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PREJUNCTIONAL ACTIONS OF PIRIBEDIL ON THE ISOLATED KIDNEY OF THE RABBIT: COMPARISON WITH APOMORPHINE

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1 The effects of piribedil on contractile responses and noradrenaline release evoked by sympathetic nerve stimulation have been studied in the isolated kidney of the rabbit. These effects were compared to those of apomorphine.

2 Electrical stimulation (2, 5 and 10 Hz) of sympathetic renal nerves produced frequency-dependent increases in perfusion pressure and noradrenaline release. Piribedil did not affect (0.1 μg/min) or diminished (1 and 10 μg/min) the stimulation-evoked increase in perfusion pressure, and increased noradrenaline release in a dose-dependent manner.

3 Increases in renal perfusion pressure and noradrenaline release induced by electrical stimulation were decreased by apomorphine (0.1 and 1 μg/min). These inhibitory effects were more marked at low frequencies of stimulation and were prevented by haloperidol (0.2 μmol/l).

4 Piribedil (0.1 and 1 μg/min) and apomorphine (0.1, 1 and 10 μg/min) did not affect the increases in renal perfusion pressure elicited by exogenously administered noradrenaline, but piribedil (10 μg/min) diminished them.

5 In the presence of desipramine (0.5 μmol/l), piribedil (0.1, 1 and 10 μg/min) produced a dose-dependent inhibition of the increases in renal perfusion pressure and noradrenaline release evoked by sympathetic nerve stimulation; the inhibitory effect of piribedil was more marked at low frequencies of stimulation and was prevented by haloperidol.

6 Piribedil increased the resting release of noradrenaline from the rabbit kidney, in contrast to apomorphine, which was without effect.

7 It is suggested that piribedil has a complex effect on sympathetic transmission. This drug exhibits an 'amphetamine-like' action, causing noradrenaline release from its postganglionic stores. This releasing effect masks an action on prejunctional inhibitory dopamine receptors. In addition, at high doses, piribedil exhibits a marked action on postjunctional sites, since it reduces the vasoconstrictor effect of exogenous noradrenaline.

Introduction

Piribedil (Pir) is usually considered as a dopamine-receptor activating agent at the level of the central nervous system (Corrodi, Farnebo, Fuxe, Hamberger & Ungerstedt, 1972) as well as at a peripheral level (Laubie, Schmitt & Falq, 1977; Buylaert, 1977; Laubie & Schmitt, 1978).

At the level of the peripheral sympathetic nervous system, Pir was shown to inhibit sympathetic transmission in femoral (Buylaert, 1977; Laubie *et al.*, 1977), cardiac, splenic, mesenteric and renal areas (Laubie & Schmitt, 1978). Since the inhibitory effects on sympathetic transmission were not due to a postjunctional action, were preferential on responses induced by low frequency stimulation and were reversed by haloperidol or pimozide, the data suggest that the effects of Pir are compatible with a reduction of noradrenaline (NA) release by an action on prejunctional inhibitory dopamine receptors.

Nevertheless, in the central nervous system, Pir also seems to activate the noradrenergic system: it induced ferocity and aggressiveness in rats, an effect antagonized by propranolol (Butterworth, Poignant & Barbeau, 1975) and decreased NA tissue levels (Corrodi *et al.*, 1972; Fuxe, 1973; Garattini, Bareggi, Marc, Calderini & Morselli, 1974; Bareggi, Markey & Paoletti, 1978).

In order to elucidate further the mechanism of action of Pir on peripheral postganglionic neurones, we have studied the effects of the drug, comparing them with those of apomorphine (Apo), on the spontaneous and

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stimulation-evoked release of NA from noradrenergic stores of the isolated perfused kidney of the rabbit.

Methods

General procedure

Male New Zealand rabbits (1.8 to 2.2 kg) were anaesthetized with urethane (1.5 to 3 g/kg, i.p.). The abdomen was opened along its midline and the left kidney dissected from its surrounding tissues. After administration of heparin (750 i.u./kg, i.v.), polyethylene catheters were tied into the renal artery and the renal vein and the kidneys were immediately perfused with a physiological saline solution (PSS) at a constant flow rate (10 ml/min) by means of a peristaltic pump. Therefore, changes in perfusion pressure were indicative of alterations of vascular resistance. After starting the perfusion, the kidneys were removed and transferred to a thermostatically controlled box and were covered with moistened cotton gauze.

Sympathetic stimulation; effects of apomorphine and/or haloperidol and of piribedil on constrictor responses and noradrenaline release

A bipolar circular electrode was placed on the renal artery. The intramural sympathetic nerves were stimulated at supramaximal voltage for 15 s with pulses of 1 ms duration at various frequencies. The intervals between stimulations were of 15 min. After control stimulations (2, 5 and 10 Hz), Apo (0.1 and 1 μg/min) and/or haloperidol (Halo, 0.2 μmol/l) or Pir (0.1, 1 and 10 μg/min) were infused in the perfusion system at a point just proximal to the renal artery, and stimulation (2, 5 and 10 Hz) was again applied during the drug(s) infusion. At the concentrations used, none of the drugs had any effect on the resting perfusion pressure. The action of the agents on the increase in perfusion pressure evoked by transmural electrical stimulation of renal sympathetic nerves was studied.

The venous effluent from the kidney was collected during sympathetic stimulation and during the following minute, in order to determine its NA concentration.

In some experiments, desipramine (0.5 μmol/l) was present in the perfusion medium from the beginning of the experiment, and we studied the effects of Pir and Halo on vasoconstrictor responses and the NA release elicited by sympathetic nerve stimulation.

Vasoconstrictor responses to exogenous noradrenaline; effects of piribedil and apomorphine

Vasoconstrictor responses to exogenous NA were elicited by injection of 100, 200 and 400 ng NA in the renal perfusion stream at a point just proximal to the artery, before and during infusions of Pir or Apo (0.1, 1 and 10 μg/min).

Measurement of endogenous noradrenaline released from the kidney during sympathetic stimulation

The NA released (pg/ml) in the venous effluent from the rabbit kidney during electrical stimulation was measured by a radiometric enzymatic method (Gauchy, Tassin, Glowinski & Cheramy, 1976). The assay required 100 μl renal effluent.

Effects of apomorphine and piribedil on resting noradrenaline release

In our experimental conditions, the resting NA levels were undetectable when the amount of the amine was measured in 100 μl effluent. In order to estimate the effects of Pir (0.1, 1 and 10 μg/min) and Apo (0.1, 1 and 10 μg/min) on the spontaneous NA release from the rabbit kidney, the NA contained in 10 ml perfusate was concentrated by absorption on microcolumns of alumina (Gauchy *et al.*, 1976) and eluted with a small volume of 0.2 N acetic acid. The NA concentration of 100 μl aliquots of the eluates was then measured as described above.

Solutions and drugs

The composition of the PSS used for perfusion of the rabbit kidney and dilution of the drugs was (mmol/l): NaCl 137, KCl 2.5, $CaCl_2$ 1.4, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.36, glucose 5.6, ascorbic acid 0.1 and Dextran T 70 0.31. This PSS was gassed with a 95% O_2 and 5% CO_2 mixture and was maintained at 37°C.

The following drugs were used: piribedil hydrochloride (Servier), haloperidol hydrochloride (Janssen-Pharm.) apomorphine hydrochloride (Sandoz), (±)-noradrenaline bitartrate (Calbiochem) and desipramine (Ciba Geigy). Drugs were dissolved in distilled water, then further diluted with PSS.

Statistical analysis of results

Student's *t* test was used to test for significant differences in the experimental results.

Results

Effects of apomorphine and haloperidol on vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline release

Electrical stimulation (2, 5 and 10 Hz) of rabbit iso-

lated kidneys produced frequency-dependent increases in perfusion pressure and NA release. Intrarenal infusion of Apo (0.1 and 1 μg/min) resulted in depression of both parameters. This reduction was more marked at low frequencies of stimulation than at higher frequencies. Halo (0.2 μmol/l) prevented both actions of Apo, but had no effect alone (Table 1).

Effects of piribedil on vasoconstrictor responses and noradrenaline release evoked by sympathetic nerve stimulation

Pir (0.1 μg/min) did not affect the vasoconstrictor responses evoked by sympathetic nerve stimulation. At higher doses (1 and 10 μg/min) Pir significantly reduced these constrictor responses.

Table 1 Influence of apomorphine (Apo) and/or haloperidol (Halo) on the increase in renal perfusion pressure (Δ RPP, mmHg) and noradrenaline (NA, pg/ml) release elicited by sympathetic stimulation in the perfused rabbit kidney

Experimental group	n		*Frequency of stimulation* (Hz) 2	5	10
Controls	7	Δ RPP	2.8 ± 0.3	15.4 ± 2.3	65.0 ± 6.2
		NA	175 ± 19	783 ± 48	2575 ± 103
Apo 0.1 μg/min	7	ΔRPP	1.0 ± 0.2***	9.0 ± 1.8*	50.6 ± 4.1*
		NA	87 ± 6***	456 ± 54**	1948 ± 118**
Apo 1.0 μg/min	7	ΔRPP	0.4 ± 0.1***	6.9 ± 1.9*	38.4 ± 6.1***
		NA	58 ± 7***	334 ± 43***	1766 ± 80***
Controls	6	ΔRPP	3.7 ± 0.5	17.8 ± 2.0	69.0 ± 7.0
		NA	92 ± 11	703 ± 95	2602 ± 142
Halo 0.2 μM	6	ΔRPP	3.1 ± 0.4	19.9 ± 1.8	78.0 ± 6.1
		NA	98 ± 8	302 ± 101	2855 ± 127
Controls	6	ΔRPP	3.5 ± 0.4	17.2 ± 2.0	56.3 ± 5.8
		NA	117 ± 9	627 ± 48	1993 ± 59
Apo 1.0 μg/min +Halo	6	ΔRPP	3.8 ± 0.3	18.9 ± 2.3	503 ± 4.7
		NA	132 ± 12	588 ± 59	1869 ± 109

Apo and/or Halo were added to the medium 15 min before the second period of nerve stimulation. Mean values are shown ± s.e. mean. n = number of experiments. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$ when compared with the respective control group.

Table 2 Influence of piribedil (Pir) on the increase in renal perfusion pressure (ΔRPP, mmHg) and noradrenaline (NA, pg/ml) release elicited by sympathetic stimulation in the perfused rabbit kidney

Experimental group	n		*Frequency of stimulation* (Hz) 2	5	10
Controls	6	ΔRPP	2.2 ± 0.5	11.7 ± 1.2	59.1 ± 2.6
		NA	98 ± 7.5	575 ± 37	2022 ± 105
Pir 0.1 μg/min	6	ΔRPP	1.9 ± 0.4	11.9 ± 0.8	55.9 ± 3.6
		NA	159 ± 13**	670 ± 41*	2449 ± 90
Pir 1.0 μg/min	6	ΔRPP	1.5 ± 0.3	8.2 ± 0.7*	46.2 ± 3.0**
		NA	265 ± 14**	825 ± 63**	2794 ± 106**
Pir 10 μg/min	6	ΔRPP	0.9 ± 0.2*	6.7 ± 0.7	38.9 ± 3.6
		NA	317 ± 24***	973 ± 79***	3098 ± 129***

After the control period, Pir was infused in successively increasing doses, over a period of 45 min for each dose and stimulation began 15 min after the start of each infusion. Mean values are shown ± s.e. mean. n = number of experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared with controls.

Table 3 Influence of piribedil (Pir) and apomorphine (Apo) on the increase in renal perfusion pressure (ΔRPP, mmHg) evoked by exogenous noradrenaline in the perfused rabbit kidney

Experimental group	n	*Dose of noradrenaline* (ng) 100	200	400
Controls	6	7.4 ± 1.2	17.5 ± 2.4	38.7 ± 3.6
Pir 0.1 μg/min	6	7.1 ± 0.8	18.3 ± 1.6	35.1 ± 2.3
Pir 1.0 μg/min	6	6.3 ± 0.5	15.4 ± 1.0	32.9 ± 3.4
Pir 10 μg/min	6	3.1 ± 0.4**	9.0 ± 0.8**	17.1 ± 1.6***
Controls	5	8.1 ± 0.9	16.9 ± 2.1	35.7 ± 2.9
Apo 0.1 μg/min	5	7.6 ± 0.6	15.1 ± 1.7	38.3 ± 2.5
Apo 1.0 μg/min	5	8.3 ± 0.7	17.2 ± 2.0	38.1 ± 3.1
Apo 10 μg/min	5	8.4 ± 0.5	14.9 ± 2.0	34.8 ± 2.1

After the control periods, Pir or Apo was infused during 45 min for each dose in increasing doses, and the contractile responses to exogenous noradrenaline was measured during the drug infusion. Mean values are shown ± s.e. mean. n = number of experiments.
** $P < 0.01$; *** $P < 0.001$ when compared with controls.

Table 4 Influence of piribedil (Pir) and/or haloperidol (Halo) in the presence of desipramine on the increase in renal perfusion pressure (ΔRPP, mmHg) and noradrenaline release (NA, pg/ml) elicited by sympathetic stimulation in the perfused rabbit kidney

Experimental group	n		*Frequency of stimulation* (Hz) 2	5	10
Controls	7	ΔRPP	15. ± 0.9	27.0 ± 1.9	46.0 ± 3.1
		NA	267 ± 30	970 ± 112	2163 ± 125
Pir 0.1 μg/min	7	ΔRPP	11.3 ± 0.7*	23.1 ± 1.1	40.7 ± 2.9
		NA	181 ± 16*	741 ± 62	2061 ± 106
Pir 1.0 μg/min	7	ΔRPP	7.9 ± 0.6**	20.0 ± 1.0*	37.3 ± 2.4*
		NA	126 ± 21**	682 ± 51*	2203 ± 134
Pir 10 μg/min	7	ΔRPP	4.2 ± 0.5***	15.9 ± 1.3***	31.8 ± 1.7**
		NA	87 ± 11***	555 ± 42**	1972 ± 104
Controls	5	ΔRPP	17.3 ± 1.4	30.2 ± 2.7	52.5 ± 3.6
		NA	283 ± 28	1010 ± 93	2254 ± 101
Halo 0.2 μM	5	ΔRPP	16.1 ± 1.0	33.3 ± 2.4	57.3 ± 3.4
		NA	258 ± 19	1100 ± 81	2301 ± 127
Controls	5	ΔRPP	16.7 ± 1.5	27.1 ± 2.0	49.1 ± 3.0
		NA	225 ± 20	859 ± 87	2025 ± 99
Pir 0.1 μg/min + Halo	5	ΔRPP	17.4 ± 1.1	26.3 ± 1.7	53.0 ± 3.2
		NA	207 ± 21	803 ± 78	2102 ± 104
Pir 1.0 μg/min + Halo	5	ΔRPP	16.3 ± 1.3	28.1 ± 2.4	47.2 ± 2.7
		NA	197 ± 24	809 ± 91	1951 ± 79
Pir 10 μg/min + Halo	5	ΔRPP	15.4 ± 1.0	24.3 ± 2.5	45.6 ± 2.8
		NA	233 ± 19	781 ± 60	1983 ± 87

Pir and/or Halo were added to the medium 15 min before the second period of stimulation. Mean values are shown ± s.e. mean. n = number of experiments. * $P < 0.05$; ** $P < 0.01$; ** $P < 0.001$ when compared with the respective control group.

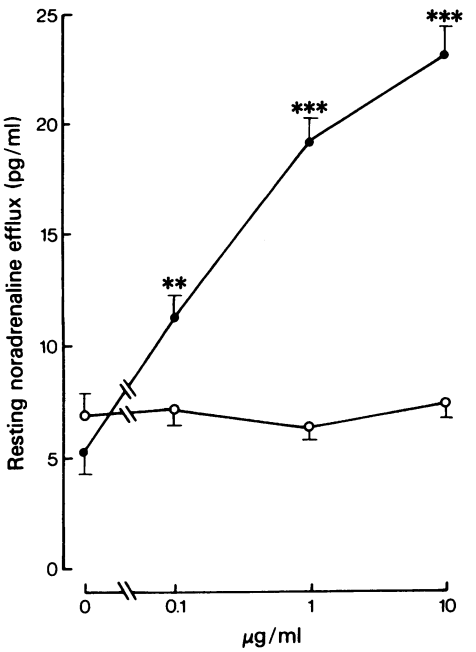

Figure 1 The effects of piribedil (●) and apomorphine (○) on the resting release of endogenous noradrenaline from the perfused rabbit kidney. Vertical lines show s.e. mean. $^{**}P < 0.01$; $^{***}P < 0.001$ compared to the resting noradrenaline release measured in the absence of any drug.

Pir (0.1, 1 and 10 μg/min) increased the NA release elicited by transmural stimulation of the renal nerves in a dose-dependent manner (Table 2).

Actions of apomorphine and piribedil on vasoconstrictor responses to exogenous noradrenaline

Apo (0.1, 1 and 10 μg/min) and Pir (0.1 and 1 μg/min) did not affect the vasoconstrictor responses to exogenously administered NA, but Pir (10 μg/min) caused a significant depression of these responses (Table 3).

Effects of piribedil on vasoconstrictor responses and noradrenaline release evoked by sympathetic nerve stimulation in the presence of desipramine

In the presence of desipramine (0.5 μmol/l), Pir (0.1, 1 and 10 μg/min) decreased the responses evoked by sympathetic nerve stimulation and caused a diminished release of NA from the rabbit kidney. The reduction of both parameters was more effective at lower frequencies of stimulation. Halo (0.2 μmol/l) treatment itself did not cause any alteration of the constrictor responses and NA release evoked by electrical stimulation, but prevented the inhibitory effects of Pir (Table 4)

Effects of apomorphine and piribedil on the resting release of noradrenaline

Apo (0.1, 1 and 10 μg/min) did not alter the resting levels of NA in the perfusate from the rabbit kidney. On the other hand, Pir, at the same doses, caused a dose-dependent increase of the spontaneous NA release from the perfused kidney (Figure 1).

Discussion

The above data show that Apo causes a reduction of the renal sympathetic nerve function via a prejunctional action: in fact, Apo reduces the vasoconstriction evoked by postganglionic sympathetic stimulation, but does not affect the renal vasoconstriction due to exogenously administered NA. Moreover, the direct measurement of endogenous NA released during sympathetic nerve stimulation shows that Apo causes a diminished liberation of the neurotransmitter, which was more marked at low frequencies of stimulation. The inhibitory effects of Apo on both constrictor responses and NA release evoked by sympathetic stimulation are abolished by Halo. This result strongly suggests that the inhibitory prejunctional action of Apo is on dopamine receptors and supports the data of Lockhandwala & Buckley (1977) who suggested, on indirect evidence, the presence of presynaptic inhibitory dopamine receptors on renal sympathetic nerves.

According to previous results from Laubie *et al.* (1977) and Laubie & Schmitt (1978), Pir reduces *in vivo* the sympathetic transmission by an action on prejunctional dopamine receptors, and acts like Apo. The results of the present study show that the effects of Pir on sympathetic transmission are more complex. In the presence of the neuronal uptake inhibitor desipramine, Pir reduces NA release evoked by sympathetic nerve stimulation. This inhibitory effect was more marked at low frequencies of stimulation, being reversed by Halo. Under these experimental conditions, the results are compatible with the concept of an effect of Pir on prejunctional inhibitory dopamine receptors and support previous data of Buylaert (1977), Laubie *et al.* (1977) and Laubie & Schmitt (1978). On the other hand, in the absence of desipramine, the effects of Pir on renal noradrenergic transmission are quite different. Pir does not diminish, but actually increases NA release due to postganglionic nerve stimulation. We tried to analyse the

reasons for this result: at the highest dose used (10 μg/min), Pir attenuates the vasoconstrictor response to exogenously administered NA. This finding may be due to a non-specific action or to blockade of postjunctional α-adrenoceptors. In the second hypothesis, Pir at the dose used, acts like an α-adrenolytic agent and may be able also to block the prejunctional α-adrenoceptors located on sympathetic nerve endings, activation of which results in diminished NA release, while receptor occupancy by an α-adrenoceptor blocking agent conversely increases the overflow of neurotransmitter during sympathetic stimulation (Langer, 1977; Westfall, 1977). Nevertheless, at lower doses (0.1 and 1 μg/min), Pir always increases NA release due to sympathetic nerve stimulation, but is devoid of postsynaptic effect. Two explanations are compatible with these results: (1) Pir preferentially blocks the prejunctional α-adrenoceptors at these doses and thus increases NA release without attenuating the effects of exogenous NA; (2) Pir exhibits an amphetamine-like action, thus releasing NA from its postganglionic stores. In our opinion, the second explanation appears more likely: in fact, the releasing action of Pir on endogenous NA stores is suppressed by desipramine, suggesting that Pir enters into sympathetic nerve endings through the neuronal uptake system and then causes the release of the neurotransmitter NA. This explanation is supported by the finding that Pir is able to increase NA release from the kidney in the absence of sympathetic stimulation. A previous study supports our data: Bareggi *et al.* (1978) showed that Pir decreases NA levels in rat brain tissue, this 'amphetamine-like' action being reduced by reserpine and as in the present work, by desipramine pretreatment.

In conclusion, the present data show that the effect of Pir on sympathetic transmission is very complex: it possesses an 'amphetamine-like' action, resulting in increased release of NA from its postganglionic stores. This releasing action masks the prejunctional inhibitory dopamine-like effect of the drug. In addition, at a high dose, Pir exhibits a marked action on postganglionic sties, since it reduces the vasoconstrictor effects of exogenously administered NA.

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