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THE EFFECTS OF PARAOXON ON BLOOD PRESSURE IN THE ANAESTHETIZED AND IN THE CONSCIOUS RAT

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1 Intravenous administration of paraoxon (150–825 μg/kg) to anaesthetized rats induced long-lasting, dose-dependent pressor effects. Only after injection of 825 μg/kg paraoxon was the pressor response followed by a depressor effect and a bradycardia that could be blocked by N-methylatropine. Intracerebroventricular injection of paraoxon into anaesthetized rats also induced pressor effects.

2 In order to elucidate the mechanism of the pressor action rats were given dexetimide, N-methylatropine, mecamylamine, phentolamine, prazosin, yohimbine, atenolol and metoprolol. If treatment with these drugs resulted in a low initial blood pressure, vasopressin was infused to elevate blood pressure to normal levels. The influence of adrenalectomy, pretreatment with reserpine and midcollicular transection was also examined.

3 The pressor effect of paraoxon was not influenced by N-methylatropine or mecamylamine. However, a combination of these drugs as well as dexetimide, phentolamine or prazosin combined with yohimbine, reduced or prevented the pressor effect.

4 In conscious rats the effects of paraoxon and the action of antimuscarinic drugs upon the pressor response were similar to those observed in anaesthetized animals.

5 Acetylcholinesterase activities were measured in various brain regions and in whole blood. Paraoxon concentrations within the CNS were also measured.

6 It is concluded that the pressor effect of paraoxon in anaesthetized and conscious rats is mediated by a central mechanism, although a contribution of peripheral acetylcholinesterase inhibition in sympathetic ganglia to this pressor effect cannot be ruled out.

Introduction

Since the initial report by Dirnhuber & Cullumbine (1955) a number of investigations have been carried out to clarify the mechanism of the pressor action produced by inhibitors of cholinesterase (e.g. Varagić, 1955; Brezenoff, 1973). Accumulation of acetylcholine (ACh) by enzyme inhibition within the CNS of rats induces pressor effects due to an increased sympathetic activity; excitation of central muscarinic receptors seems to be responsible. Other drugs acting on cholinoceptors, such as carbachol (Brezenoff & Jenden, 1970; Brezenoff, 1972), oxotremorine (Dage, 1979; Weinstock, Zavadil, Chicueh & Kopin, 1979) and ACh (Krstic & Djurkovic, 1978; Krstic, 1978) administered intravenously, into the ventricles or into the hypothalamus, produced comparable effects on blood pressure to those of physostigmine.

A few authors have described the actions of irreversible cholinesterase inhibitors in rats (Dirnhuber & Cullumbine, 1955; Kuga & Erdmann, 1967; Vetterlein & Haase, 1979). In some studies (e.g. Kuga & Erdmann, 1967; Stamenović & Varagić, 1970) the doses that were used of various receptor blocking agents, like hexamethonium, were relatively low and therefore could not prevent the action of the cholinesterase inhibitors. Moreover, application of nicotinic receptor and α-adrenoceptor antagonists resulted in a substantial decrease in blood pressure and heart rate. Since pressor effects can be more pronounced if the initial blood pressure is low, the potency of blocking agents might be reduced when treatment with these antagonists results in a low initial blood pressure.

The present study was undertaken with paraoxon. This irreversible cholinesterase inhibitor will reduce enzyme activity for much longer than physostigmine, the pressor effects of which were reported to be short-lasting (e.g. Nakagawa, 1968). Moreover, the effects by physostigmine cannot be attributed to inhibition of cholinesterase only (Cox & Lomas, 1972; Bartolini, Bartolini & Domino, 1973).

Paraoxon was administered to both anaesthetized

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and conscious rats in order to study its action. Treatment with antimuscarinic, antinicotinic drugs, α- and β-adrenoceptor blocking agents was carried out to elucidate the mechanism of action. When treatment resulted in a low initial blood pressure, arterial pressure was elevated to normal values by a continuous vasopressin infusion. Acetylcholinesterase (AChE) activities in various brain regions and whole blood and paraoxon concentrations within the brain were also measured.

Methods

Male Wistar, normotensive rats (250–300 g) were anaesthetized with pentobarbitone (75 mg/kg, i.p.). The animals were artificially respired and the rectal temperature kept at 37°C by heat-controlled operating tables. Blood pressure was recorded from a cannulated femoral artery by means of a Statham transducer (P23 Db) connected to a Hellige (HE 17) recorder. Heart rate was established from pulse waves in the femoral artery. The right femoral vein was cannulated for administration of drugs. When necessary, blood pressure of animals was increased to normal levels by the infusion of vasopressin (1.5×10^{-2} iu kg^{-1} min^{-1}) into the left femoral vein. For intracerebroventricular (i.c.v.) administration of paraoxon, a hole was drilled into the skull 1.5 mm lateral and 1 mm caudal of the bregma. Injections were performed by means of a Hamilton syringe to a depth of 4 mm. At the end of the experiment methylene blue was injected in order to indicate the location of administration.

Adrenalectomy

Adrenalectomy was carried out under hexobarbitone anaesthesia (150 mg/kg, i.p.). Anaesthesia lasted for about 20 min. The animals were treated with desoxycorticosterone (DOCA): 15 mg DOCA kg^{-1} (s.c.) during the first two days, and 7 mg DOCA kg^{-1} (s.c.) during the following 6–8 days (Peters, 1959). Experiments were carried out 8–10 days after the extirpation. In separate experiments the action of paraoxon was studied after acute bilateral adrenalectomy.

Reserpine-treated rats

Reserpine was administered for 2 successive days (5 mg/kg a day, i.p.). The depletion of catecholamines was confirmed with tyramine (100 mg/kg, i.v.). In these animals, the increase of mean arterial pressure and heart rate was significantly ($P < 0.05$) reduced after the administration of tyramine and amounted to 7 ± 1 mmHg and 1 ± 2 beats/min, respectively (control values: 32 ± 5 mm Hg and 63 ± 9 beats/min, $n = 6$).

Decerebrate animals

In separate experiments, paraoxon was tested in rats after midcollicular transection as described by Henning, Rubenson & Trolin (1972).

Conscious rats

On the day prior to the experiments, rats were anaesthetized with hexobarbitone (150 mg/kg, i.p.). Anaesthesia lasted for about 20 min. Catheters were inserted into a femoral vein and a femoral artery led subcutaneously and exteriorized through a small skin incision between the ears. The catheters were previously filled with heparinized saline (100 iu/ml) and the open end was closed.

Distribution of paraoxon and acetylcholinesterase activity within the CNS

After infusion of paraoxon, the anaesthetized rats were decapitated and the brain isolated on ice. It was dissected along the midline in two halves, which include hemisphere, mesencephalon and rhombencephalon. The concentration of paraoxon was determined in the left half of the brain by high performance liquid chromatography according to the method described previously (de Neef, Porsius & van Rooy, 1981b). In the right half of the brain, AChE activity was measured according to the method described by Ellman, Courtney, Andres & Featherstone (1961). In separate experiments enzyme activities were measured in the following brain regions: pons plus medulla oblongata, hypothalamus, cerebellum and the right hemisphere. ChE activity in whole blood was also determined.

Drugs

The following drugs were used: arecoline HBr (Merck); atenolol HCl (I.C.I.); desoxycortisone trimethylacetate (Ciba Geigy); dexetimide HCl (= d-benzethimide HCl) (Janssen Pharmaceutics); gallamine triethiodide (Sigma); hexobarbitone sodium (Bayer); mecamylamine HCl (Sigma); N-methylatropine nitrate (Merck); metoprolol HCl (Hässle AB); paraoxon (Sigma); pentobarbitone sodium (Abbott); phentolamine HCl (Ciba Geigy); prazosin HCl (Pfizer); reserpine (Ciba Geigy); vasopressin (Sandoz) and yohimbine HCl (Sigma).

Paraoxon was dissolved in dimethylformamide (DMF). For intravenous administration the solution was diluted with saline so that the final injection fluid contained not more than 3% DMF. In control experiments, it was established that DMF (3% in saline) did not affect blood pressure or heart rate. For i.c.v. application, paraoxon was dissolved in ethanol and

polyethyleneglycol (both 15% w/v) since DMF (3%) did not allow a more concentrated solution of paraoxon to be prepared. I.c.v. administration of the vehicle did not influence haemodynamics. Yohimbine and prazosin were dissolved in 5% w/v glucose solution. All other drugs were dissolved in saline. Neither saline nor glucose (5% in water) influenced blood pressure or heart rate after intravenous administration. Doses of drugs refer to salts. Paraoxon was infused intravenously for 1 min. I.c.v. injections (volume 10 μl) were applied for 15 s.

Doses used: 28, 83, 150, 275 and 825 μg = 10^{-7}, 3×10^{7}, 5.5×10^{-7}, 10^{-6} and 3×10^{-6} mol paraoxon/kg.

All receptor blocking agents were tested in separate experiments. Statistical analysis was performed by means of Student's *t* tests. $P < 0.05$ was considered significant. Mean values are presented as mean ± s.e.mean.

Results

Intact anaesthetized rats

Paraoxon (150–825 μg/kg, i.v.) increased systolic as well as diastolic blood pressure; 83 μg/kg paraoxon had no significant ($P > 0.05$) effect on blood pressure (Figure 1). The maximum effect was observed 2–4 min after administration of 275 or 825 μg/kg paraoxon. The activity persisted for some 40 min. The pressor response to 825 μg/kg paraoxon was followed by a reduction in blood pressure. Heart rate fell immediately upon the start of the infusion. N-methylatropine (0.5 mg/kg, i.v.) prevented both the bradycardia and the hypotensive effect of 825 μg paraoxon/kg and prolonged the pressor effect (40 min). N-methylatropine 0.5 mg/kg also blocked the hypotensive effect and the bradycardia induced by arecoline (15 μg/kg, i.v.) indicating the involve-

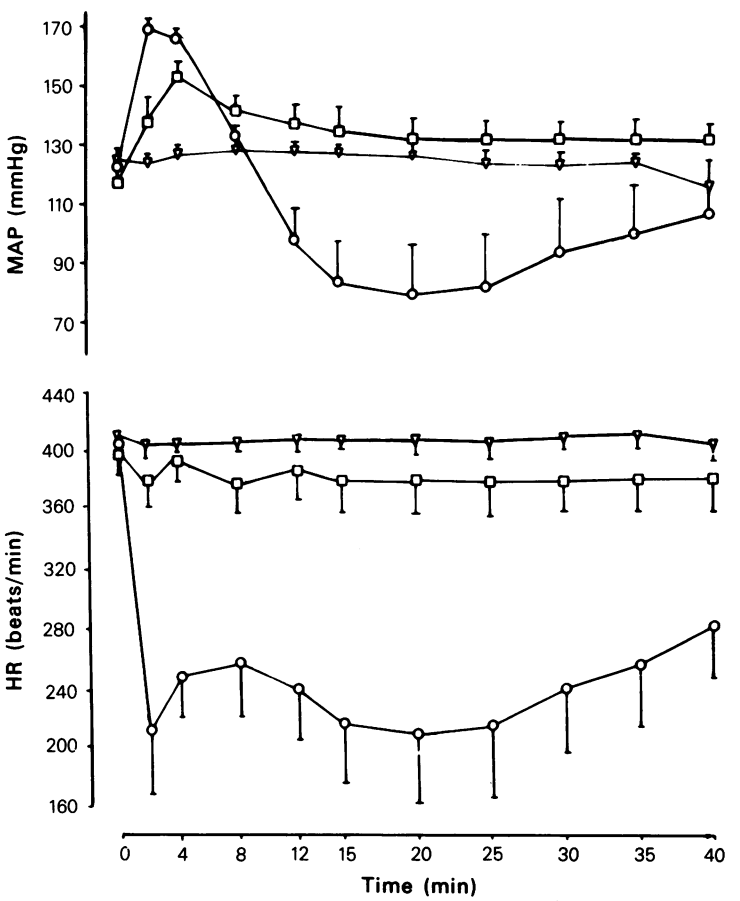

Figure 1 Effect of i.v. administered paraoxon: 83 μg/kg (▽); 275 μg/kg (□); 825 μg/kg (○); upon mean arterial pressure (MAP) and heart rate (HR) in anaesthetized rats (mean of $n = 4-6$); vertical lines show s.e.mean. Paraoxon was infused for 1 min.

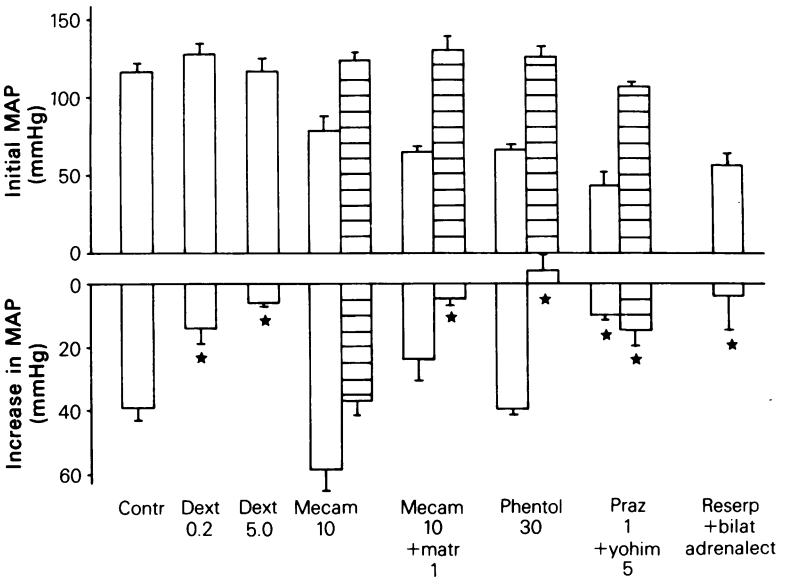

Figure 2 The effects of treatment with dexetimide (Dext, 0.2 and 5.0 mg/kg), mecamylamine (Mecam, 10 mg/kg), alone or combined with N-methylatropine(matr 1 mg/kg), phentolamine (Phentol, 30 mg/kg), prazosin (Praz, 1 mg/kg) together with yohimbine (yohim, 5 mg/kg) and sympathectomy (reserpine = reserp together with bilateral adrenalectomy) upon the initial mean arterial pressure (MAP) (upper histograms) and upon the pressor action of paraoxon (275 μg/kg = control) (lower histograms) in anaesthetized rats. Hatched columns represent the effect in the presence of an infusion of vasopressin (1.5×10^{-2} iu kg^{-1} min^{-1}). Maximal values are represented as mean of $n = 4-7$; vertical lines show s.e.mean. *Siginificantly different from control ($P < 0.05$).

ment of peripheral muscarinic receptors in the response to paraoxon.

Mecamylamine (10 mg/kg), phentolamine (30 mg/kg) and yohimbine (5 mg/kg) together with prazosin (1 mg/kg) decreased initial mean arterial pressure (MAP) significantly ($P < 0.05$, see Figure 2). Mecamylamine (10 mg/kg), atenolol (1 mg/kg), metoprolol (1 mg/kg), phentolamine (30 mg/kg) and yohimbine (5 mg/kg) together with prazosin (1 mg/kg) reduced the initial heart rate (all $P < 0.05$) to 288 ± 6, 307 ± 5, 309 ± 7, 323 ± 17, 324 ± 18 beats/min ($n = 4-6$), respectively (control: 398 ± 14 beats/min, $n = 6$).

After infusion of paraoxon, only dexetimide (0.2 and 5 mg/kg) and yohimbine (5 mg/kg) together with prazosin (1 mg/kg) diminished the pressor effect of paraoxon significantly (Figure 2). N-methylatropine (0.5–5 mg/kg), gallamine (2 mg/kg), atenolol (1 mg/kg), metoprolol (1 mg/kg), phentolamine (30 mg/kg) and mecamylamine (10 mg/kg) alone or in any combination, were ineffective. Paraoxon increased heart rate significantly ($P < 0.05$) after treatment with mecamylamine by 107 ± 15 beats/min, or after phentolamine by 85 ± 18 beats/min ($P < 0.05$); changes in heart rate were variable with yohimbine plus prazosin. Heart rate was not influenced significantly ($P > 0.05$) when paraoxon was administered after treatment with phentolamine (30 mg/kg) combined with atenolol (2 mg/kg) and N-methylatropine (0.5 mg/kg) or after treatment with prazosin (1 mg/kg) together with yohimbine (5 mg/kg), atenolol (2 mg/kg) and N-methylatropine (0.5 mg/kg), though a similar rise in blood pressure was produced by the cholinesterase inhibitor.

Chronic bilateral adrenalectomy did not influence the pressor effect of paraoxon. Additional treatment with phentolamine (30 mg/kg) though reducing initial blood pressure and heart rate to 42 ± 6 mmHg and 260 ± 27 beats/min ($n = 4$; $P < 0.05$), respectively, did not affect the pressor effect of paraoxon significantly ($P > 0.05$). In reserpinized rats, initial MAP and heart rate was 73 ± 3 mmHg and 318 ± 3 beats/min ($n = 4$), respectively. The pressor effect of paraoxon was not changed in these animals. Acute bilateral adrenalectomy in reserpinized rats induced an additional, significant ($P < 0.05$) reduction in blood pressure (Figure 2) and heart rate (238 ± 12 beats/min, $n = 5$). Treatment with reserpine combined with adrenalectomy reduced the pressor effect of paraoxon almost completely.

Since the injection of nicotine- and α-adrenoceptor blocking agents resulted in a low initial blood pressure, vasopressin was infused continuously in order to elevate arterial pressure to normal values. Vaso-

pressin did not change the effects of the antagonists upon heart rate. Although mecamylamine (10 mg/kg) did not prevent the pressor action of paraoxon after the elevation of MAP to normal values by vasopressin, additional treatment with N-methylatropine (0.5 mg/kg) prevented the pressor effect. Moreover, phentolamine (30 mg/kg) prevented the action of paraoxon in this model. Yohimbine (5 mg/kg), together with prazosin (1 mg/kg) reduced the pressor effect of 275 μg/kg paraoxon significantly ($P<0.05$), although heart rate increased by 74 ± 13 beats/min ($P<0.05$, $n=5$).

As mentioned before, treatment with N-methylatropine (0.5 mg/kg) prevented the hypotensive and bradycardic effect produced by 825 μg/kg paraoxon. The pressor effect was significantly reduced by dexetimide (0.2 mg/kg, Table 1) though even a high dose of dexetimide (5 mg/kg) failed to abolish the action of paraoxon (Table 1). I.c.v. injection of 83 μg/kg paraoxon had no effect on blood pressure.

I.c.v. injection of 275 μg/kg paraoxon produced a pressor effect (Figure 3) which reached its maximum after 6 min and amounted to 28 ± 5 mmHg ($n=6$). Heart rate was not changed. Treatment with N-methylatropine (0.5 mg/kg, i.v.) was ineffective (Figure 3).

Decerebrate rats

After midcollicular transection, mean arterial pressure fell to 87 ± 8 mmHg and heart rate to 308 ± 14 beats/min ($n=6$). Upon i.v. administration of 275 μg/kg paraoxon, MAP but not heart rate increased significantly ($P<0.05$) by 79 ± 10 mmHg ($n=6$). As in intact anaesthetized animals, blood pressure was elevated for at least 40 min.

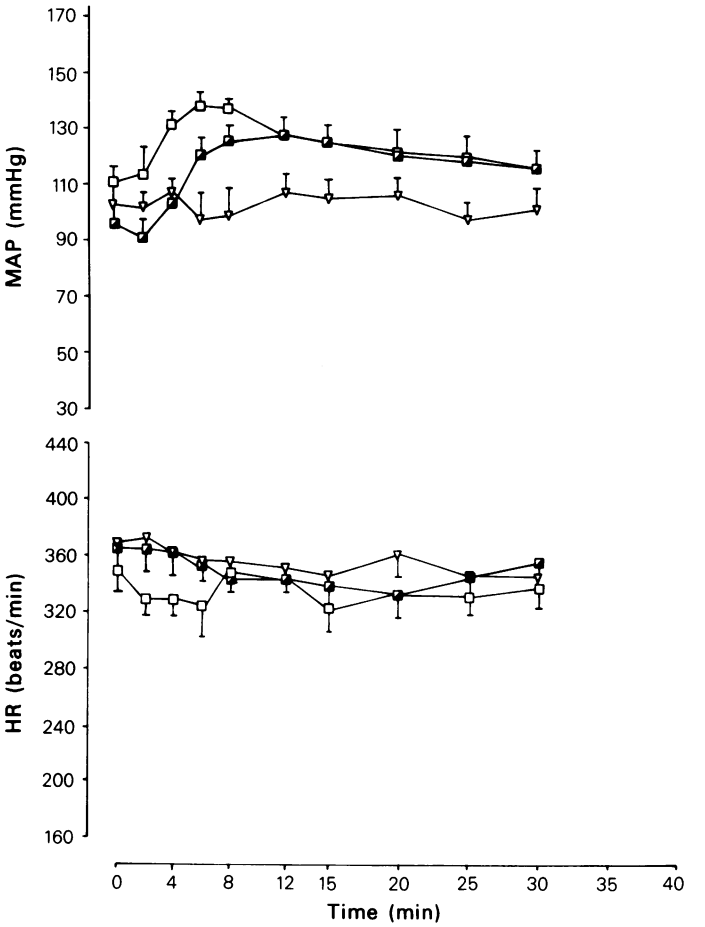

Figure 3 Effect of i.c.v. administered paraoxon: 83 μg/kg (▽); 275 μg/kg (□) upon mean arterial pressure (MAP) and heart rate (HR) in anaesthetized rats, and the effect on the latter dose of pretreatment with N-methylatropine 0.5 mg/kg, i.v. (◪). Values are mean of $n=5-6$; vertical lines show s.e.mean. Paraoxon was infused for 15 s.

Table 1 The effects of various receptor blocking agents upon mean arterial pressure (MAP) and heart rate (HR) in anaesthetized rats and their antagonistic activities upon the action of paraoxon (825 μg/kg)

Treatment	*Dose* (mg/kg)	*Initial MAP* (mmHg)	*Change in MAP*[1] (mmHg)	*Initial HR* (beats/min)	*Change in HR* (beats/min)	n
Paraoxon (Px) (825 μg/kg)		122±7	+54±7 / −45±10	407±18	−263±35	6
Px + N-methylatropine	0.5	115±5	+56±4	390±13	+ 33±19*	4
Px + dexetimide	0.2	126±5	+15±5*	394± 8	− 1±14*	6
Px + dexetimide	5	130±2	+ 8±1*	392±22	0± 0*	6

Maximal values are presented as mean ± s.e.mean and were measured within 20 min after the start of the infusion of paraoxon.
*Indicates significantly ($P < 0.05$) different from control (825 μg/kg paraoxon).
[1]Pressor and depressor effect (if present).

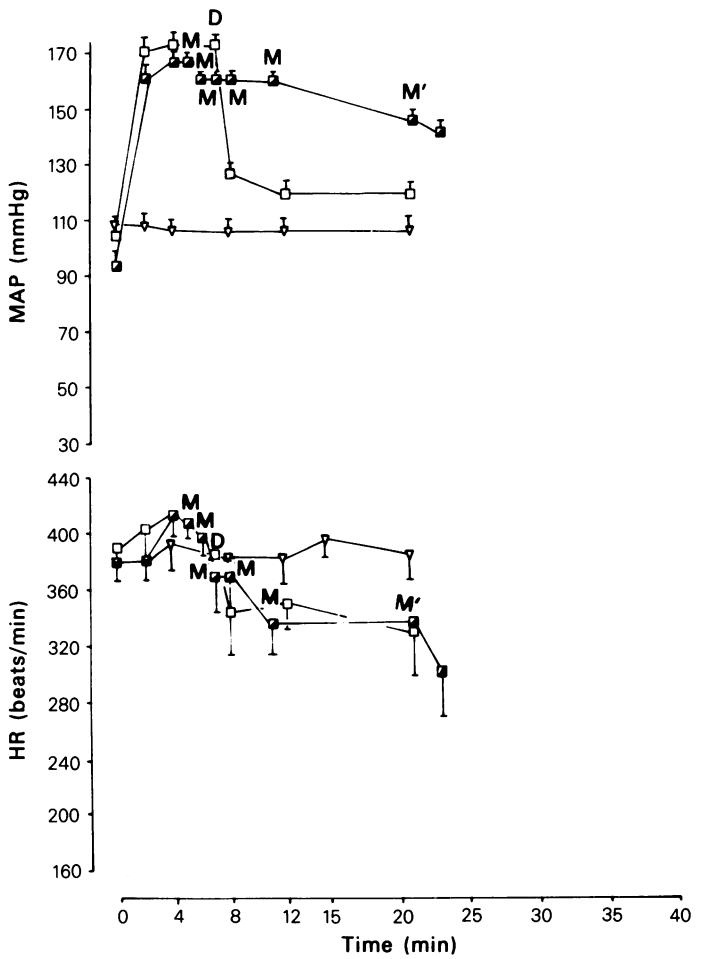

Figure 4 Effects of i.v. administered paraoxon: 83 μg/kg (▽); 275 μg/kg (□) upon mean arterial pressure (MAP) and heart rate (HR) in conscious rats. Dexetimide (0.2 mg/kg at D, □) and N-methylatropine (0.2 mg/kg at M and 0.5 mg/kg at M[1] ◪) were administered intravenously during the pressor effect. Values are mean of $n = 4-6$; vertical lines show s.e.mean. Paraoxon was infused for 1 min.

Conscious rats

In conscious animals, initial MAP and heart rate were of the same order of magnitude as those of pentobarbitone-anaesthetized animals. Paraoxon 83 μg/kg did not change MAP or heart rate significantly (Figure 4). Paraoxon 275 μg/kg induced a pressor response while heart rate was not influenced significantly. Cumulative administration of N-methylatropine (0.2 mg/kg) during the pressor response to 275 μg/kg paraoxon reduced heart rate while blood pressure was maintained at a high level. A single dose of dexetimide (0.2 mg/kg) reduced the elevated blood pressure while heart rate diminished similarly as after the administration of N-methylatropine (Figure 4).

Paraoxon concentrations and acetylcholinesterase activities in anaesthetized rats

At 2, 5, 10, 15 and 40 min after infusion of 83–825 μg/kg paraoxon, drug concentrations and AChE activities were measured in whole brain tissue. Concentrations and enzyme inhibitions were dose-dependent and maximal 2 min after dosing (Table 2). Although paraoxon was rapidly eliminated, AChE remained inhibited. Inhibition of whole brain AChE by 58 ± 10% (at 2 min) had no effect on blood pressure (83 μg/kg paraoxon), while inhibition by 83–100% (measured at 2 min) induced dose-dependent pressor effects (150–825 μg/kg paraoxon).

Enzyme activities were also measured in different brain regions and in whole blood after infusion of 28–825 μg/kg paraoxon (Table 3). The inhibitions were dose-dependent in all parts and of the same order of magnitude after infusion of a particular dose. Inhibition of AChE within brain regions by more than ±77% (150 μg/kg paraoxon) induced pressor responses. ChE activity in the blood was low but was inhibited by paraoxon dose-dependently. Blood pressure was not affected when ChE in blood was inhibited up to 60 ± 6% (83 μg/kg, Table 3).

Discussion

A number of authors have described the effect of paraoxon on the cardiovascular system (Dirnhuber & Cullumbine, 1955; Kuga & Erdmann, 1967; Vetterlein & Haase, 1979). As far as we know, no action other than the inhibitory effect on cholinesterase has been established. Our study demonstrates that the effects of paraoxon on blood pressure and on AChE activity remained throughout the period of observation while paraoxon itself was rapidly eliminated from the brain. Therefore, it seems likely that the

Table 2 Acetylcholinesterase (AChE) inhibition and drug concentrations in whole brain tissue after i.v. administration of different doses of paraoxon (Px) at various times (2, 5, 10, 15, 40 min) after dosing

	2 min			5 min			10 min			15 min			40 min		
Dose Px (μg/kg)	*Px (ng/g tissue)*	*AChE inhib (%)*	n	*Px (ng/g tissue)*	*AChE inhib (%)*	n	*Px (ng/g tissue)*	*AChE inhib (%)*	n	*Px (ng/g tissue)*	*AChE inhib (%)*	n	*AChE inhib (%)*	n
825	1061 ± 87	100 ± 0	5	820 ± 141	100 ± 0	5	450 ± 76	100 ± 0	5	182 ± 67	100 ± 0	5	99 ± 0	4
275	238 ± 40	99 ± 2	5	232 ± 57	99 ± 1	5	79 ± 11	98 ± 1	5	n.d.	n.e.		97 ± 0	4
150	76 ± 12	83 ± 9	9	72 ± 18	91 ± 4	9	n.d.	n.e.	5	n.d.	n.e.		90 ± 2	5
83	n.d.	58 ± 10	5	n.d.	44 ± 14	5	n.d.	n.e.		n.d.	n.e.		38 ± 8	5
28	n.d.	n.e.		n.d.	n.e.		n.d.	n.e.		n.d.	n.e.		17 ± 4	5

Control enzyme activity is 8.09 ± 0.30 μmol min^{-1} g^{-1} tissue ($n = 9$).
Values are presented as mean ± s.e.mean.
n.d. = not detectable (< 50 ng/g tissue)
n.e. = not established

Table 3 Acetylcholinesterase (AChE) inihibition in different brain regions and in whole blood 40 min after i.v. administration of different doses of paraoxon (Px)

	Control activity		*AChE inhibition (%)*			
	(μmol min^{-1} g^{-1} tissue)	n	Px 83 μg/kg n = 5	150 μg/kg n = 4	275 μg/kg n = 4	825 μg/kg n = 4
Pons-medulla	5.52 ± 0.46	19	21 ± 22	77 ± 5	94 ± 1	100 ± 1
Hypothalamus	5.56 ± 0.93	13	37 ± 12	77 ± 2	88 ± 5	99 ± 1
Cerebellum	2.00 ± 0.72	19	24 ± 10	77 ± 2	91 ± 1	97 ± 2
Hemisphere	7.53 ± 0.65	13	20 ± 14	80 ± 5	96 ± 1	99 ± 0
Blood	0.30 ± 0.04*	15	60 ± 6	n.e.	92 ± 3	97 ± 3

Values are presented as mean ± s.e.mean
*μmol min^{-1} ml^{-1}
n.e. = not established

paraoxon-induced cardiovascular effects are due only to a reduction of cholinesterase activity.

The pressor effect induced by the highest dose of paraoxon was followed by a N-methylatropine-sensitive depressor effect and bradycardia. However, even the higher dose of N-methylatropine did not influence the maximal pressor response to paraoxon. Paraoxon increases blood pressure by stimulation of central muscarinic receptors since dexetimide inhibited the effect in a dose-dependent manner. This lipophilic drug possesses potent and specific antimuscarinic properties (Janssen, Niemegeers, Schelkens, Demoen, Lenaerts, Van Nueten, Van Wijngaarden & Brugmans, 1971; Laduron, Verwimp & Leysen, 1979). The reduction of the pressor effect by dexetimide is analogous to the inhibitory action of atropine on the hypertensive effects by physostigmine (Varagić, 1955; Hornykiewicz & Kobinger, 1956; Medaković & Varagić, 1957; Brezenoff, 1973).

As the effects of paraoxon and of antimuscarinic drugs are comparable with those in intact anaesthetized animals, anaesthesia does not appear to influence the present results.

Midcollicular transection did not diminish the pressor effect of paraoxon. Hence the site of action is probably located within the pontomedullary region. This agrees with the conclusions of Varagić (1955), Brezenoff (1973) and Brezenoff & Rusin (1974). However, since injection of cholinomimetic agents into the posterior hypothalamic nucleus also produces pressor effects (Brezenoff, 1972), this region could also be involved.

The results indicate that the rise in blood pressure could be attributed to an increase in sympathetic outflow. Neither previous bilateral adrenalectomy for 8 days nor pretreatment with reserpine influenced the pressor effect of paraoxon. The lack of effect of adrenalectomy agrees with various other studies (Dirnhuber & Cullumbine, 1955; Hornykiewicz & Kobinger, 1956; Medaković & Varagić, 1957; Lesić & Varagić, 1961; Kaul & Grewal, 1972; Brezenoff, 1973), though other authors (Lesić & Varagić, 1961; Kuga & Erdmann, 1967; and Stamenović & Varagić, 1970) found that reserpine prevented the pressor effect of physostigmine or paraoxon. However, a combination of adrenalectomy and reserpine pretreatment blocked the pressor effect of paraoxon. Since treatment with atenolol and metoprolol had no inhibitory effect, β-receptors do not play an important role in the pressor response to paraoxon. A combination of the α_1- and α_2-adrenoceptor blocking agents yohimbine and prazosin reduced the pressor effect. While α-receptor blocking agents diminish the pressor action of other anticholinesterase agents (Dirnhuber & Cullumbine, 1955; Veragić, 1955; Medaković & Varagić, 1957; Kuga & Erdmann, 1967: Pscheidt, Votava & Himwich, 1967; Brezenoff, 1973), in the present experiments phentolamine did not influence the pressor effect of paraoxon.

Treatment with various receptor blocking agents (e.g. phentolamine or mecamylamine) reduces the initial blood pressure. Since pressor effects might be more pronounced when the initial blood pressure is low, in our experiments arterial pressure was elevated to normal values by vasopressin. In these circumstances phentolamine reduced the pressor effect by paraoxon significantly.

Mecamylamine did not affect the pressor action of paraoxon even when arterial pressure was elevated with vasopressin. N-methylatropine alone or in combination with mecamylamine failed to reduce the pressor effect of paraoxon significantly. Mecamylamine combined with N-methylatropine during vasopressin infusion prevented the action of paraoxon. Hence, the pressor effect may be mediated by ganglionic muscarinic receptors when nicotinic receptors are blocked, as suggested by Gokhale, Gulati & Joshi (1964) for physostigmine. On the other hand, when muscarinic receptors in the sympathetic ganglia are blocked the pressor effect is mediated by ganglionic nicotinic receptors. Previous-

ly Fleisch, Flacke & Gillis (1969) reported that the ACh-induced tachycardia in dogs could be prevented only by the simultaneous administration of a nicotinic and a muscarinic antagonist.

High doses of dexetimide did not abolish the pressor effect of paraoxon, infusion of paraoxon into pithed rats in which the brain was destroyed also induced pressor effects (De Neef & Porsius, 1981a). Indeed, intravenous and i.c.v. application were equieffective. These findings suggest that a peripheral mechanism facilitates the centrally evoked effect. The peripheral effect of paraoxon in normotensive rats was investigated in a separate study (De Neef & Porsius, 1982).

Brain AChE inhibition by paraoxon and paraoxon pressor effects were both dose-dependent. Enzyme inhibition was maximal after 2 min. Changes in blood pressure occurred only when enzyme activity was reduced by more than ±77%. A relationship between AChE inhibition within the CNS and the effect on blood pressure in the cat also exists (De Neef & Porsius, 1980). In the rat changes in blood pressure only occurred when 76 ng paraoxon per g brain tissue was present. Higher concentrations of paraoxon had stronger effects on enzyme activity and induced stronger pressor effects. Thus, inhibition of AChE in the CNS points towards a central role of acetylcholine in the action of paraoxon.

The results of this study indicate that besides a centrally mediated pressor effect a peripheral contribution to the action of paraoxon is likely. Stimulation of central muscarinic receptors, probably due to accumulated acetylcholine, increases preganglionic activity. The central site of action is located within the pontomedullary region. Inhibition of peripheral AChE activity will facilitate ganglionic transmission. Thus, postganglionic sympathetic activity will be enhanced as a result of the increased preganglionic activity and the facilitated ganglionic transmission the latter mediated both via the muscarinic and nicotinic ganglionic receptors. The pressor response to paraoxon is only prevented when both types of receptors are blocked.

Reprint requests to A.J.P. please.

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