaving each concentration to equilibrate for 20–30 min.

In some experiments the twitch response was alternated with single doses of NA, histamine or acetylcholine and the effect of phentolamine on these observed.

The ability of various receptor antagonists to reverse the inhibitory effect of phentolamine was determined by adding phentolamine ($0.3 \mu M$) to the organ baths and recording the inhibition obtained. The antagonist was then added and its effect on the phentolamine inhibition recorded.

In a separate series of experiments, rats and rabbits were dosed with reserpine 5 mg kg^{-1} subcutaneously 24 h before the removal of the vasa deferentia and the effect of this treatment on the responses to phentolamine and clonidine determined.

[^{3}H]NA release from rat occipital cortex
The methods have been decribed previously (Ennis 1983). Briefly, slices (250 × 250 μm) of the occipital cortex from two rats were preloaded with [^{3}H]NA (10^{-7} M) and superfused with previously oxygenated (95% O_2, 5% CO_2) Krebs Henseleit solution at 37 °C at a rate of 0.4 ml min^{-1}. Two pulses of Krebs Henseleit solution containing 25 mM K$^+$, obtained by iso-osmotic replacement of NaCl by KCl, were administered for 4 min, at 42(S_1) and 66(S_2) min after the start of the superfusion. Fractions of the superfusate were collected every 4 min and the radioactivity in each fraction together with that remaining in the tissue at the end of the experiment was determined by liquid scintillation counting. Modifying drugs were added to the superfusing medium immediately after S_1. The fractional release of tritium was calculated, i.e. the radioactivity in each superfusate fraction was divided by the radioactivity present in the slices at the start of that collection period. Spontaneous release was taken as the fractional release occurring immediately before S_1 and immediately before S_2. The percentage radioactivity released above spontaneous levels by the two pulses of K$^+$ solution was expressed as the ratio S_2/S_1 for both control and drug-treated slices. The S_2/S_1 ratio for drug treated slices was expressed as a percentage of the control S_2/S_1 ratio.

Concentration-response curves for yohimbine versus clonidine or phentolamine were analysed by the method of Arunlakshana & Schild (1959). Linear regression analysis was used to calculate the intercept and the slope of the line. Student's *t*-test was used for statistical analysis.

Drugs used were: acetylcholine bromide (Sigma), ascorbic acid, atropine sulphate (BDH), clonidine HCl (Boehringer), cimetidine (Smith, Kline and French), desmethylimipramine HCl (Ciba-Geigy), histamine acid phosphate (BDH), indoramin HCl (Wyeth), 1-[^{3}H]noradrenaline; spec. act. 32 Ci mmol^{-1} (Amersham International), (−)-noradrenaline bitartrate (Koch-Light), methysergide bimaleate (Sandoz), pargyline HCl (Sigma), phentolamine mesylate (Ciba) (±)-propranolol HCl, reserpine, yohimbine HCl (Sigma).

Reserpine was dissolved in dimethylsulphoxide (DMSO). All other drugs were dissolved in distilled water and diluted to the required concentration with Krebs solution.

RESULTS
Rat and rabbit vasa deferentia
The unstimulated rabbit vas deferens developed spontaneous contractile activity; electrical stimulation of both the rat and the rabbit vasa deferentia produced individual contractile responses defined as twitch responses. These remained constant for more than 3 h. They were inhibited by clonidine with an IC50 (concn producing a 50% inhibition of the twitch response) of 2.7 ± 0.3 nM (rat n = 6) and 6.0 ± 0.9 nM (rabbit n = 4). Clonidine completely inhibited the responses in vasa from both species at concentrations >50 nM. Phentolamine completely inhibited the response of the rabbit vas deferens at concentrations >1 μM, the IC50 being 0.15 ± 0.04 μM (n = 5); its inhibitory effect was reversed by washing, 15–30 min being required for return to the control value recorded before addition of the drug. With the rat vas deferens, phentolamine increased the size of the twitch at all concentrations used (0.1–10 μM). The inhibition by clonidine of the twitch response in vasa from both species was antagonized competitively by yohimbine and inhibition of the twitch response of the rabbit vas by phentolamine was also antagonized competitively by yohimbine (Fig. 1). The pA$_2$ values and Schild plot slopes are given in Table 1. Yohimbine alone did not inhibit the twitch response of vasa from either species and small increases in size of the twitch response were seen at concentrations above 1 μM. The α_1-adrenoceptor antagonist, indoramin (0.1–1 μM), which itself had no effect on the twitch responses of rat or rabbit vasa, did not antagonize the inhibitory effects of clonidine or phentolamine. Atropine, propranolol, methysergide or cimetidine (10 μM) did not reverse the inhibitory effect of phentolamine.

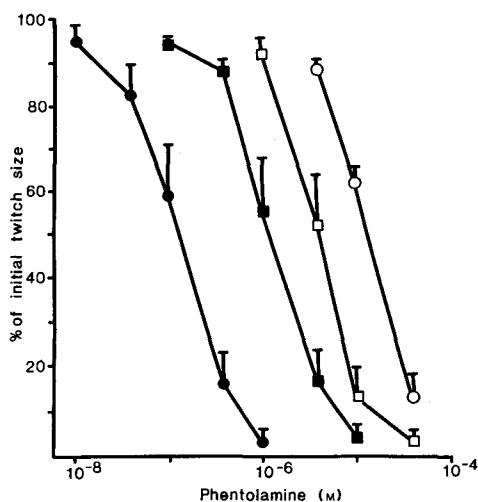As well as inhibiting the twitch response in the

FIG. 1. Dose-response curves for phentolamine in the rabbit vas deferens in the presence of increasing concentrations of yohimbine. ● Control: ■ yohimbine 10^{-7} M, □ yohimbine 3×10^{-7} M, ○ yohimbine 10^{-6} M (n = 5).

rabbit vas deferens, phentolamine (0·3–1 μM) inhibited the contractions induced by NA (10 μM), but not those by acetylcholine (50 μM) or histamine (100 μM) in tissues from both species. The phentolamine-induced inhibition of the NA contractions was not reversed by yohimbine (0·1–1·0 μM). The NA uptake$_1$ inhibitor, DMI (0·3–1 μM), completely inhibited the twitch response of rabbit and rat vasa and this effect was antagonized by yohimbine (0·1–1·0 μM).

Reserpine (5 mg kg^{-1} 24 h before removal of the vasa deferentia) had no significant effect on twitch height of either rabbit or rat vasa but completely antagonized the inhibitory effect of DMI in these tissues. There was no significant difference between the IC50 for clonidine or phentolamine on the rabbit vas deferens after reserpine pretreatment when compared with controls; IC50 = 5·3 ± 0·05 nM (n = 4) and 0·13 ± 0·3 μM (n = 4) respectively after reserpine pretreatment. However, in the rat vas

Table 1. pA$_2$ (95% confidence limits) values for yohimbine.

	Clonidine	n	Phentolamine	n
Rat vas deferens	7·6 (7·4–7·7) Slope = 0·83	6	—	
Rabbit vas deferens	7·7 (7·5–8·1) Slope = 0·84	4	7·9 (7·6–8·5) Slope = 1·06	5
Rat cortex	7·7 (7·3–8·1) Slope = 1·10	4	8·1 (7·7–8·9) Slope = 1·01	6

deferens the dose response curve to clonidine was moved significantly to the left after reserpinization: IC50 for clonidine was 1·2 ± 0·3 nM (n = 4) $P < 0·5$ when compared to control IC50 of 2·7 ± 0·3 nM (n = 6).

[³H]NA release from slices from rat occipital cortex
After 30 min of superfusion the fractional release rate of tritium was constant at 0·56 ± 0·06% min^{-1}. Addition of 25 mM K$^+$ produced an increase in the release of radioactivity to a maximum of 1·54 ± 0·09% min^{-1} which persisted for 12 min. The S$_2$/S$_1$ ratio was 0·83 ± 0·03 (n = 40). The K$^+$-evoked, but not the spontaneous, release was calcium-dependent.

Phentolamine (1–100 nM), added to the superfusing medium 20 min before S$_2$, produced a concentration-related inhibition of K$^+$-evoked tritium release. The maximal effect obtained was 50% inhibition and this was observed at 100 nM phentolamine. The IC50 was 8·7 ± 2·0 nM (n = 5). Concentrations of phentolamine above 1 μM produced a significant ($P < 0·02$) enhancement of tritium release. These results are shown in Table 2. Clonidine (3–1000 nM) produced a concentration-related inhibition of tritium release. The maximum inhibition obtained was 70% at 1 μM and the IC50 was 75·0 ± 8·9 nM.

Table 2. S$_2$/S$_1$ ratios for the K$^+$-evoked release of tritium from slices of rat occipital cortex preloaded with [³H]NA, Effect of phentolamine, clonidine and DMI.

Treatment	Concn (M)	Mean S$_2$/S$_1$ ratio ± s.e.m.	n
Control		0·76 ± 0·03	10
Phentolamine	10^{-9}	0·63 ± 0·04	4
	10^{-8}	0·48 ± 0·09*	6
	10^{-7}	0·40 ± 0·05**	10
	10^{-6}	0·93 ± 0·04**	4
	10^{-5}	1·08 ± 0·08**	4
Clonidine	10^{-7}	0·49 ± 0·06**	6
DMI	5×10^{-7}	0·99 ± 0·06**	10
DMI + phentolamine	$5 \times 10^{-7} + 10^{-7}$	1·06 ± 0·13†	8
DMI + clonidine	$5 \times 10^{-7} + 10^{-7}$	1·14 ± 0·17†	6

Significantly different from controls *$P < 0·05$, **$P < 0·01$.
† Not significantly different from DMI controls.

Yohimbine (3–100 nM) produced parallel rightward shifts in the concentration effect curve to clonidine and in the inhibition part of the concentration-effect curve to phentolamine. The pA$_2$ values are shown in Table 1. Yohimbine alone had *no effect on K$^+$-induced tritium release up to*

100 nM. Above this concentration enhancement of release was observed (S_2/S_1 ratio at 300 nM = $1\cdot00 \pm 0\cdot06$, n = 4).

Indoramin had no significant effect on the response to phentolamine. Phentolamine (10 nM) produced $22 \pm 0\cdot5\%$ inhibition and in the presence of indoramin (100 nM) $26 \pm 7\cdot5\%$ inhibition.

DMI ($0\cdot5\,\mu$M) produced an increase in the K$^+$-evoked release of tritium ($S_2/S_1 = 0\cdot99 \pm 0\cdot06$). In the presence of DMI ($0\cdot5\,\mu$M) there was no inhibitory effect of phentolamine (10 nM) or clonidine (100 nM). Both compounds produced a small increase in release compared with DMI alone. These results are shown in Table 2.

DISCUSSION
Phentolamine and clonidine inhibited the K$^+$-evoked release of [^{3}H]NA from slices of rat occipital cortex and inhibited the electrically-induced twitch response of the rabbit vas deferens. Clonidine, but not phentolamine, inhibited the twitch response of the rat vas deferens. The effects of both compounds were competitively antagonized by yohimbine with similar pA_2 values against each compound suggesting that the same receptor was involved in the response to both compounds. In contrast, indoramin ($0\cdot1$–$1\,\mu$M), a selective α_1-adrenoceptor antagonist (Rhodes & Waterfall 1978), had no significant effect on the inhibition induced by both compounds.

The NA uptake$_1$ inhibitor, DMI (Titus & Spiegel 1962), produced an enhancement of K$^+$-evoked [^{3}H]NA release. The inhibitory effect of both phentolamine and clonidine was not observed when the superfusing media contained DMI. The prevention of the inhibitory effect of clonidine on electrically-evoked NA release by uptake inhibitors has previously been reported by Pelayo et al (1980) and by Reichenbacher et al (1982) who both reported that the size of the inhibitory response to clonidine was dependent on the biophase concentration of NA.

There are few published reports on the effect of low concentrations of phentolamine on [^{3}H]NA release from rat cortical slices. Wemer et al (1979) found a concentration-related increase of tritium release in response to phentolamine from 30 nM–10 μM, however, those authors included DMI (10 μM) routinely in their superfusion experiments and that will significantly increase the biophase concentration of NA, thus masking the inhibitory effect of phentolamine.

The possibility that DMI prevented the effect of phentolamine by blocking the uptake of phentolamine into the nerve terminal is unlikely, since phentolamine only competes for the NA uptake site at concentrations above 1 μM (Broadhurst et al 1983) i.e. over 100 times the IC50 for the inhibition of [^{3}H]NA release.

It was not possible to determine the effect of DMI on the response to either phentolamine or clonidine in the rat or rabbit vasa because DMI itself inhibited the twitch response in both preparations.

To show that the inhibition of the rabbit vas deferens twitch response was not due to NA uptake inhibition, the effect of phentolamine was determined in vasa taken from rabbits given reserpine 5 mg kg^{-1} subcutaneously 24 h before removal of the vasa. In tissues from reserpinized rabbits, DMI no longer inhibited the twitch response; in contrast, phentolamine still inhibited the twitch response and its effect and that of clonidine were not significantly different from controls.

A non-specific postsynaptic depressant effect of phentolamine cannot explain the inhibitory action in the rabbit vas deferens since contractions produced in response to exogenous acetylcholine or histamine were not inhibited by phentolamine, whereas the electrically-evoked twitch response was inhibited. The contraction produced by exogenous NA was antagonized by phentolamine, demonstrating the postsynaptic α_1-adrenoceptor-blocking property of phentolamine. This effect was not reversed by yohimbine.

Indoramin is a potent histamine H$_1$-antagonist as well as an α_1-adrenoceptor antagonist (Alps et al 1972), so the phentolamine inhibition is not related to an action at H$_1$-receptors or α_1-adrenoceptors, nor is the effect related to stimulation of muscarinic, 5-HT-, H$_2$- or β-receptors, since antagonists at these receptor sites did not affect the inhibitory response to phentolamine. These antagonists themselves have no significant effect upon the twitch responses of the rabbit vas deferens.

In conclusion, the similarity between the inhibitory response to phentolamine and clonidine in the rabbit vas deferens and rat occipitoparietal cortex slices suggests that in these preparations both compounds were acting as agonists at a similar receptor. The antagonism of the responses by the selective α_2-adrenoceptor antagonist yohimbine indicates that α_2-adrenoceptors may be involved in the response. Why this agonist affect of phentolamine should be confined to some tissues and not others cannot be explained at present.

Note added in proof: since the preparation of this

manuscript Angus & Lew (1984) have published similar results suggesting that phentolamine may act as a presynaptic α_2-adrenoceptor agonist in the vasculature and right atria of the rabbit thus providing further corroboration that phentolamine has some agonist activity.

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