Br. J. Pharmac. (1981), **73**, 799–805

COMPARATIVE STUDIES ON THE EFFECTS OF TOLMESOXIDE (Rx71107), A TOLMESOXIDE METABOLITE (Rx71112) AND NIFEDIPINE IN ISOLATED BLOOD VESSELS

ERICH MIKKELSEN & OLE LEDERBALLE PEDERSEN*

Department of Clinical Pharmacology, University of Aarhus, DK-8000 Aarhus C, and
*Department of Internal Medicine I, Aarhus Amtssygehus, DK-8000 Aarhus C, Denmark

1 The effect was studied of tolmesoxide (Rx71107), a tolmesoxide metabolite (Rx71112) and nifedipine on active tension in human isolated crural veins and in rat thoracic aorta. The effects of tolmesoxide and nifedipine on ^{22}Na and ^{45}Ca net influx in noradrenaline-induced contractions were investigated in rat thoracic aorta.

2 Tolmesoxide, Rx71112 and nifedipine caused a concentration-related inhibition of noradrenaline (NA)- and potassium (K^+)-induced contractions in human veins. Nifedipine was by far the most potent drug in these respects. Tolmesoxide (4.7 μM–4700 μM) and Rx71112 (22 μM–220 μM) inhibited the NA-induced contraction more effectively than the K^+-induced concentration. The reverse was true for nifedipine (0.0023–3 μM).

3 Nifedipine had a similar potency in inhibiting the NA-induced contraction in rat aorta and human veins, whereas the inhibitory effect of tolmesoxide was more pronounced in rat aorta than in human veins.

4 Tolmesoxide was a weak antagonist of the contractile effects of the cumulative addition of calcium on human veins. In concentrations up to 470 μM, tolmesoxide was completely devoid of inhibitory effects on ^{22}Na and ^{45}Ca net influx in rat aorta. Rx71112 (220 μM) had an inhibitory effect on ^{22}Na net influx but no significant effect on ^{45}Ca net influx.

5 Nifedipine was an effective antagonist of the contractile effects of cumulative addition of calcium in human veins and had a concentration-related inhibitory effect on both ^{22}Na and ^{45}Ca uptake in rat aorta.

6 The results indicate that tolmesoxide and Rx71112 cause dilatation of human crural veins *in vitro* at concentrations that do not interfere with the net transmembranal movements of Ca and are thus clearly different from the effects of nifedipine.

Introduction

Tolmesoxide (4.5-dimethoxy-2-methylphenyl methyl sulphoxide) has been shown to lower arterial pressure in several animal species (Doxey, 1978) presumably by its direct relaxant effect on vascular smooth muscle. *In vivo* studies in man demonstrated that tolmesoxide dilates forearm arteries or veins equally well (Collier, Lorge & Robinson, 1978). Preliminary investigations on isolated non-human vessels indicate that the main metabolite of tolmesoxide (Rx71112, 4.5-dimethoxy-2-methyl phenyl methyl sulphone) isolated from human plasma also has a direct vasodilatory effect (unpublished observations). The precise mechanism of vasodilatation by these drugs has not yet been clarified and the *in vitro* effects of tolmesoxide and its metabolite have not previously been studied in human isolated vessels.

The aim of the present study was to investigate the effect of tolmesoxide and Rx71112 on human isolated veins and to compare it with that of the calcium antagonist, nifedipine. We also investigated the interaction between these drugs and extracellular calcium and the influence of tolmesoxide and nifedipine on ^{22}Na and ^{45}Ca net influx and active tension in the isolated aorta of the rat.

Methods

Human crural veins were obtained from 14 patients (5 men and 9 women) aged 27–70 years who were undergoing surgery for varicose veins. The patients were anaesthetized with thiopentone, nitrous oxide and halothane, and received pethidine and atropine for premedication. Immediately after the operation, specimens of macroscopically normal veins were isolated and cut into rings (outer diameter 1–2 mm,

0007-1188/81/080799-07 $01.00

length about 4 mm) which were mounted in a 30 ml organ bath containing aerated Krebs solution maintained at 37°C (Mikkelsen, Andersson & Bengtsson, 1978). During a stabilizing period of 1–2 h, the resting tension was repeatedly adjusted to about 10 mN. Maximum contractions were induced either by noradrenaline (NA) 18 μM or by potassium (K^+) 127 mM until the responses were reproducible and then the vasodilator drugs were tested. Isometric tension was recorded by means of a Grass Ft 03 transducer connected to a Beckmann R 611 polygraph.

In some experiments, preparations of thoracic aorta isolated from normotensive Wistar rats were used. The animals were killed by a blow on the neck, the thoracic aorta was removed and cut into rings (length about 4 mm) and mounted in organ baths in the same way as the human preparations (as described above). The resting tension was adjusted to an optimal value of approx. 20 mN.

Assessment of drug effects in the isometric tension studies

The inhibitory effect of tolmesoxide, Rx71112 and nifedipine on human vein preparations was tested by adding the drugs 15 min before giving a maximally effective dose of NA or K^+. In some experiments, the relaxant effect was studied by adding the drugs to the organ bath whilst the tissue was contracted by either NA or K^+.

The role of extracellular calcium on the action of tolmesoxide and nifedipine on K^+- or NA-evoked contractions in human veins was studied after 30 min preincubation in 'Ca^{2+}-free medium' (see below). Then calcium was added cumulatively either in the absence or in the presence of the vasodilating drugs. Equipotent concentrations of tolmesoxide and nifedipine, calculated from concentration-response curves in human veins, were chosen for this investigation.

In rat aorta, cumulative noradrenaline concentration-response curves were first established, the concentration being increased when a steady response was obtained on the preceding dose. The tissue was then repeatedly washed with normal Krebs solution, until the initial tension was resumed. Successive cumulative concentration-response curves were established with noradrenaline until they were reproducible, after which the procedure was repeated in the presence of the vasodilator drugs. The median effective concentration (EC_{50}) was calculated from each individual experiment. According to Fleming, Westfall, de la Lande & Jellett (1972), distribution of equi-effective doses of agonists is generally normal on a log scale and the statistics were therefore computed on the logarithms of EC_{50} values.

In order to prevent reuptake of noradrenaline and block β-receptors, cocaine 5 μM and propranolol 3.4 μM were always present in the organ bath during these determinations.

Assessment of drug effects on ^{45}Ca and ^{22}Na net influx in rat aorta

The effect of tolmesoxide and nifedipine on ^{22}Na and ^{45}Ca uptake in rat aorta was investigated by a modification of the isotope technique previously described by Dahl-Hansen & Clausen (1973) and Case & Clausen (1973).

Groups of ring preparations from aortae were equilibrated in aerated Krebs solution (37°C) for 60 min followed by a period of 15 min in normal Krebs solution (37°C) with or without tolmesoxide or nifedipine. The concentrations of tolmesoxide and nifedipine used were equipotent in inhibiting the NA-induced contraction in the rat aorta. The tissues were then transferred to a solution containing ^{45}Ca and ^{22}Na (1 μCi/ml) and 18 μM NA but otherwise having the same composition as the preincubation medium. The time of incubation in this medium was 15 min. Finally, the preparations were washed in ice cold Krebs solution (3 × 20 min). To block the active Na^+/K^+ transport at this stage, ouabain 1 mM was added to the wash-out solution. The tissues were blotted on dry filter paper, weighed and dissolved in 2 ml 5% trichloracetic acid (TCA) for 18 h at 55°C. Then the tissues were homogenized and after centrifugation at 2000 rev/min for 10 min, 1.5 ml of the clear supernatant was transferred to 10 ml scintillation mixture for counting in a Packard Tricarb liquid scintillation-spectrometer, model 3320, using channels set for the simultaneous determination of ^{22}Na and ^{45}Ca. The results were corrected for quenching by external standardization; 100 μl of the incubation medium was counted with 1.4 ml TCA and the specific activity of ^{22}Na and ^{45}Ca was used as reference.

The ^{22}Na and ^{45}Ca taken up and retained during the wash period were calculated as follows:

$$^{22}\text{Na content } \mu\text{mol/g wet wt.} = \frac{\text{ct/min in aorta}}{\text{g wet wt.}} \times \frac{[\mu\text{mol}]\text{ Na in incub. media}}{\text{ct/min in incub. media}}$$

^{45}Ca content was calculated in the same way and expressed as [nmol]/g wet wt.

Drugs, isotopes and solutions

The drugs used were: tolmesoxide (Rx71107, Reckitt & Colman), tolmesoxide sulphone metabolite (Rx71112, Reckitt & Colman), nifedipine (Adalat, Bayer AG), noradrenaline bitartrate (DAK), and ouabain (DAK). ^{22}Na (500 μCi/mg) was obtained from the Radiochemical Centre, Amersham, and ^{45}Ca (500 μCi/g) from the Danish Atomic Energy Commission, Isotope laboratory Riso.

The drugs were added to the organ bath in volumes of 0.03–0.3 ml. Stock solutions of noradrenaline were stabilized with ascorbic acid (100 μM)..

The Krebs solution had the following composition (mM): NaCl 119, KCl 4.6, $NaHCO_3$ 20, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 11, pH 7.4. The solution used for depolarization contained KCl 127 mM and no NaCl, but was otherwise of the same composition. In 'calcium-free medium', $CaCl_2$ was omitted from the Krebs solution, and 0.01 mM EGTA was added to remove any small amounts of contaminating calcium. Twice-distilled water and analytical grade chemicals were used. The solutions were prepared on the day of the experiment.

Statistical evaluation

The results were expressed as mean ± s.e. mean. Student's t test was used and $P < 0.05$ was considered significant.

Results

Effects of tolmesoxide, tolmesoxide metabolite and nifedipine on human isolated crural veins

Veins contracted by NA or K^+ were relaxed by tolmesoxide, Rx71112 or nifedipine. With all drugs at their lowest concentrations the relaxation was slow and gradual and a plateau was difficult to obtain. Consequently, concentration-response curves were not constructed.

A reduction occurred in the amplitude of NA- and K^+-induced contractions when the relaxant drugs were added beforehand. Figure 1 shows the concentration-related inhibitory effect of tolmesoxide and Rx71112, both of which were more effective in inhibiting the NA-induced contraction than that induced by K^+. Because of isolubility, Rx71112 could not be tested in concentrations as high as those used for tolmesoxide and therefore its relative potency could not be accurately assessed. By contrast, nifedipine inhibited the K^+-induced contractions significantly more than NA-induced responses (Figure 1); nifedipine was by far the most potent relaxant drug in these experiments.

In calcium-free medium, when equipotent amounts of nifedipine and tolmesoxide were added, tolmesoxide caused only a minor depression and displacement of the cumultative Ca^{2+} concentration-response curves in human veins contracted by NA or K^+. By contrast, nifedipine almost abolished the response to potassium (Figure 2a) and also reduced the maximum response to NA in the presence of 4 mM Ca^{2+} to an average of 57% of control (Figure 2b).

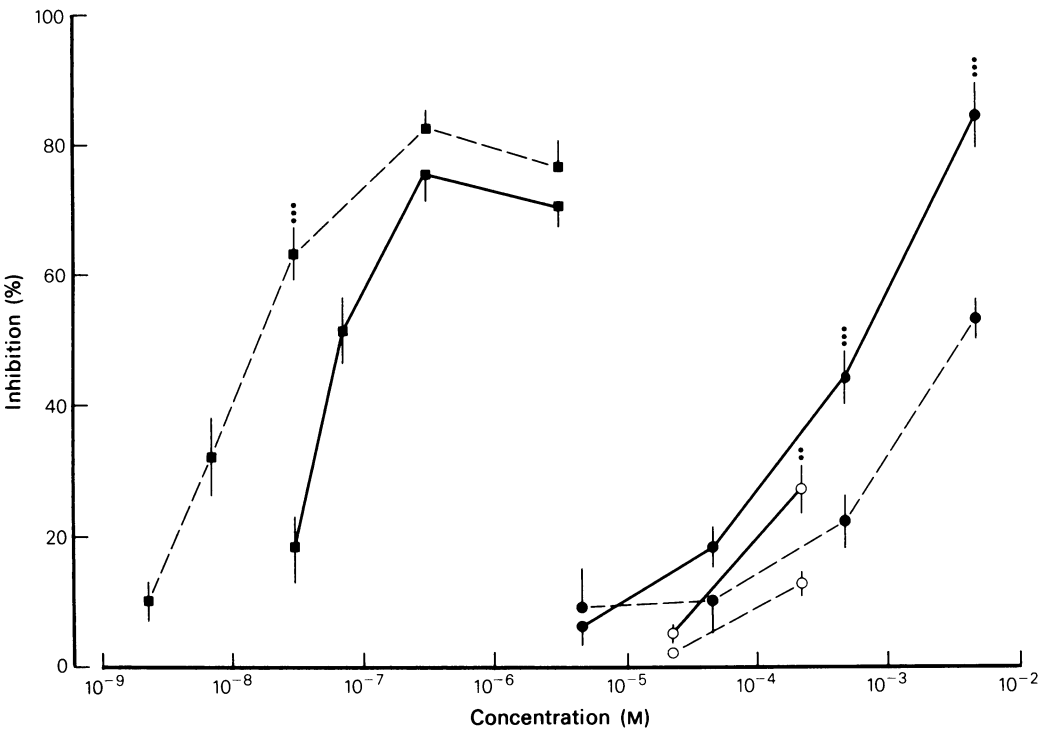

Figure 1 Inhibitory effect of nifedipine (■), tolmesoxide (●) and Rx71112 (○) on the response of human isolated crural veins to potassium 127 mM (broken line) and noradrenaline 18 μM (continuous line). Each point: mean of n = 7; vertical lines show s.e. mean. ***$P < 0.001$; **$P < 0.01$.

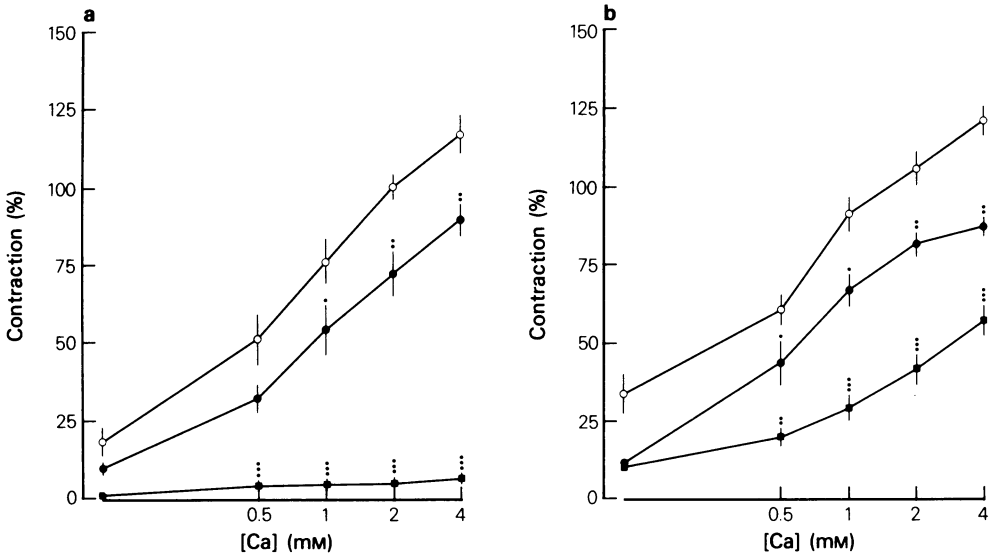

Figure 2(a) Responses of human isolated crural veins contracted by potassium (127 mM) to increasing concentrations of Ca^{2+} after exposure to Ca^{2+}-free medium for 30 min. Control: absence of drugs (○); tolmesoxide, 940 μM (●); nifedipine, 0.036 μM (■). Mean of n = 6–7; vertical lines show s.e. mean: (b) Responses of human crural veins contracted by noradrenaline (18 μM) to increasing concentrations of calcium after 30 min exposure to Ca^{2+}-free medium. Control: absence of drugs (○); tolmesoxide, 470 μM (●); nifedipine, 0.072 μM (■). Mean of n = 6–7; vertical lines show s.e. mean. The contractile response measured in normal Krebs solution was used as the reference point for 100% contraction in both (a) and (b).

Comparison of the responses in human veins and rat aorta to tolmesoxide and nifedipine

In Figure 3, the results of the inhibition studies in NA-contracted human veins are compared with the effect of nifedipine and tolmesoxide on rat aorta. As can be seen, in both vessels nifedipine had almost the same potency in inhibiting the NA-contractions and also caused a similar depression of the maximal contraction to NA. By contrast, tolmesoxide produced a larger inhibitory effect in rat aorta than in human vein at the two highest concentrations used.

Inhibitory potency on the concentration-effect curve of noradrenaline in rat isolated aorta

Nifedipine (0.003 to 0.3 μM) was the most potent drug in inhibiting the contractile response to noradrenaline in rat thoracic aorta, shifting the cumulative NA concentration-response curve to the right and causing a reduction in the maximum contractile effect (Figure 4a). The EC_{50} for noradrenaline was 4.3 ± 0.6 × 10^{-7} M and in the presence of nifedipine (0.03 and 0.3 μM) the EC_{50} increased to 1.0 ± 0.3 × 10^{-6} M and 8 ± 1.6 × 10^{-7} M, whilst the maximum contractions were reduced to 51% and 29% of control, respectively.

Tolmesoxide (47 and 470 μM) also reduced the maximum response to noradrenaline (by 24% and 54%) and increased the EC_{50} from 4.5 ± 0.6 × 10^{-7} M to 8.0 ± 1.6 × 10^{-7} M and 1.0 ± 0.15 × 10^{-6} M, respectively. Rx71112 (220 μM) increased the EC_{50} to 9.8 ± 1.0 × 10^{-7} M and reduced the maximum response to noradrenaline by an average of (28%) (Figure 4b). Based on this limited study, Rx71112 appears to be less potent than tolmesoxide.

Effects of nifedipine and tolmesoxide on ^{22}Na and ^{45}Ca net influx in rat aorta

Table 1 summarizes the results of these studies. Tolmesoxide had no significant effect on ^{22}Na and ^{45}Ca uptake in concentrations (47 and 470 μM) that had inhibitory effects on NA-induced contractions. By contrast, nifedipine showed a dose-related, significant inhibitory effect on ^{45}Ca uptake; ^{22}Na uptake was also significantly inhibited at the hightest concentrations used. Rx71112 (220 μM) reduced ^{22}Na uptake, but had no effect on ^{45}Ca uptake.

Discussion

The present study shows that tolmesoxide had a concentration-related relaxant effect on human veins *in vitro*. This is in accordance with plethysmographic investigations on forearm haemodynamics in healthy subjects, in which tolmesoxide was found to dilate

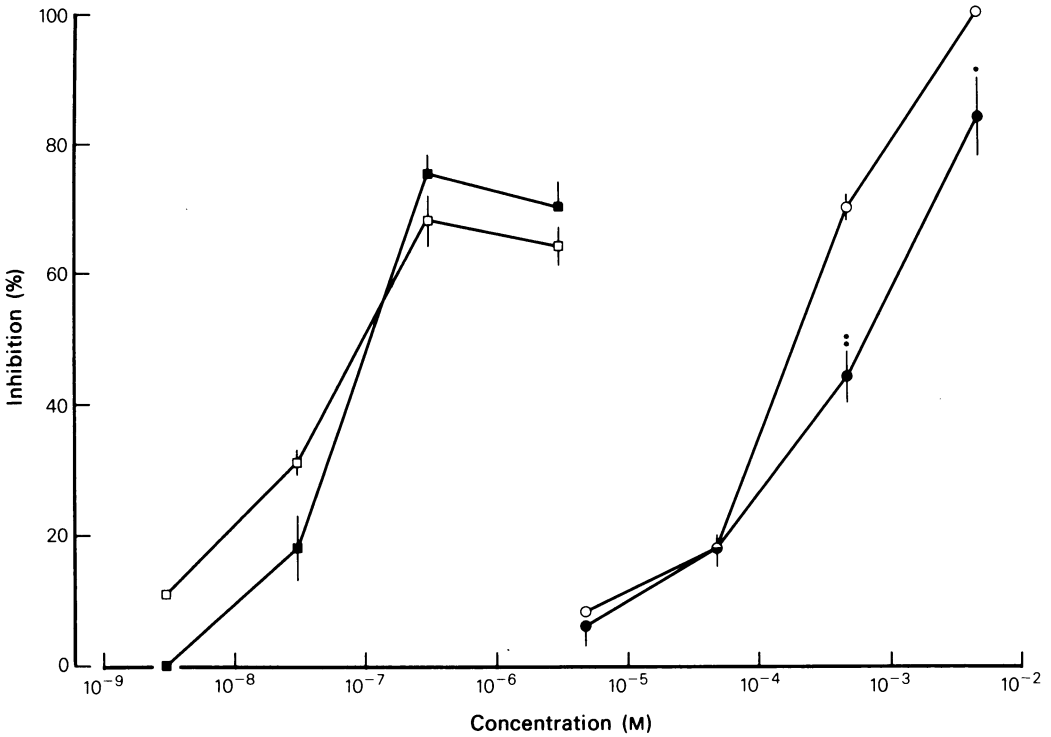

Figure 3 Inhibitory effect of nifedipine (□, ■) and tolmesoxide (○, ●) on human isolated crural veins (filled symbols) and rat aorta (open symbols) contracted by noradrenaline (18 μM). Each point is mean of $n = 7$; vertical lines show s.e. mean. $^{*}P < 0.05$; $^{**}P < 0.001$.

equally well human arteries or veins (Collier *et al.*, 1978). The drug concentrations necessary to obtain a measurable inhibitory effect on the NA-induced vasoconstruction *in vivo* were of similar magnitude to the concentrations which were effective *in vitro* (1–10 μg/ml). However, to obtain more pronounced responses *in vitro*, relatively large concentrations of tolmesoxide were required. In a previous study, dihydralazine was found to have a very low potency *in vitro* in spite of its definite clinical effectiveness (Lederballe Pedersen, Mikkelsen & Andersson, 1979). There are several possible explanations for this

Table 1 Effect of tolmesoxide, Rx71112 and nifedipine on ^{22}Na and ^{45}Ca net influx in rat thoracic aorta contracted by noradrenaline (18 μM).

Additions	*Concentrations* (μM)	*^{22}Na influx* (μmol/g)	*^{45}Ca influx* (nmol/g)
Control		1.78 ± 0.08 (7)	184 ± 8.5 (7)
Tolmesoxide	4.7	1.79 ± 0.05 (7)	168 ± 10.6 (7)
	47	1.83 ± 0.09 (7)	171 ± 11.9 (7)
	470	1.65 ± 0.12 (7)	172 ± 15.9 (7)
Control		1.85 ± 0.19 (5)	202 ± 11.4 (5)
Rx71112	220	1.31 ± 0.11 (5)*	195 ± 11.0 (5)
Control		1.66 ± 0.18 (6)	187 ± 9.0 (6)
Nifedipine	0.003	1.40 ± 0.10 (6)	154 ± 24.6 (6)
	0.03	0.80 ± 0.16 (6)*	130 ± 13.8 (6)*
	0.3	0.80 ± 0.09 (6)**	101 ± 5.3 (6)**

Values are mean ± s.e. mean. The number of experiments is given in parentheses.
$^{*}P < 0.05$; $^{**}P < 0.01$.

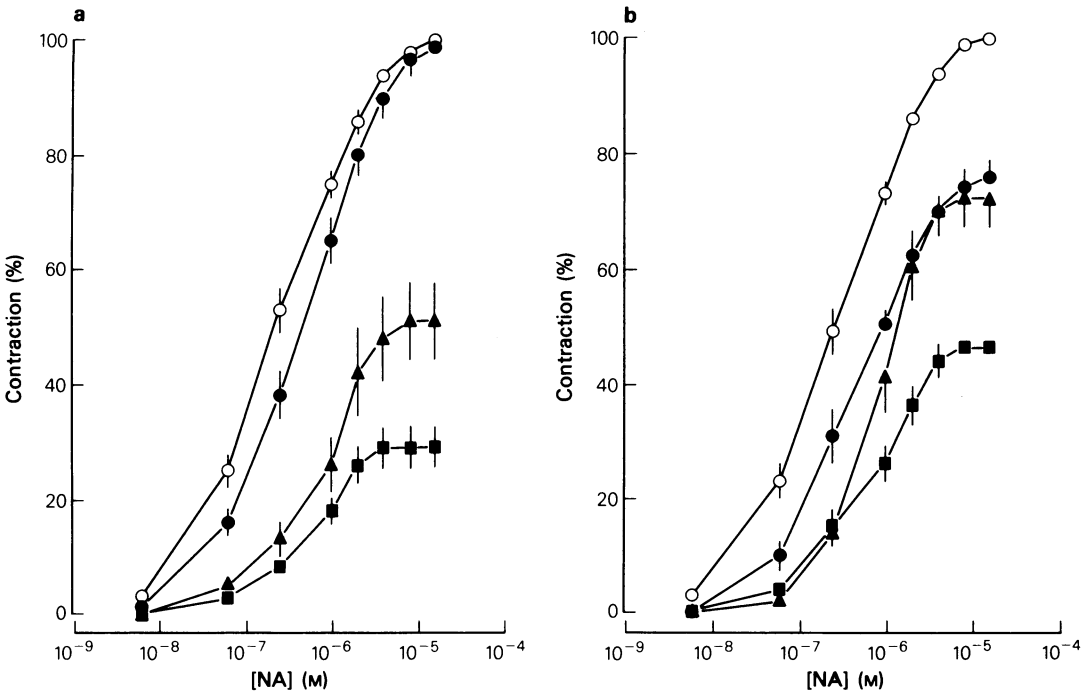

Figure 4(a) Effect of nifedipine, (0.003–0.3 μM) on the concentration-effect curve to noradrenaline in rat thoracic aorta: control (○); nifedipine 0.003 μM (●), 0.03 μM (▲) and 0.3 μM (■). (b) Effect of tolmesoxide, 47 μM (●) and 470 μM (■) and Rx71112 (220 μM) (▲) on the concentration-effect curve to noradrenaline in rat thoracic aorta. Control: (○); each point is mean of n = 19–20. Noradrenaline + drug: (filled symbols); each point is mean of n = 6–7; vertical lines shown s.e. mean in (a) and (b).

contrasting picture such as: (a) biotransformation with the occurrence of even more potent metabolites; (b) *in vivo* accumulation in vascular tissue; or (c) additive central effects of the drug.

In the present studies, the effect of the main metabolite (Rx71112) was investigated. Although little information is available on the amounts of Rx71112 occurring in human plasma after chronic oral administration of tolmesoxide, Rx71112 is not likely to be mainly responsible for the effects since it was less potent than tolmesoxide on rat aorta and at best, no more potent on human vein.

The mode of action of tolmesoxide at a cellular level has not yet been clearly defined. The relaxant effect of the drug might partly be due to a blockade of Ca influx across the muscle cell membrane. The present study shows that such an action cannot be essential for the effect of tolmesoxide, as the drug had only a slight antagonistic action on the contractile effects of cumulative addition of calcium. This contrasts with the inhibitory effects of nifedipine, which were only slightly affected by increasing concentrations of extracellular calcium. The K^+-induced contraction was particularly sensitive to the effects of nifedipine as it remained almost completely abolished even on addition of an excess of extracellular Ca^{2+}, whereas it was possible to establish a more pronounced contraction in NA-exposed vessels. The discrepancy between the effect of nifedipine on K^+ and NA found in normal Krebs solution as well as in calcium-free medium with cumulative addition of calcium is well understood, as the K^+ contraction is mainly dependent on extracellular and the NA contraction on intracellular calcium stores (Peiper, Griebel & Wende, 1971). Drugs with a specific blocking effect on Ca^{2+} entry will therefore show a stronger inhibitory effect on the K^+ contraction than on the NA contraction (Mikkelsen *et al.*, 1978).

The differences between tolmesoxide and nifedipine in mode of action was demonstrated in the ion flux studies in rat isolated aorta. Nifedipine was found to have a concentration-related inhibitory effect on ^{22}Na and ^{45}Ca net influx, whereas tolmesoxide exerted no such effect using concentrations that caused relaxation.

Investigations of vasoactive drugs have shown that data obtained in one type of vessel cannot be extrapolated to vessels isolated from other species or to other vascular regions within the same animal or man, until comparative investigations have been carried

out (see e.g. Müller-Schweinitzer, 1976; Mikkelsen, Andersson & Lederballe Pedersen, 1979).

In the present study, rat aorta and human veins responded similarly to nifedipine. At low concentrations, tolmesoxide produced identical degrees of relaxation in human veins and rat aorta, but at the highest concentration used, tolmesoxide caused a larger relaxation of rat aorta than human veins.

Thus, rat thoracic aorta seems to be well-suited for the more specific ion flux studies. These experiments showed that tolmesoxide affected neither ^{22}Na nor ^{45}Ca net influx significantly. Thus, unlike nifedipine, the vasodilator effect of tolmesoxide cannot be explained by a specific inhibition of cellular Ca^{2+} entry. However, since only net influx of the isotopes was studied, these findings do not rule out the possibility that tolmesoxide affects transmembranal Na^+ and Ca^{2+} movements, for instance by concomitant effects on influx and efflux. Rx71112 may have some effect on ^{22}Na net influx without affecting the ^{45}Ca net influx significantly. The reason for such findings remains obscure.

Further elucidation of the cellular action of tolmesoxide and its metabolite will demand additional investigations, such as specific efflux studies and studies including blockade of the Na^+/K^+ pump.

This study was supported by the Danish Medical Research Council (grant no. 512–15533),

The skilful technique assistance of Ms Hanne Larsen, Ms Deborah Chason and Mr Borge Poulsen is gratefully acknowledged.

Reckitt & Colman kindly supplied us with tolmesoxide and tolmesoxide metabolite. Nifedipine was supplied by Bayer AG.

References

CASE, R.M. & CLAUSEN, T. (1973). The relation between calcium exchange and enzyme secretion in the isolated rat pancreas. *J. Physiol.* **235**, 75–102.

COLLIER, J.G., LORGE, R.E. & ROBINSON, B.F. (1978). Comparison of effects of tolmesoxide (Rx71107), diazoxide, hydrallazine, prazosin, glyceryl trinitrate and sodium nitroprusside on forearm arteries and dorsal hand veins of man. *Br. J. clin. Pharm.*, **5**, 35–44.

DAHL-HANSEN, A.B. & CLAUSEN, T. (1973). The effect of membrane stabilizers and ouabain on the transport of Na^+ and K^+ in rat soleus muscle. *Biochim. biophys. Acta.*, **318**, 147–153.

DOXEY, J.C. (1978). Tolmesoxide, a drug that lowers blood pressure by a direct relaxant effect on vascular smooth muscle. *Br. J. Pharmac.*, **63**, 111–118.

FLEMING, W.W., WESTFALL, F.P., de la LANDE, I.S. & JELLETT, L.B. (1972). Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. *J. Pharmac. exp. Ther.*, **181**, 339–345.

LEDERBALLE PEDERSEN, O., MIKKELSEN, E. & ANDERSSON, K.-E. (1979). Comparison of the *in vitro* effects of prazosin, nifedipine, and dihydralazine in isolated human mesenteric and crural vessels. *Archs int. Pharmacodyn.*, **241**, 224–234.

MIKKELSEN, E., ANDERSSON, K.-E. & BENGTSSON, B. (1978). Effects of verapamil and nitroglycerin on contractile responses to potassium and noradrenaline in isolated human peripheral veins. *Acta pharmac. tox.*, **42**, 14–22.

MIKKELSEN, E., ANDERSSON, K.-E. & LEDERBALLE PEDERSEN, O. (1979). Verapamil and nifedipine inhibition of contractions induced by potassium and noradrenaline in human mesenteric arteries and veins. *Acta pharmac. tox.*, **44**, 110–119.

MÜLLER-SCHWEINITZER, E. (1976). Responsiveness of isolated canine cerebral and peripheral arteries to ergotamine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **292**, 113–118.

PEIPER, U., GRIEBEL, L. & WENDE, W. (1971). Activation of vascular smooth muscle of rat aorta by noradrenaline and depolarization: two different mechanisms. *Pflügers Arch.*, **330**, 74–89.

(Received April 9, 1980. Revised February 9, 1981.)