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THE PRESSOR EFFECTS OF PARAOXON IN THE PITHED RAT

J.H. DE NEEF & A.J. PORSIUS

Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands

1 In pithed rats paraoxon 825 μg/kg induced a short-lasting pressor effect. Lower doses of the drug were ineffective.

2 The pressor effect was prevented by N-methylatropine, dexetimide and α-receptor blocking agents but not by mecamylamine.

3 When blood pressure of pithed rats was elevated either by the continuous infusion of vasopressin or by electrical stimulation of the pithing rod, both 275 and 825 μg/kg paraoxon induced further pressor effects. The effectiveness of various receptor blocking agents was similar to that observed in pithed rats without vasopressin.

4 It is concluded that the pressor effect of paraoxon is mediated by ganglionic muscarinic receptors. Stimulation of these receptors by accumulated acetylcholine results in an increase in postganglionic sympathetic activity and causes pressor effects.

5 The peripheral action of paraoxon is compared with its action in intact anaesthetized animals.

Introduction

Although in most species the cardiovascular effects of centrally applied cholinomimetic agents are variable, in rats they are consistent (for review see Philippu, 1981). Stimulation of central muscarinic receptors in the rat increases preganglionic sympathetic activity resulting in pressor effects. These effects are also brought about by such cholinomimetic agents as oxotremorine or pilocarpine (e.g. Dage, 1979) and by acetylcholinesterase (AChE) inhibitors like physostigmine (e.g. Brezenoff & Rusin, 1974). The observed pressor effects after intravenous administration of lipophilic AChE inhibitors are generally attributed to their central action.

In pithed rats AChE inhibitors, e.g. sarin (Dirnhuber & Cullumbine, 1955) or physostigmine (Varagić, 1955; McEwen, 1968) also induce pressor responses, although higher doses are required. Recently we suggested that a peripheral component of the pressor effect induced by paraoxon in intact anaesthetized rats (De Neef, Jordaan & Porsius, 1982) may also exist. The present study was undertaken to investigate the mechanism of this peripherally mediated pressor effect. Paraoxon was infused into pithed rats and its actions studied. In separate experiments the blood pressure of pithed animals was elevated either by a continuous infusion of vasopressin or by electrical stimulation of the pithing rod. The cardiovascular effects of paraoxon were then subjected to pharmacological analysis.

Methods

Male Wistar, normotensive rats (250–300 g) were anaesthetized with pentobarbitone (75 mg/kg, i.p.). A pithing rod was pushed through the orbit into the vertebral canal and animals were artificially respired. Rectal temperature was kept at 37°C by heat controlled operating tables. Blood pressure was measured from a cannulated femoral artery by means of a Statham transducer (P23 Db) connected to a Hellige (HE 17) recorder and expressed as mean arterial pressure (MAP). Heart rate was determined from pulse waves in the femoral artery. The right femoral vein was cannulated for the intravenous administration of drugs.

In separate experiments the blood pressure of pithed animals was increased to values of intact rats by the infusion of vasopressin (1.5×10^{-2} iu $kg^{-1} min^{-1}$) into the left femoral vein. In another series of experiments rats were subjected to electrical stimulation of the thoracic spinal cord (C7-L2) as described by Gillespie & Muir (1967). Continuous stimulation (4 Hz; 2 ms; 90 V) raised blood pressure and heart rate which remained constant at the elevated values for at least 30 min.

The action of paraoxon was also studied after acute bilateral adrenalectomy and in reserpine-treated, pithed rats. Reserpine was administered for 2 consecutive days (5 mg/kg a day, i.p.). The depletion of

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catecholamines was checked by the inability of tyramine (100 μg/kg, i.v.) to increase MAP and heart rate significantly ($P<0.05$): 4±1 mmHg and 4±4 beats/min ($n=4$) respectively (control values: 35±3 mmHg and 140±9 beats/min, ($n=5$).

Drugs

The following were used: dexetimide HCl (=d-benzethimide HCl) (Janssen Pharmaceutics); mecamylamine HCl (Sigma); N-methylatropinium nitrate (Merck); paraoxon (Sigma); pentobarbitone sodium (Abbott); phentolamine HCl (Ciba Geigy); prazosin HCl (Pfizer); reserpine (Ciba Geigy); vasopressin (Sandoz); and 1,1-dimethyl-4-carboxypiperdine methylester iodide (abbreviation: QS-4-Me) which was synthesized and donated by Lambrecht & Mutschler (1973). This quarternary isocareidine ester induces pressor effects by stimulation of muscarinic receptors in sympathetic ganglia (Porsius, Wilffert, Lambrecht, Moser & Mutschler, 1981).

Paraoxon was dissolved in dimethylformamide (DMF). Before administration the solution was diluted with saline so that the final injection fluid did not contain more than 3% DMF. Yohimbine and prazosin were dissolved in 5% w/v glucose solution. All other drugs were dissolved in saline. In control experiments neither saline nor DMF (3% in saline) or glucose (5% in distilled water) influenced blood pressure or heart rate. Doses of drugs refer to salts. Paraoxon was infused intravenously for 1 min: doses used: 275 μg = 10^{-6} mol and 825 μg = 3×10^{-6} mol/kg.

Statistical analysis was performed by means of Students' t tests. $P<0.05$ was considered significant. Mean values are presented as mean±s.e.mean.

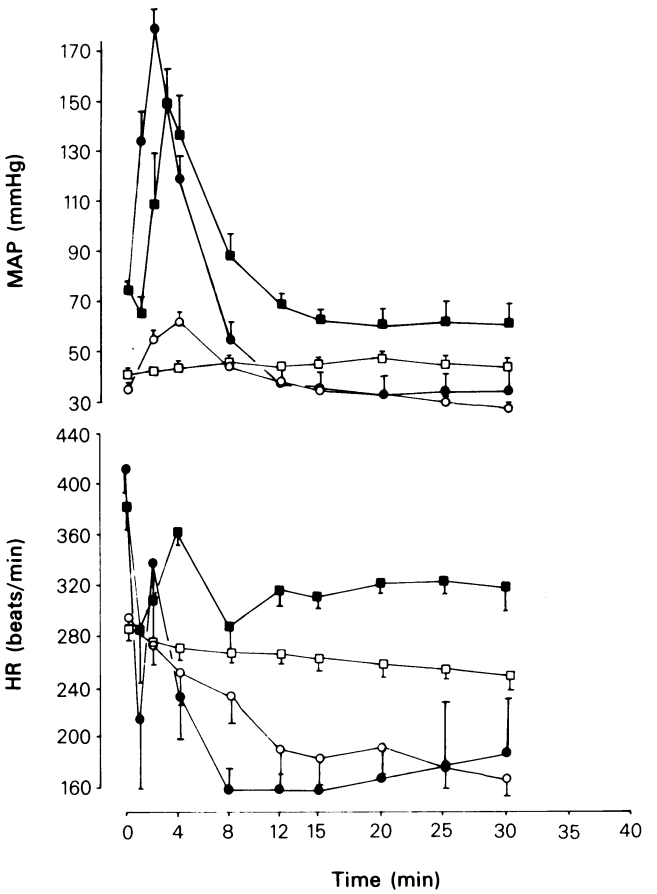

Figure 1 Effect of intravenous administration of paraoxon (□) 275 μg/kg; (○) 825 μg/kg) upon mean arterial pressure (MAP) and heart rate (HR) in pithed rats. Paraoxon was infused for 1 min. Closed symbols represent the effects in pithed rats after the elevation of blood pressure by electrical stimulation of the pithing rod (mean of $n=4$–19; vertical lines show s.e.mean).

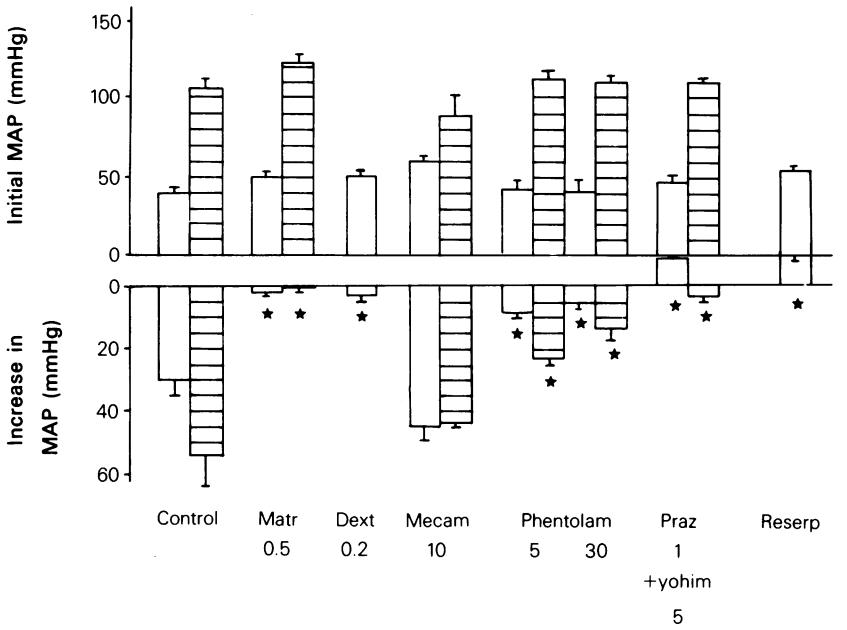

Figure 2 The effects of intravenous treatment with N-methylatropine (Matr, 0.5 mg/kg), dexetimide (Dext, 0.2 mg/kg), mecamylamine (Mecam, 10 mg/kg), phentolamine (Phentolam, 5 and 30 mg/kg), prazosin (Praz, 1 mg/kg) combined with yohimbine (yohim, 5 mg/kg) and sympathectomy (reserpine, Reserp) upon mean arterial pressure (MAP) and their influence on the pressor response to paraoxon (825 μg/kg = control; i.v.) in the pithed rat (open column). The influence of these treatments on the pressor effect produced by paraoxon (825 μg/kg) during a vasopressin infusion (hatched columns) is shown. Maximal values are presented as mean of $n = 4-19$; vertical lines show s.e.mean. *Significantly different from control ($P < 0.05$).

Results

Pithed rats

In pithed rats initial MAP and heart rate were 40 ± 3 mmHg and 293 ± 9 beats/min ($n = 19$), respectively. In these animals paraoxon at the highest applied dose (825 μg/kg) induced a short-lasting pressor response while heart rate diminished gradually (Figure 1). Systolic as well as diastolic pressure were maximal 4 min after administration. Smaller doses of paraoxon (i.e. 275 μg/kg) were ineffective on blood pressure and heart rate.

N-methylatropine (0.5 mg/kg), mecamylamine (10 mg/kg), phentolamine (30 mg/kg), and yohimbine (5 mg/kg) combined with prazosin (1 mg/kg) all decreased initial heart rate significantly ($P < 0.05$) to 245 ± 8, 267 ± 7, 197 ± 9 and 258 ± 9 beats/min ($n = 4-6$), respectively from the control value. The pressor effect of paraoxon (825 μg/kg) was significantly reduced by N-methylatropine (0.5 mg/kg), by dexetimide (0.2 mg/kg), by the combined effect of yohimbine and prazosin, by phentolamine (5 and 30 mg/kg) and by pretreatment with reserpine (Figure 2), although heart rate increased significantly ($P < 0.05$) after treatment with phentolamine (30 mg/kg, increase: 97 ± 7 beats/min, $n = 4$). In separate experiments it was demonstrated with the muscarinic agonist QS-4-Me (90 μg/kg) that N-methylatropine (0.5 mg/kg) blocked ganglionic muscarinic receptors (increase in MAP = 7 ± 5 mmHg; control: 55 ± 6 mmHg, $n = 6$).

Neither mecamylamine nor acute bilateral adrenalectomy affected the pressor effect produced by paraoxon.

Pithed rats with elevated blood pressure

Following treatment with vasopressin. In controls infusion of vasopressin (1.5×10^{-2} iu $min^{-1} kg^{-1}$) increased blood pressure to levels found in anaesthetized normotensive rats, i.e. 107 ± 6 mmHg ($n = 11$). Heart rate was not influenced by infusion of vasopressin (i.e. 332 ± 16 beats/min, $n = 11$). Both blood pressure and heart rate were maximal 4–8 min after the start of the infusion and were constant for at least 30 min. Under these conditions, 825 μg/kg paraoxon induced a further rise followed by a fall in

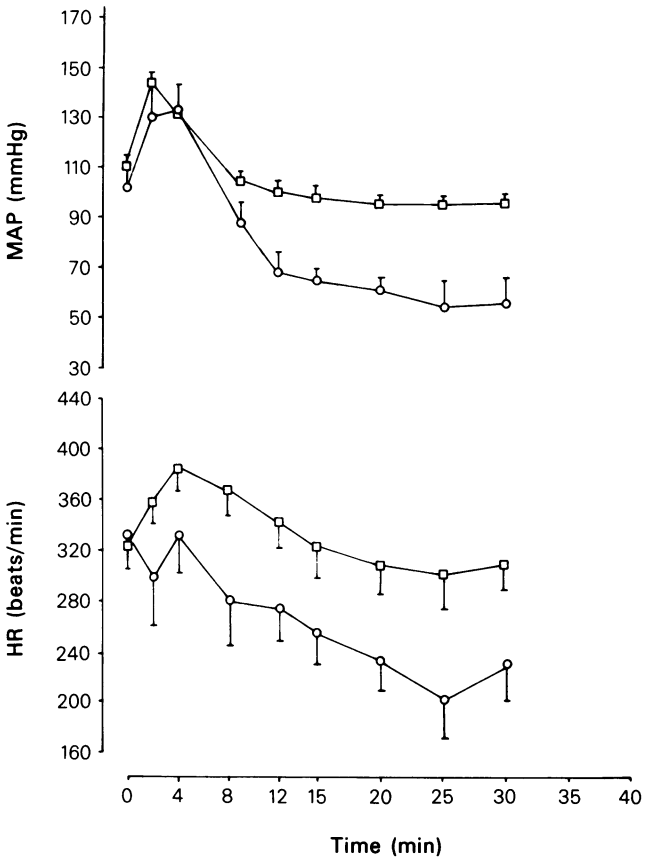

Figure 3 Effect of intravenously administered paraoxon (□) 275 μg/kg; (○) 825 μg/kg) upon mean arterial pressure (MAP) and heart rate (HR) in pithed rats after the elevation of blood pressure by means of a vasopressin infusion (mean of $n = 6-11$; vertical lines show s.e.mean).

blood pressure, while heart rate decreased gradually (Figure 3). It is striking that the maximal pressor response to 825 μg/kg paraoxon is significantly stronger with than without vasopressin infusion (Figure 2). Additionally, 275 μg/kg paraoxon which would otherwise have been ineffective caused a short-lasting pressor effect accompanied by a bradycardia during vasopressin infusion. The maximal rise in blood pressure was observed 2–4 min after beginning infusion with paraoxon (Figure 3).

The initial heart rate decreased upon treatment with phentolamine (5 and 30 mg/kg) and with yohimbine and prazosin (5 + 1 mg/kg) (all $P < 0.05$) to 261 ± 8, 215 ± 5 and 268 ± 6 beats/min ($n = 4-6$), respectively. Phentolamine (5 and 30 mg/kg) yohimbine (5 mg/kg) together with prazosin (1 mg/kg), reduced the pressor effect produced by 825 μg/kg paraoxon (Figure 2). Only N-methylatropine prevented both the pressor and the depressor effect. After the infusion of paraoxon a tachycardia was observed in the presence of phentolamine (30 mg/kg). This increase in cardiac frequency was 173 ± 54 beats/min ($n = 4$, $P < 0.05$). Both the tachycardia and the increase in MAP were abolished by additional treatment with atenolol (2 mg/kg). Mecamylamine (up to 10 mg/kg) did not affect the pressor response to paraoxon.

By electrical stimulation of the rod. Electrical stimulation of the pithing rod increased both initial MAP and heart rate significantly ($P < 0.05$) to 78 ± 2 mmHg and 396 ± 23 beats/min, respectively ($n = 9$). Upon infusion of 275 μg/kg paraoxon a short-lasting pressor response was observed while heart rate decreased (Figure 1). The maximal increase in MAP amounted to 74 ± 17 mmHg ($n = 5$) 3 min after administration. Treatment with N-methylatropine (0.5 mg/kg) reduced the pressor ef-

fect ($P<0.05$) (increase in MAP: 12 ± 2 mmHg, $n=5$). Administration of 825 μg/kg gave rise to a stronger pressor effect which amounted to 105 ± 6 mmHg ($n=4$) 2 min after dosing. After the initial rise, blood pressure fell below the initial value and was accompanied by a severe bradycardia (Figure 1).

Discussion

Paraoxon 275 μg/kg induces a long-lasting pressor effect both in the intact anaesthetized and in the conscious rat (De Neef, Jordaan & Porsius, 1982). However, only higher doses of paraoxon were able to produce pressor effects in the pithed rat with low initial blood pressure, as was demonstrated previously by Varagic (1955) and McEwen (1968) using physostigmine.

The pressor effect produced by paraoxon was abolished by antimuscarinic agents: dexetimide (0.2 mg/kg) blocks the pressor response to stimulants of ganglionic muscarinic receptors (Porsius *et al.*, 1981). Our results show that N-methylatropine (0.5 mg/kg) also blocks the pressor response to QS-4-Me, which indicates that ganglion muscarinic receptors are blocked by this antimuscarinic drug. Moreover, the same dose of N-methylatropine prevents the rise in blood pressure to paraoxon. Mecamylamine did not prevent the pressor effect of paraoxon. Hence in pithed rats the pressor response seems to be mediated by ganglionic muscarinic receptors. In contrast, the action of paraoxon in anaesthetized animals is mediated by ganglionic nicotinic receptors and is only mediated by ganglionic muscarinic receptors when nicotinic receptors are blocked (De Neef, Jordaan & Porsius, 1982).

The pressor effect produced by paraoxon seems to be mediated by an increase in sympathetic activity since it was reduced by treatment with reserpine or with α-receptor blocking agents. The result contradicts the study of McEwen (1968). This author could not prevent the pressor effect of physostigmine with phentolamine (up to 5 mg/kg). The paraoxon-induced tachycardia after treatment with α-antagonists is probably due to blockade of presynaptic cardiac α-receptors and the inhibition of the reuptake of noradrenaline by these agents (Farnebo & Hamberger, 1971; Kirpekar & Puig, 1971; Cubeddu, Barnes, Langer & Weiner, 1974). Under these circumstances the catecholamine stimulates β_1-adrenoceptors in the heart. Therefore, treatment with phentolamine was combined with atenolol in order to keep heart rate constant. However, this additional treatment did not influence the inhibitory effect of phentolamine.

Adrenalectomy did not reduce the pressor effect produced by paraoxon. Hence the release of catecholamines from the adrenals is not essential for the action of paraoxon as was found by McEwen (1968) using physostigmine.

When the low initial blood pressure of pithed rats is raised by a vasopressin infusion to values of normotensive rats, administration of the highest dose of paraoxon (825 μg/kg) induced pressor and depressor effects. The response to this dose closely resembles that in intact anaesthetized animals (De Neef, Jordaan & Porsius, 1982). In intact rats, however, the increase blood pressure was initiated by a stimulation of muscarinic receptors in the CNS, since N-methylatropine was ineffective. In contrast, this antimuscarinic drug abolished the pressor effect in pithed rats significantly ($P<0.05$). The observed bradycardia is probably the result of an interaction with postganglionic muscarinic receptors since N-methylatropine prevented the fall in cardiac frequency.

We demonstrated that in intact animals the potency of various antagonists was dependent on the initial blood pressure (De Neef, Jordaan & Porsius, 1982). Phentolamine (30 mg/kg) reduced blood pressure but did not prevent the pressor effect by paraoxon in intact animals. After elevation of the initial low arterial pressure to normal values by vasopressin, phentolamine abolished the action of paraoxon. In the pithed model, however, the antagonist was as potent in blocking the effect of 825 μg/kg paraoxon as in pithed rats with vasopressin.

In the pithed rat ganglionic transmission operates at a low level. By electrical stimulation of the pithing rod this transmission will increase and under these circumstances administration of 275 and 825 μg/kg paraoxon induced strong pressor effects. The potentiation of physostigmine-induced pressor responses by raising blood pressure following stimulation of the rod has been demonstrated previously by Drew & Leach (1974). It seems likely that the potentiation is due to the release of extra acetylcholine from preganglionic sympathetic nerves. Since AChE is inhibited simultaneously, considerably more acetylcholine will accumulate within the synaptic cleft, inducing an increase in sympathetic outflow. These findings corroborate the facilitation of ganglionic transmission that was also demonstrated after DFP (Holaday, Kamijo & Koelle, 1954; Volle, 1961).

The results of the present study demonstrate that even in pithed rats, paraoxon induces pressor effects. Due to the fact that in pithed rats ganglionic transmission operates at a low level, a strong inhibition of AChE at sympathetic ganglia is necessary to induce any effect upon blood pressure. This pressor effect, however, is more pronounced when preganglionic activity is increased by electrical stimulation of the

pithing rod. Accumulated ACh probably stimulates ganglionic muscarinic receptors which gives rise to an increased postganglionic sympathetic activity and consequently pressor responses. The cause for the enhanced potency of paraoxon in pithed rats with vasopressin remains unclear and is the subject of further investigation. The results support our earlier conclusion that the pressor effect of paraoxon in anaesthetized rats is mediated by both a central and a peripheral mechanism.

Reprint requests to A.J.P., please.

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