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TOLMESOXIDE, A DRUG THAT LOWERS BLOOD PRESSURE BY A DIRECT RELAXANT EFFECT ON VASCULAR SMOOTH MUSCLE

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1 The blood pressure of conscious normotensive, deoxycorticosterone acetate (DOCA) hypertensive and 1 kidney Goldblatt hypertensive rats and renal hypertensive and normotensive cats was reduced by tolmesoxide (4,5-dimethoxy-*o*-tolyl methyl sulphoxide).

2 Tachycardia accompanied the hypotension. In rats the increase in heart rate was abolished by concurrent administration of propranolol. Tachycardia did not occur in pithed rats.

3 Vasoconstriction induced by sympathetic stimulation, noradrenaline, tyramine, angiotensin or vasopressin was antagonized by tolmesoxide.

4 The antagonism of vasoconstrictor responses produced by tolmesoxide was unaffected by β-adrenoceptor, muscarinic or histamine antagonists.

5 It is concluded that the lowering of pressure produced by tolmesoxide results from a direct relaxant effect on vascular smooth muscle.

Introduction

Tolmesoxide (4,5-dimethoxy-*o*-tolyl methyl sulphoxide) is a member of a series of aryl sulphoxides which were synthesized as potential anti-hypertensive agents.

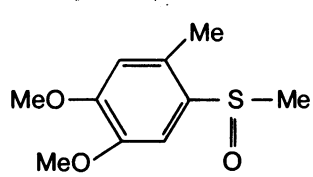

Figure 1 Chemical structure of tolmesoxide.

The studies described here demonstrate that, in animals, tolmesoxide has a blood pressure lowering effect which appears to result from a direct relaxant effect on vascular smooth muscle.

Methods

Conscious rats

Experiments were carried out on male normotensive, one kidney Goldblatt-hypertensive and metacorticoid hypertensive rats (Sprague Dawley C.F.Y.). Blood pressure was recorded from aortic catheters implanted under halothane anaesthesia by the technique of Weeks & Jones (1960). Experiments were performed not less than 48 h after surgery. In experiments where animals were dosed orally, food was removed 18 h before dosing. Blood pressure was measured under conditions of minimal restraint with a Bell and Howell pressure transducer (type 327-1-221) linked via a carrier preamplifier to an E & M Physiograph.

One kidney Goldblatt hypertension was induced in rats (160–180 g) by means of silver clips (0.25 mm aperture) placed on the right renal artery. The left kidney was removed during the same operation. The rats were used during the fifth week after nephrectomy.

Metacorticoid hypertension was induced in rats (80–110 g) by a modification of the method of Stanton & White (1965).

Conscious cats

Aortic blood pressure was recorded from unrestrained normotensive and renal hypertensive cats as described by Finch (1974). The animals were fasted overnight before dosing orally with tolmesoxide in gelatin capsules.

Pithed rats

Pithed rat preparations were used to study the effects of tolmesoxide on pressor responses evoked either by stimulation of the entire sympathetic outflow (Gilles-

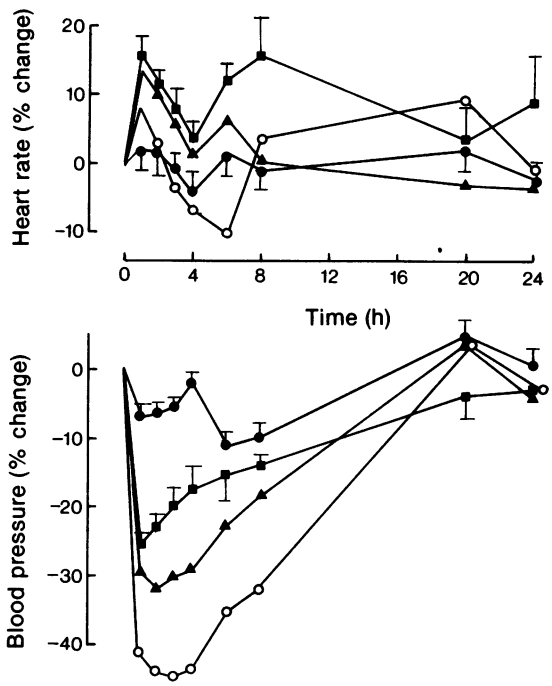

Figure 2 Dose-response effect of tolmesoxide on the mean blood pressure and heart rate of conscious DOCA hypertensive rats. Controls (●), tolmesoxide 20 mg/kg given orally (■), 50 mg/kg (▲) and 100 mg/kg (○). Mean results from a minimum of 10 rats are shown; vertical lines show s.e. means.

pie & Muir, 1967) or intravenous injections of noradrenaline or angiotensin. Stimulation at frequencies of 1–6 Hz, 0.5 ms duration and 20 V was applied for periods of 15 seconds. Tubocurarine (1 mg/kg) was given intravenously before stimulation was started.

Anaesthetized cats

Cats (2–3 kg) were anaesthetized initially with halothane and anaesthesia maintained with chloralose (75 mg/kg, i.v.). The femoral artery and vein were cannulated and a pressure transducer was connected to the arterial cannula. The effects of tolmesoxide on blood pressure, heart rate and pressor responses to noradrenaline, tyramine and angiotensin were studied.

Diuretic studies

Male rats (150–250) which had been fasted overnight were used in this study. Control rats were hydrated orally with distilled water (25 ml/kg body weight). Two animals were housed in each cage with 6 pairs of rats per group, except in the control group which consisted of 12 pairs of rats. Urine was collected over a period of 5 h and total sodium and potassium excretion was determined with an EEL 450 flame photometer.

Rat isolated mesenteric vessels

Rat isolated perfused mesenteric arteries were set up as described by McGregor (1965). Constrictor responses to either noradrenaline (3–10 μg) or vasopressin (0.3–0.6 u) were elicited; time cycles of 5 and 10 min respectively were used. Drugs were added to the perfusion fluid in the required concentration and a contact time of 20 min allowed for the maximum effect to become apparent.

Tolmesoxide is a neutral compound which exists as two optical isomers. The racemic mixture was used in the present work. Other drugs used were: angiotensin (Hypertensin, CIBA); atropine sulphate (Burroughs Wellcome); diazoxide (Allen & Hanburys); isoprenaline sulphate (Burroughs Wellcome); mepyramine maleate (May & Baker); noradrenaline bitartrate (BDH); prazosin hydrochloride (Pfizer); propranolol hydrochloride (ICI); salbutamol sulphate (Allen & Hanburys); tubocurarine chloride (Burroughs Wellcome); tyramine hydrochloride (Koch Light Labs) and vasopressin (Parke Davis). With the exception of diazoxide all drugs were dissolved in 0.9% w/v NaCl solution (saline) before use. Diazoxide was dissolved in dilute alkali at pH 11. Unless otherwise stated all doses in the text refer to the respective salt.

Results

Effects of tolmesoxide on conscious animals

Rats. Tolmesoxide administered orally lowered the mean arterial blood pressure of DOCA hypertensive rats in a dose-related manner (Figure 2). The fall in blood pressure was rapid and was well maintained for 1–8 hours. The hypotensive effect was accompanied by a tachycardia at all doses, although at 100 mg/kg orally the initial tachycardia preceded a secondary fall in heart rate (Figure 2).

The magnitude of the hypotensive effect produced by tolmesoxide (50 mg/kg orally) was greater in deoxycorticosterone acetate (DOCA) hypertensive rats than in either one kidney Goldblatt hypertensive rats or normotensive rats (Table 1).

Cats. Tolmesoxide (50 mg/kg, orally) reduced the blood pressure of both normotensive and renal hypertensive cats (Table 1). The reduction in blood pressure was accompanied by an increase in heart rate. The magnitude of the hypotensive response in renal hyper-

tensive cats was very similar to that seen in renal hypertensive rats (Table 1).

Repeated administration to DOCA hypertensive rats. The effects of daily intraperitoneal administration of tolmesoxide on the blood pressure and heart rate of DOCA hypertensive rats were studied over a period of 5 days. On the first day the time course of a single intraperitoneal injection of tolmesoxide (50 mg/kg) was studied. The fall in blood pressure produced by tolmesoxide was rapid in onset; the peak response occurring 30–60 min after the injection of the compound. At the peak hypotensive response the blood pressure of the treated rats was significantly lower ($P < 0.01$) than that of the control group of animals. Blood pressure had returned to control values 5 h after the injection of tolmesoxide. The reduction in blood pressure was associated with a significant ($P < 0.05$) increase in heart rate. On the next 4 days the magnitude of the hypotensive effect of tolmesoxide was not significantly different ($P > 0.05$) from that seen on the first day although the duration of effect was reduced. Heart rate, although elevated on days 2–5, was not significantly different ($P > 0.05$) from that of the control group of rats. On withdrawal of tolmesoxide, blood pressure increased and heart rate fell (Figure 3).

Effect of propranolol on the tachycardia produced by tolmesoxide. In conscious normotensive rats the hypotensive effect of tolmesoxide (100 mg/kg, orally) was accompanied by a significant ($P < 0.001$) elevation of heart rate (Table 2). Propranolol (2 mg/kg, s.c.) did not significantly ($P > 0.05$) affect the resting

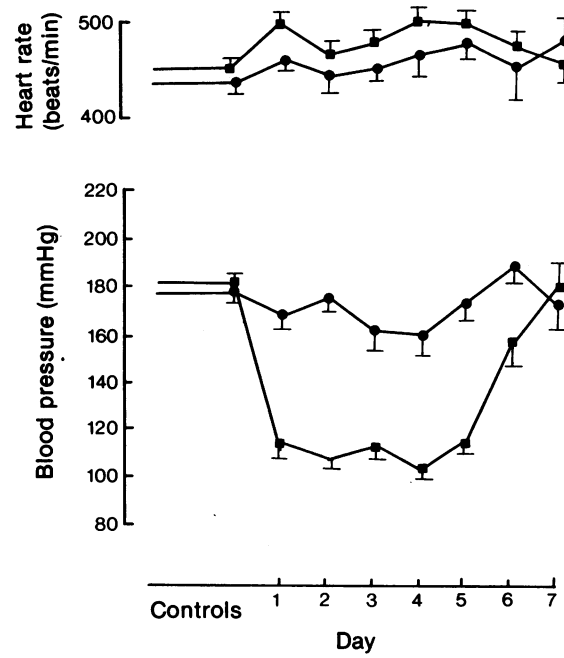

Figure 3 The effect of daily administration of tolmesoxide on mean arterial blood pressure and heart rate of conscious DOCA hypertensive rats. Tolmesoxide (50 mg/kg, i.p., ■) was injected on days 1–5 and blood pressure measured 30 min after injection. No drug was injected on days 6 and 7. A control group of rats (●) was included in the study. Mean results from a minimum of 7 rats are shown; vertical lines show s.e. means.

Table 1 Effect of tolmesoxide (50 mg/kg, orally) on the mean blood pressure and heart rate of conscious normotensive and hypertensive rats and cats

Model	*Pre-drug*		*Post-drug*	
	Mean blood pressure (mmHg)	*Heat rate (beats/min)*	*Mean blood pressure (mmHg)*	*Heart rate (beats/min)*
Normotensive rat ($n = 9$)	121 ± 2	384 ± 10	96 ± 2 (2) $P < 0.001$	439 ± 13 $P < 0.01$
Goldblatt rat ($n = 11$)	189 ± 4	391 ± 13	156 ± 4 (2) $P < 0.001$	429 ± 18 NS
DOCA rat ($n = 11$)	173 ± 4	422 ± 11	117 ± 3 (2) $P < 0.001$	463 ± 14 $P < 0.01$
Normotensive cat ($n = 5$)	91 ± 5	157 ± 14	77 ± 5 (4) $P < 0.01$	202 ± 26 $P < 0.05$
Renal cat ($n = 4$)	140 ± 13	189 ± 23	101 ± 6 (1) $P < 0.05$	238 ± 36 $P < 0.05$

Values shown are means ± s.e. mean of mean blood pressure and heart rate before drug and at the maximum hypotensive response; time in hours shown in parentheses. NS—values not significantly different from pre-drug values ($P > 0.05$) using Student's t test for paired data.

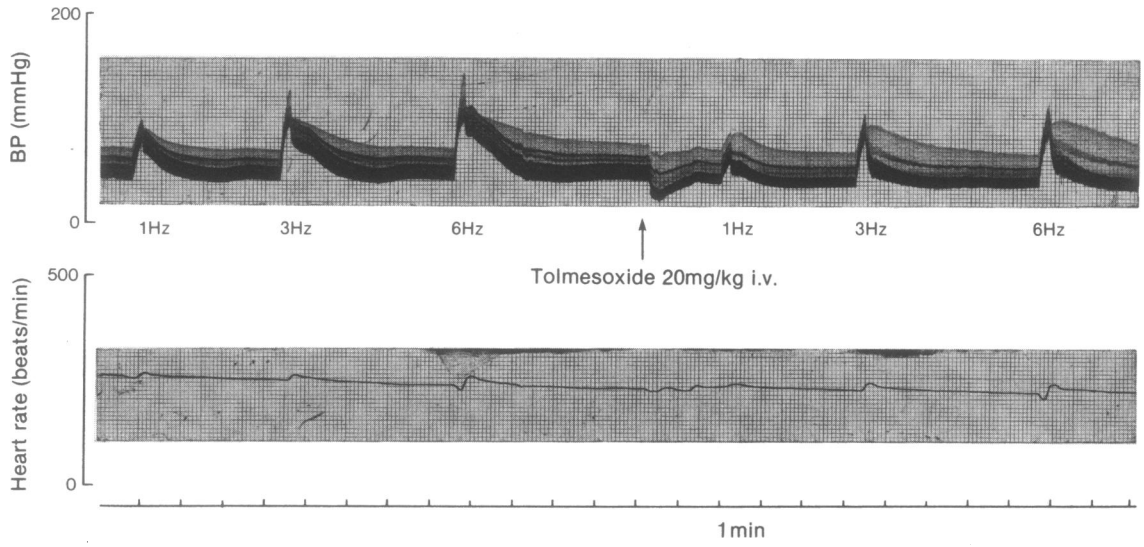

Figure 4 Effects of tolmesoxide on blood pressure (BP), heart rate and pressor responses in the pithed rat. Pressor responses were induced by stimulation of the entire sympathetic outflow (20 V, 0.5 ms for 15 s at frequencies of 1, 3 and 6 hertz).

blood pressure of normotensive rats but heart rate was significantly ($P < 0.05$) reduced. When tolmesoxide and propranolol were administered together to a group of normotensive rats, propranolol prevented the tachycardia but had no effect on the reduction in blood pressure produced by tolmesoxide (Table 2). Thus, this combination of drugs resulted in significant reductions in both resting blood pressure ($P < 0.001$) and heart rate ($P < 0.05$).

The effect of tolmesoxide on vasoconstrictor responses

Pithed rats. Tolmesoxide (20 mg/kg, i.v.) reduced the blood pressure of pithed rats without affecting heart rate. The pressor responses elicited by stimulation of the entire sympathetic outflow were reduced by this dose of tolmesoxide but cardiac acceleration induced by sympathetic stimulation was unaffected (Figure 4).

The pressor responses evoked by either sympathetic stimulation of intravenous injections of noradrenaline and angiotensin were significantly less ($P < 0.01$) in rats which had been pretreated for 1 h with tolmesoxide (50 mg/kg, i.p.) than in control animals (Figure 5).

In a separate experiment, pretreatment of rats with either propranolol (2 mg/kg, s.c.), mepyramine (3

Table 2 The effect of tolmesoxide on the mean blood pressure and heart rate of conscious normotensive rats and the effect of concurrent administration of propranolol

Treatment	*Pre-drug*		*1 hour post-drug*	
	Mean blood pressure (mmHg)	*Heart rate (beats/min)*	*Mean blood pressure (mmHg)*	*Heart rate (beats/min)*
Tolmesoxide 100 mg/kg, orally	133 ± 4	395 ± 8	88 ± 2 $P < 0.001$	446 ± 15 $P < 0.001$
Propranolol 2 mg/kg, s.c.	132 ± 2	391 ± 15	139 ± 5 NS	318 ± 13 $P < 0.05$
Tolmesoxide 100 mg/kg, orally + propranolol 2 mg/kg, s.c.	129 ± 4	391 ± 19	96 ± 4 $P < 0.001$	333 ± 16 $P < 0.05$

Mean results from a minimum of 6 rats ± s.e. mean are shown. NS—values not significantly different ($P > 0.05$) from pre-drug values using Student's *t* test for paired data.

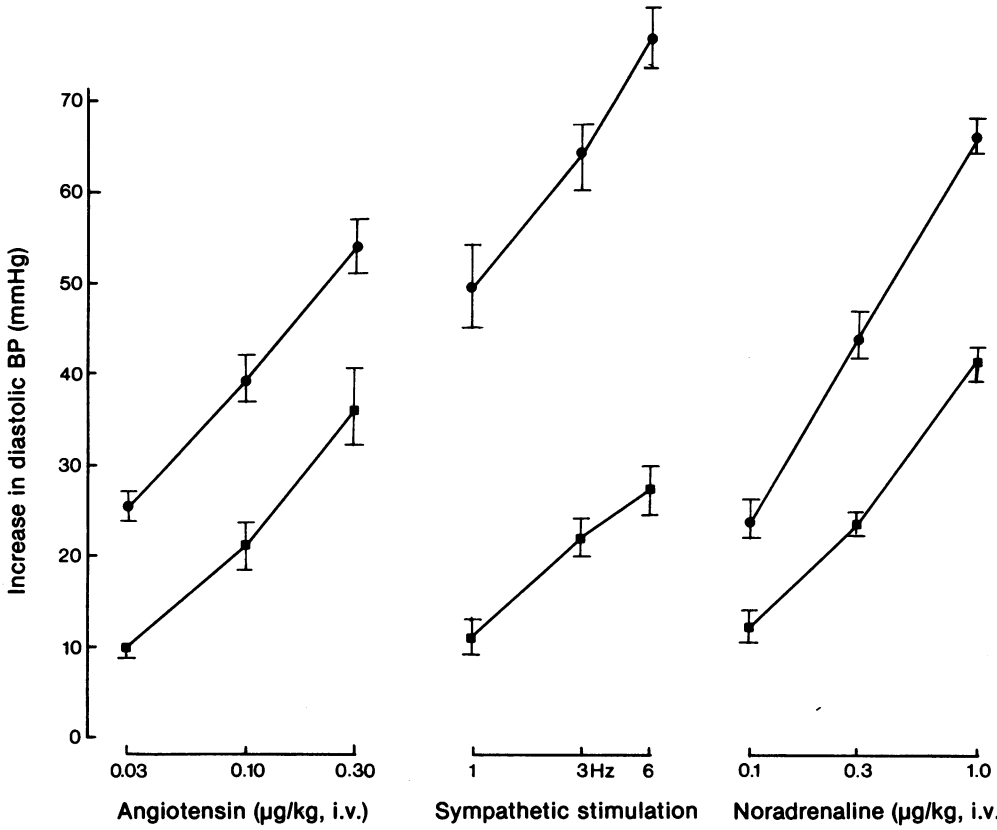

Figure 5 The effect of tomesoxide on pressor responses induced by sympathetic stimulation, noradrenaline and angiotensin in the pithed rat. Tolmesoxide (50 mg/kg, i.p.) 60 min pretreatment (■), controls (●). The results are the mean of 5 experiments; vertical lines show s.e. means.

Table 3 Effect of tolmesoxide and diazoxide on urine and ion excretion in normotensive water-loaded rats

Drug	*Oral dose (mg/kg)*	*Total 5 h excretion*			*Mean blood pressure (mmHg)*
		Na^+ (μEq/100 g)	*K^+ (μEq/100 g)*	*Urine (ml/100 g)*	
Control ($n = 12$)	—	53 ± 8	48 ± 6	3.1 ± 0.1	121 ± 4
Tolmesoxide ($n = 6$)	50	66 ± 12 NS	103 ± 14 $P < 0.01$	3.4 ± 0.3 NS	96 ± 2 $P < 0.001$
Tolmesoxide ($n = 6$)	100	65 ± 11 NS	84 ± 11 $P < 0.005$	1.6 ± 0.2 $P < 0.001$	90 ± 6 $P < 0.001$
Diazoxide ($n = 6$)	100	7 ± 3 $P < 0.001$	16 ± 2 $P < 0.001$	0.3 ± 0.1 $P < 0.001$	94 ± 5 $P < 0.001$

In the diuretic studies the mean results of n pairs of rats ± s.e. mean are shown. All rats water loaded 25 ml/kg, orally.
The mean blood pressure in the table was that recorded at the maximum hypotensive response in normotensive rats. The results are the mean from a minimum of 8 rats ± s.e. mean.
NS—values not significantly different ($P > 0.05$) from those in the control group using Student's t test for unpaired data.

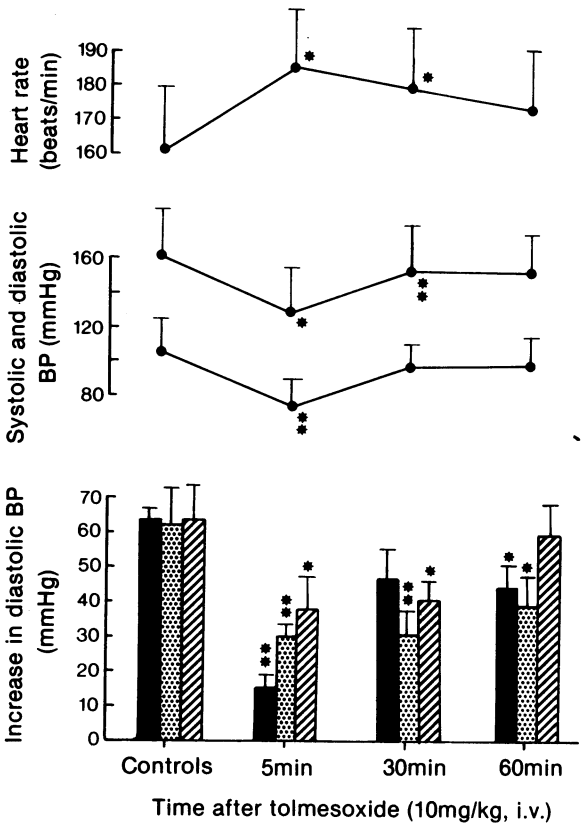

Figure 6 Effects of tolmesoxide (10 mg/kg, i.v.) on arterial blood pressure (BP), heart rate and pressor responses in the cat anaesthetized with chloralose. Pressor responses were induced by intravenous injections of noradrenaline (1 μg/kg) solid columns, tyramine (50 μg/kg) stippled columns and angiotensin (0.1 μg/kg) hatched columns. $^{*}P < 0.05$, $^{**}P < 0.01$ using Student's t test for paired data.

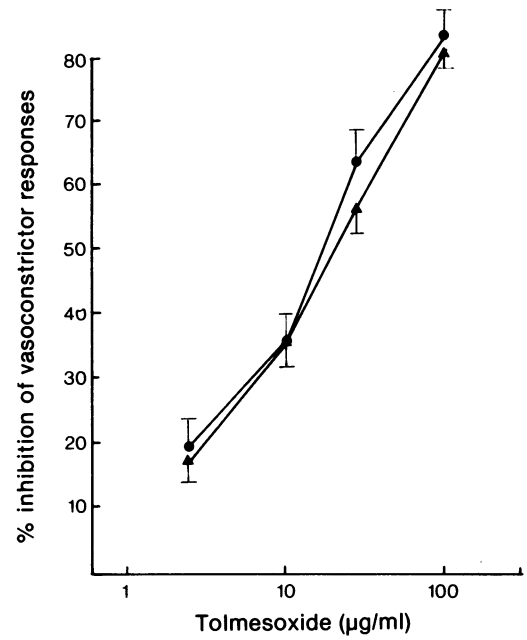

Figure 7 The effect of tolmesoxide on submaximal vasoconstrictor responses induced by noradrenaline (▲) and vasopressin (●) in the rat perfused mesentery preparation. Results are the mean of 5 experiments; vertical lines show s.e. means.

mg/kg, i.p.) or atropine (0.5 mg/kg, i.p.) had no effect on the extent of the antagonism of noradrenaline produced by tolmesoxide in pithed rats. At these doses propranolol, mepyramine and atropine did not themselves alter the noradrenaline response.

Anaesthetized cats. The blood pressure of cats anaesthetized with chloralose was reduced by tolmesoxide (10 mg/kg, i.v.). The hypotensive response was accompanied by an increase in heart rate (Figure 6). Pressor responses evoked by intravenous injections of noradrenaline, tyramine and angiotensin were reversibly antagonized by tolmesoxide (Figure 6).

Rat mesenteric vasculature. The vasoconstrictor effects produced by submaximal concentrations of noradrenaline and vasopressin in the rat isolated perfused mesenteric vasculature were antagonized by tolmesoxide (Figure 7). The antagonism of the noradrenaline vasoconstrictor responses produced by tolmesoxide was unaffected by propranolol (0.1 μg/ml). Noradrenaline vasoconstrictor responses were unaffected by isoprenaline and salbutamol in concentrations of up to 1 and 10 μg/ml respectively. Noradrenaline vasoconstrictor responses were selectively antagonized by prazosin (1–10 ng/ml); vasopressin responses were unaffected.

The effect of tolmesoxide on diuresis in the rat

The effects of tolmesoxide on urine and electrolyte excretion were studied in rats and the effects seen were compared with those produced by diazoxide, a vasodilator which is known to produce marked antidiuretic effects in rats (Taylor & Rubin, 1964). Tolmesoxide and diazoxide both reduced urine excretion in conscious rats (Table 3). The effects of these compounds on ion excretion were very dissimilar. Tolmesoxide given orally at 50 or 100 mg/kg had no significant effect on sodium excretion whereas potassium excretion was significantly elevated. Dia-

zoxide (100 mg/kg, orally) caused marked retention of both sodium and potassium. In a separate experiment the hypotensive effects of diazoxide and tolmesoxide were compared in conscious normotensive rats. It can be seen in Table 3 that the falls in blood pressure seen with diazoxide and tolmesoxide were of the same order of magnitude.

Discussion

The experiments described here demonstrate that tolmesoxide reduces arterial blood pressure in conscious cats and rats as a result of a direct relaxant effect on vascular smooth muscle. The reductions in blood pressure produced by tolmesoxide were more marked in hypertensive than normotensive animals. In rats the reductions in blood pressure produced by tolmesoxide were greater in DOCA hypertension than renal hypertension (1 Kidney Goldblatt). In terms of hypotensive effect, tolmesoxide was equipotent with diazoxide, but was ten times less potent than hydralazine (Doxey & Leach, unpublished observations). On repeated administration to DOCA hypertensive rats there was no tolerance to the peak hypotensive response to tolmesoxide although the duration of this response was reduced.

In conscious cats and rats the hypotension was accompanied by an increase in heart rate. It has been shown previously (Fielden, Owen & Taylor, 1976) that, in rats, propranolol prevented the tachycardia produced by vasodilators. It would appear therefore that the increase in heart rate seen with this type of molecule is due entirely to a reflex increase in sympathetic nervous activity. In normotensive rats the hypotensive effects of tolmesoxide were unaffected by co-administration of propranolol, the tachycardia however was abolished. Thus the hypotensive effect of tolmesoxide was not mediated via β-adrenoceptors and the tachycardia was a reflex response to the direct vasorelaxant action of the compound. This contention was supported by the finding that tolmesoxide did not increase the heart rate of pithed rats.

The actions of tolmesoxide in the central nervous system appear to be of little importance in the hypotensive action of the compound. The reduction in blood pressure and antagonism of vasoconstriction induced by stimulation of the entire sympathetic outflow (Gillespie & Muir, 1967) produced by tolmesoxide in pithed rats correlated well with its hypotensive effect in conscious rats. Cardiac acceleration induced by sympathetic stimulation was unaffected by tolmesoxide indicating its lack of effect on sympathetic ganglia and adrenergic neurones. It is concluded from these studies that tolmesoxide is acting on vascular smooth muscle.

In pithed rats tolmesoxide was equally effective in inhibiting vasoconstriction produced by sympathetic stimulation, noradrenaline and angiotensin. α-Adrenoceptor antagonists selectively antagonize sympathetic stimulation and noradrenaline responses whereas angiotensin responses are unaffected (Doxey & Leach, unpublished observations). The non-specific suppression of pressor responses seen with tolmesoxide is characteristic of directly acting vasodilators (Rubin, Roth, Taylor & Rosenkilde, 1972). The inhibition of noradrenaline-induced vasoconstriction produced by tolmesoxide in pithed rats was unaffected by pretreatment with propranolol, atropine or mepyramine demonstrating its lack of effect on β-adrenoceptor, muscarinic or histamine receptors. The fact that tolmesoxide lowered the blood pressure of normotensive, one kidney Goldblatt and DOCA hypertensive rats is additional evidence for a vasodilator mechanism of action (Sturtevant, 1958; Stanton & Cooper, 1966; Ebihara & Martz, 1970).

Confirmation of conclusions drawn from the pithed rat studies was seen in anaesthetized cats and in isolated mesenteric vessels. In cats the reduction in arterial blood pressure produced by tolmesoxide was associated with an increase in heart rate and a non-specific antagonism of pressor responses induced by intravenous injections of noradrenaline, tyramine and angiotensin. In isolated vascular preparations tolmesoxide non-selectively antagonized both noradrenaline and vasopressin-induced constrictor responses. In contrast, prazosin, a competitive postsynaptic α-adrenoceptor antagonist (Cambridge, Davey & Massingham, 1977; Doxey, Smith & Walker, 1977) at concentrations that abolished noradrenaline vasoconstrictor responses had no effect on the vasoconstriction produced by vasopressin. The inhibition of noradrenaline vasoconstriction produced by tolmesoxide was unaffected by propranolol, and β-adrenoceptor agonists themselves did not inhibit noradrenaline constrictor responses. Thus *in vitro* experiments confirmed that tolmesoxide was neither a β-adrenoceptor agonist nor an α-adrenoceptor antagonist.

The effects of tolmesoxide on urine and electrolyte excretion were studied in conscious rats and the results compared with those produced by diazoxide. Diazoxide is a directly acting vasodilator that causes severe retention of urine and electrolytes in rats (Taylor & Rubin, 1964). At equivalent hypotensive doses the reduction in urine excretion produced by tolmesoxide was less than that produced by diazoxide and in marked contrast to diazoxide, the excretion of sodium and potassium was not reduced. A satisfactory explanation of why tolmesoxide reduces urine excretion in rats without a concomitant reduction in either sodium or potassium excretion has not been obtained.

In conclusion the studies described here indicate that tolmesoxide reduces the blood pressure of nor-

motensive and hypertensive animals by a direct relaxant effect on vascular smooth muscle. This contention is supported by studies in normotensive volunteers where tolmesoxide was shown to dilate arteries of the forearm by a direct action (Collier, Lorge & Robinson, 1977).

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