Br. J. Pharmac. (1979), **67**, 255–263

URETHANE AND CONTRACTION OF VASCULAR SMOOTH MUSCLE

BURTON M. ALTURA & JULIO WEINBERG

Department of Physiology, SUNY Downstate Medical Center, Brooklyn, New York 11203, U.S.A.

1 *In vitro* studies were undertaken on rat aortic strips and portal vein segments in order to determine whether or not the anaesthetic, urethane, can exert direct actions on vascular smooth muscle.

2 Urethane was found to inhibit development of spontaneous mechanical activity. This action took place with a urethane concentration as little as one tenth of that found in anaesthetic plasma concentrations, i.e., 10^{-3} M.

3 Urethane (10^{-3} to 10^{-1} M) dose-dependently attenuated contractions induced by adrenaline, angiotensin and KCl. These inhibitory actions were observed with urethane added either before or after the induced contractions.

4 Ca^{2+}-induced contractions of K^+-depolarized aortae and portal veins were also attenuated, dose-dependently, by urethane.

5 All of these inhibitory effects were completely, and almost immediately, reversed upon washing out the anaesthetic from the organ baths.

6 A variety of pharmacological antagonists failed to mimic or affect the inhibitory effects induced by urethane.

7 These data suggest that plasma concentrations of urethane commonly associated with induction of surgical anaesthesia can induce, directly, relaxation of vascular muscle.

Introduction

Urethane has been used as a hypnotic/narcotic agent since 1885 (Schmiedeberg, 1885) and is the anaesthetic of choice in many physiological and pharmacological experiments. Though widely used, it is known to lower blood pressure (Hillebrand, Van Der Meer & Ariëns, 1971; Buñag & Mullenix, 1972; Brezenoff, 1973), and to decrease cardiovascular responsiveness to noradrenaline (Buñag & Mullenix, 1972; Brezenoff, 1973) and angiotensin (Volicer & Loew, 1971; Buñag & Mullenix, 1972). Its well-known blood pressure lowering effect is thought to be a result either of a loss of blood plasma into the peritoneal cavity (Van Der Meer, Versluys-Broers, Tuynman & Burr, 1975), or of direct cardiac effects (Giles, Quiroz & Burch, 1969). A direct effect on peripheral blood vessels was first suggested in 1886 (Huchard, 1886). But, to our knowledge, no systematic quantitative studies have been undertaken on either arterial or venous smooth muscles to investigate this possibility.

It was, therefore, of interest to determine whether urethane would have any direct action on blood vessels or influence on: (1) spontaneous mechanical activity of isolated blood vessels; and (2) drug-induced contractions of isolated vascular smooth muscles. The present studies indicate that concentrations of urethane used to induce surgical anaesthesia can induce, directly, potent depressant effects on arterial and venous smooth muscle.

Methods

Thoracic aortae and portal veins were obtained from male Wistar rats, weighing 300 to 400 g, after decapitation. Only male rats were employed in these studies in order to avoid the influence of sex and sex hormones on the reactivity of blood vessels to catecholamines (Altura, 1972). Helically cut aortic strips (1.4 to 1.6 mm in width by 25 mm in length) were set up isometrically, *in vitro*, under a resting tension of 1.5 g, similar to that described by Altura & Altura (1974). Segments of portal veins (10 to 12 mm long) were tied at both ends and arranged isometrically, *in vitro*, under a resting tension of 0.5 g (Altura & Altura, 1975). The preparations were equilibrated for 2 h in muscle chambers containing Krebs-Ringer bicarbonate (KRB) solution, the composition of which was (mmol/l): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25.0 and glucose 10.0. The KRB solution was bubbled continuously with a 95% O_2 and 5% CO_2 mixture and kept at 37°C (pH 7.4 to 7.5). The loading tensions

0007-1188/79/100255-09$01.00

were maintained and periodically adjusted throughout the experiments. The incubation media were routinely changed every 10 to 15 min as a precaution against interfering metabolites (Altura & Altura, 1970). Grass model 7C polygraphs, equipped with Grass model 7 DAF driver amplifiers, Grass model 7 PID pre-amplifiers and Grass model FT.03C force-displacement transducers were used as recording equipment (Altura & Altura, 1970).

After the 2 h incubation period the following experiments were carried out:

(1) Some vessel strips were exposed to KRB solutions containing urethane in concentrations of 1.0×10^{-5} M to 1×10^{-1} M for 10 min periods to determine whether or not it affected base-line tension and/or changed spontaneous mechanical activity. After the 10 min period of preincubation a single dose of agonist (adrenaline, angiotensin or KCl) was added to the organ-bath and the contraction elicited was compared with the control responses obtained before and after contact with urethane. Only one agonist was used for each preparation and submaximal equipotent doses were chosen for all three agonists. After ascertaining the complete contractile response, the preparation was washed rapidly three times with KRB and an interval of 30 to 45 min (with fresh KRB solution changed at every 10 to 15 min) elapsed before another single dose of agonist was added to the organ bath. During this time no changes assignable to urethane wash-out were observed. Furthermore, the control responses as well as the control cumulative dose-response curves (below) are reproducible to an error of no more than 7%.

(2) Other vascular strips were exposed to progressively increasing doses of adrenaline, angiotensin, or KCl in order to obtain complete cumulative dose-response curves (DRC) in the absence and presence of urethane. Only one agonist was used for each preparation. Two control agonist DRCs were obtained before incubating the preparations with urethane. Preincubations of 10 min with urethane (5.0×10^{-2} M or 1.0×10^{-1} M) were followed by complete agonist-cumulative DRCs in the presence of urethane.

(3) In additional experiments, aortae and portal veins were exposed to a calcium-free Krebs-Ringer solution for 30 min with successive washings in this solution every 10 min (Altura & Altura, 1974). After the 30 min period the vascular strips were exposed to a calcium-free high potassium depolarizing Krebs-Ringer solution (Altura & Altura, 1974) for 45 min, with successive washings in this solution every 15 min. A complete cumulative contractile DRC for $CaCl_2$ was then obtained for each preparation (Altura & Altura, 1974; 1975). At the end of the DRC, $CaCl_2$ was washed out with normal KRB solution, reincubated in KRB for 60 min, and the calcium-free, high K^+-depolarizing procedure was repeated again, as described above. A second cumulative DRC for $CaCl_2$ was then obtained in the presence of urethane (5.0×10^{-2} M or 1.0×10^{-1} M) added to the muscle chamber 10 min before the first dose of $CaCl_2$.

(4) In some experiments aortae were exposed to submaximal doses of agonists and after a stable contraction was obtained, urethane was added to the muscle chamber in progressively increasing cumulative doses, in order to observe whether it could relax the already established contractions.

(5) In still another group of experiments, aortic strips ($n = 4$) and portal veins ($n = 4$) were exposed separately to various specific pharmacological antagonists for 10 min or to a prostaglandin synthetase inhibitor (indomethacin, 1.0 μg/ml) for 20 min. After the preincubation periods the aortae were stimulated with a submaximal single dose of KCl ($\cong ED_{50}$). Both KCl-contracted aortae and spontaneously contracting portal veins were then exposed to urethane (5.0×10^{-2} M) in order to observe whether: (a) the inhibitory actions of urethane would be affected by α-adrenoceptor blockade (phentolamine, 0.1 μg/ml), β-adrenoceptor blockade (propranolol hydrochloride 0.5 μg/ml), 5-hydroxytryptamine receptor blockade (methysergide, 0.5 μg/ml), or histamine receptor blockade (diphenhydramine hydrochloride, 0.5 μg/ml); and (b) prostaglandins could have any intermediate role in the inhibitory responses observed with urethane. All concentrations of these drugs described above produce specific antagonism to their respective agonists on rat aorta and portal veins studied *in vitro* (Altura & Altura, 1974; 1975; Altura, Edgarian & Altura, 1976).

Single agonist dose-induced responses were measured as total developed tension. Cumulative dose-induced contractile responses were measured as differences between basal tension in the absence of agonist and elicited tensions with each cumulative dose of agonist. In the veins, the average value between base line tension and maximum contractile-spike tension was taken as the parameter for each experimental condition in order to evaluate the differential tensions. Results are given in mg of developed isometric contractile tension or in percent of maximum control responses.

Data are expressed as means ± s.e. mean for each experimental group. Where appropriate, the means (± s.e. means) were compared by Student's t test or paired t test, and considered significantly different if $P < 0.05$.

The following chemicals and drugs were used: urethane (ethyl carbamate Sigma Chem. Co.), epinephrine (adrenaline chloride, Parke Davis and Co.), angiotensin (Hypertensin, Ciba-Geigy), potassium chloride (Fisher Scientific Co., A.C.S. certified),

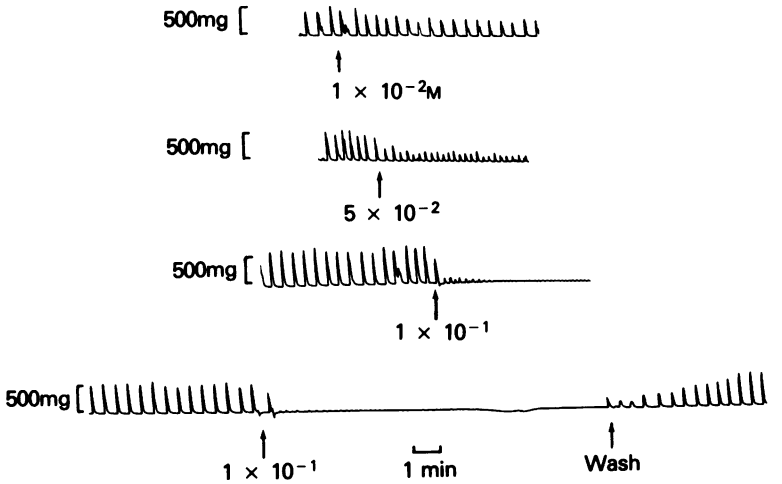

Figure 1 Influence of various concentrations of urethane on development of spontaneous mechanical activity in isolated portal vein of the rat. Arrows indicate point at which tissues were exposed to urethane (M concentrations are shown) or washed in KRB. Values on left represent tension.

calcium chloride (Fisher Scientific Co., A.C.S. certified), phentolamine (Regitine, Ciba Pharmaceutical Co.), methysergide (Sandoz Pharmaceuticals), atropine sulphate (Mann Research Laboratories), propranolol hydrochloride (Aldrich Chemical Co.,) indomethacin (gift from Merck Sharp and Dohme), and diphenhydramine hydrochloride (Benadryl, Parke Davis and Co.). The concentrations of drugs and chemicals are expressed as final molar concentrations in the organ bath.

Results

Influence of different concentrations of urethane on spontaneous mechanical activity and basal tension

Figure 1 shows recordings of typical changes in spontaneous mechanical activity of portal veins after addition of urethane. The inhibitory effect on portal vein spontaneous contractions was observed with concentrations as low as 1.0×10^{-3} M and dose-dependently increased with higher concentrations, as shown in Table 1. In most of our experiments, in which high concentrations of urethane (e.g. 1.0×10^{-1} M) were used, we observed an immediate abolition of venous spontaneous mechanical activity, as shown in Figure 1. In a few other experiments, this inhibitory effect took longer to become manifest, e.g., 3 to 4 min and was completely reversed upon one single wash with KRB. No significant effect was observed on the frequency of portal vein spontaneous contractions, except with high concentrations (1.0×10^{-1} M) (Table 1).

In a few of our experiments with aortic strips, we noted spontaneous contractions, similar to those seen previously (Altura & Altura, 1975; 1978a). In these experiments, urethane, in concentrations of $5.0 \times$

Table 1 Effect of urethane on spontaneous contractions of rat hepatic-portal veins

Urethane (M)	n	*Contractile tension (mg)*	*Frequency (contractions/min)*
0	33	423.5 ± 29.3	2.59 ± 0.13
1.0×10^{-4}	3	425.0 ± 28.7	2.57 ± 0.57
1.0×10^{-3}	10	280.0 ± 40.0*	2.31 ± 0.17
5.0×10^{-3}	6	150.0 ± 39.7*	2.73 ± 0.17
1.0×10^{-2}	15	190.0 ± 27.5*	2.39 ± 0.25
2.5×10^{-2}	5	125.0 ± 30.5*	2.82 ± 0.34
5.0×10^{-2}	28	64.7 ± 9.0*	2.76 ± 0.22
1.0×10^{-1}	23	5.0 ± 2.0*	1.43 ± 0.53†

Values are mean ± s.e. mean.
* Significantly different from paired controls ($P < 0.01$).
† Significantly different from paired controls ($P < 0.03$).

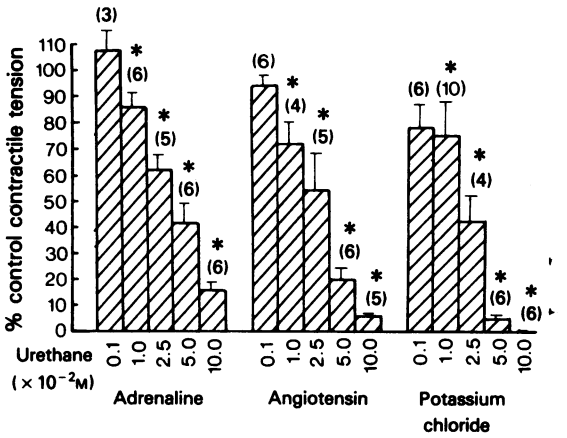

Figure 2 Differential sensitivity of equi-potent adrenaline, angiotensin, and KCl-induced contractions on rat aortic strips to inhibition by urethane ($\times 10^{-2}$ M). Numbers in parentheses indicate different number of preparations used. Bars represent one s.e. Asterisks indicate experimental mean values which are significantly different from paired controls (without urethane) ($P < 0.05$, paired t test). Last value for KCl = 0 ± 0 with 10×10^{-2} M urethane.

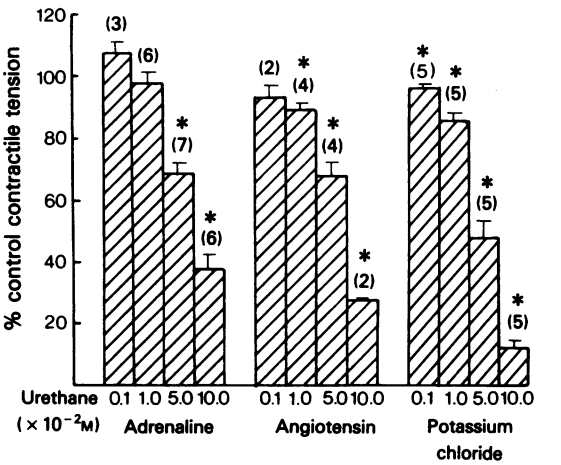

Figure 3 Differential sensitivity of equi-potent adrenaline, angiotensin, and KCl-induced contractions on rat portal veins to inhibition by urethane ($\times 10^{-2}$ M). Bars show one s.e. Asterisks indicate experimental mean values which are significantly different from paired controls (without urethane) ($P < 0.05$, paired t test).

10^{-2} M and 1.0×10^{-1} M, markedly depressed or completely abolished the spontaneous mechanical activity; this effect being reversed upon washing out with normal KRB.

Influence of different concentrations of urethane on single agonist dose-induced contractions of rat aortae and portal veins

Figures 2 and 3 indicate that a 10 min preincubation period of rat aortae and portal veins with different concentrations of urethane, before the addition of single submaximal doses of adrenaline, angiotensin or potassium chloride, caused inhibition of the drug-induced contractile responses in a dose-related manner. The three agonists exhibited the following relative order of sensitivity to urethane inhibition, for both aortae and portal veins: KCl > angiotensin > adrenaline. These inhibitory effects on drug-induced responses were completely and rapidly reversed upon washing in KRB solution.

Figure 4 shows that cumulative doses of urethane, added to the muscle chamber after angiotensin-, or potassium-induced contractions were established in rat aortae, cause a dose-dependent relaxation.

Influence of urethane on adrenaline, angiotensin, and potassium concentration-effect curves of rat aortae and portal veins

Figures 5, 6 and 7 show complete concentration-effect curves for adrenaline, angiotensin and potassium chloride in rat aortae and portal veins in control conditions (no urethane), and in the presence of urethane. Table 2 complements the graphic data, and with Figures 5 to 7, indicates that the following parameters were changed, dose-dependently by urethane, for all three agonists: (a) the concentration-effect curves were significantly shifted to the right in a non-parallel manner for the aortae and in a parallel manner for the veins; (b) threshold doses were increased significantly; (c) the ED_{50}s were increased significantly (except for 5×10^{-2} M urethane with KCl responses on veins); and (d) the maximum developed tensions were decreased significantly (except for 5×10^{-2} M urethane with adrenaline and KCl responses on veins).

Effects of urethane on calcium chloride-induced contractions of potassium-depolarized rat aortic strips and portal veins

Urethane caused dose-dependent shifts to the right of the calcium chloride concentration-effect curves (Figure 8). For example, the threshold concentrations and the ED_{50}s for calcium chloride-induced contractions were significantly increased and the maximum developed tensions were decreased dose-dependently by urethane (Table 2).

Failure of pharmacological antagonists and of a prostaglandin synthetase inhibitor to mimic or to affect urethane inhibition

Use of phentolamine, propranolol, methysergide, atropine, and diphenhydramine in concentrations

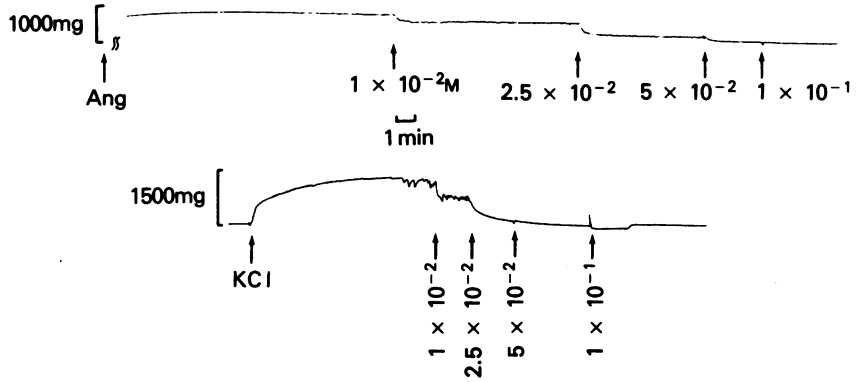

Figure 4 Urethane-induced relaxation of angiotensin (Ang) and KCl-induced contractions in rat aortic strips. Arrows indicate the points at which urethane was added in cumulative concentrations.

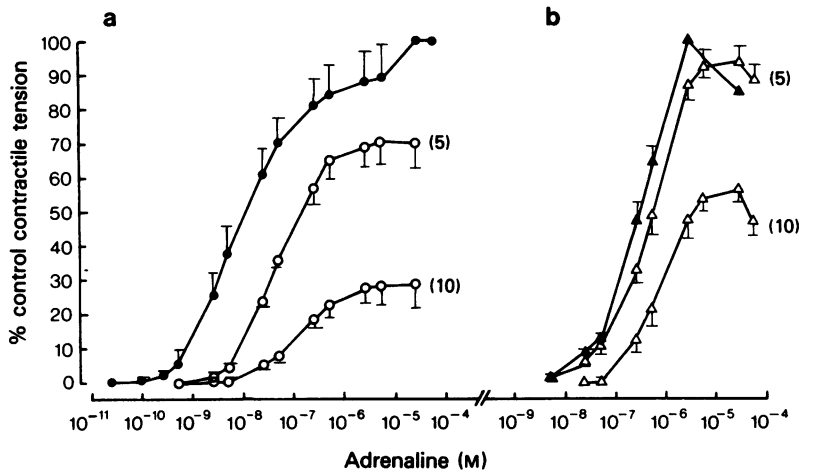

Figure 5 Influence of urethane on adrenaline concentration-effect curves in rat aortic strips (a) and portal vein (b). Mean values are shown; vertical lines indicate s.e. mean; $n = 4$ for aortae and 6 for veins. All experimental dose-response curves are significantly different from paired controls ($P < 0.05$). In (a), (●) = controls, (○) = urethane 10^{-2}M; in (b), (▲) = controls, (△) = urethane 10^{-2} M.

which specifically antagonize ED_{50} responses of their respective agonists, did not change the spontaneous mechanical activity of the portal veins nor prevent (or modify) the inhibitory effects of urethane on either portal veins or KCl-contracted aortae. Although indomethacin attenuated the portal vein spontaneous contractions 30 to 50%, it did not alter the inhibitory effects of urethane either in the veins or in the aortae ($n = 4$ animals).

Discussion

Our results show that urethane inhibits, dose-dependently, the spontaneous mechanical contractions of rat aortae and portal veins, decreases the sensitivity of rat aortae and portal veins to different vasoactive agonists and attenuates the contractile responses of aortic and venous smooth muscle to vasoactive stimuli. Since these effects are observed with urethane concentrations in the range of those found in plasma during anaesthesia e.g. 1.5 to 2.5 × 10^{-2} M (Boyland & Rhoden, 1949; Beickert, 1951; Bailey & Christian, 1952), it seems reasonable to suggest that the fall in blood pressure, the peripheral vasodilatation associated with urethane anaesthesia (Hillebrand *et al.*, 1971; Buñag & Mullenik, 1972; Brezenoff, 1973), and the decreased responsiveness to pharmacological agents described here and elsewhere (Owen, 1971; Volicer & Loew, 1971; Buñag & Mullenix, 1972; Brezenoff, 1973; Miller & Wiegman, 1977), are due, at least in part, to a direct action of urethane on vascular smooth muscle.

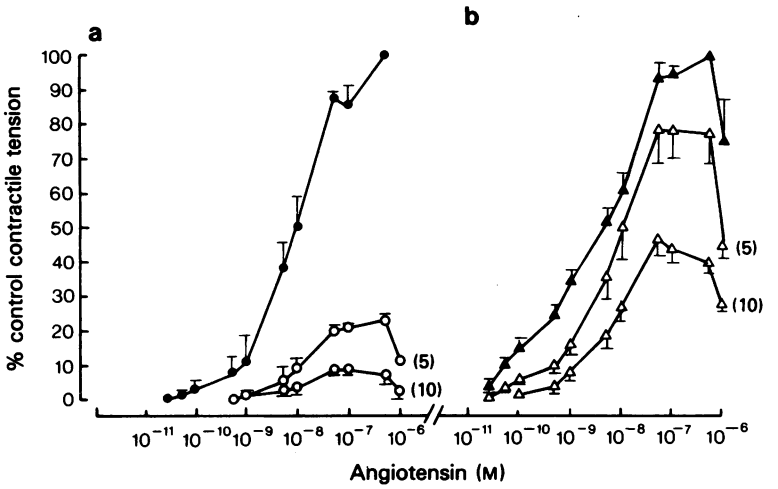

Figure 6 Influence of urethane on angiotensin II amide concentration-effect curves in rat aortic strips (a) and portal veins (b). Mean values are shown; vertical lines indicate s.e. mean, $n = 4$ for aortae and 6 for veins. All experimental dose-response curves are significantly different from paired controls ($P < 0.05$). In (a), (●) = control, (○) = urethane 10^{-2} M; in (b), (▲) = controls, (△) = urethane 10^{-2} M.

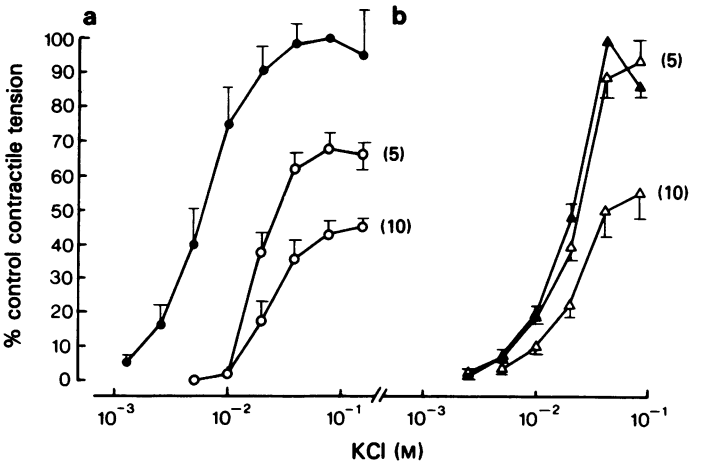

Figure 7 Influence of urethane on KCl concentration-effect curves in rat aortic strips (a) and portal veins (b). Mean values are shown; vertical lines indicate s.e. mean; $n = 6$ each for aortae and veins. All experimental dose-response curves (except for 5×10^{-2} M on vein) are significantly different from paired controls ($P < 0.05$). In (a), (●) = controls, (○) = urethane $\times 10^{-2}$ M; in (b), (▲) = controls, (△) = urethane $\times 10^{-2}$ M.

Urethane inhibition of mechanical activity cannot be assigned to an osmolar effect because it differs from the inhibitory effects caused by sucrose, mannitol or urea hypertonicity on rat aortic and portal venous smooth muscle (Johansson & Johnson, 1968; Altura, Edgarian & Altura, 1976; Altura & Altura, 1978a). High doses of urethane (e.g. 1.0×10^{-1} M) completely abolish spontaneous contractions of portal veins. Nevertheless, this preparation still responds dose-dependently to adrenaline, angiotensin and potassium depolarization. This observation could be used to suggest that urethane, even in this high concentration, cannot block completely the transmembrane calcium-ion movements, as do calcium antagonists such as verapamil, lanthanum, SKF 525A, or cinnarizine (Godfraind & Kaba, 1972; Bohr, 1973; Godfraind, 1976). However, urethane may exert a partial inhibition on the membrane permeability to extracellular calcium ions, since it attenuates, markedly, Ca^{2+}-induced contractions (Figure 8). This

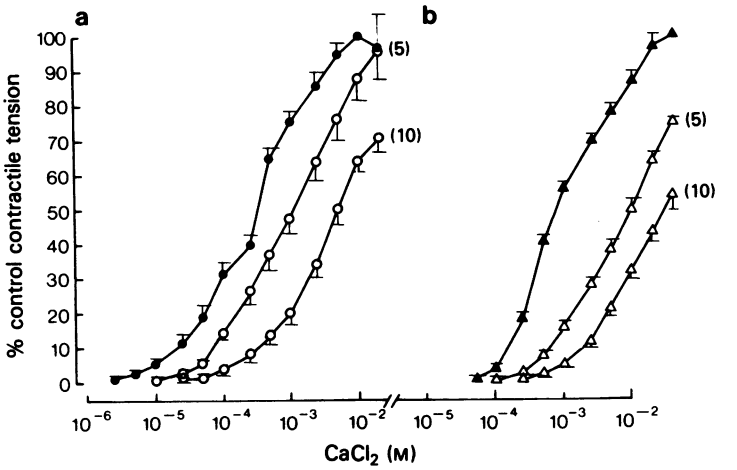

Figure 8 Influence of urethane on $CaCl_2$-induced contractions of potassium-depolarized rat aortae (a) and portal veins (b) (see Methods). Mean values are shown, vertical lines indicate s.e. mean; n = 5–10 each. All experimental DRC are significantly different from paired controls ($P < 0.01$). In (a), (●) = controls, (○) = urethane × 10^{-2} M; in (b), (▲) = controls, (△) = urethane × 10^{-2} M.

would explain the abolition of the spontaneous contractions, since these are dependent on $[Ca^{2+}]_o$ (see recent review by Altura & Altura, 1978a). In the case of portal veins, this could also be used to explain the increase in threshold concentration observed for all agonists, and the parallel displacement of all DRCs to the right. However, it is also possible, that urethane could accelerate the rate of Ca^{2+} sequestration or calcium efflux. Further experiments to test these tenets are in progress.

In the aortae, all DRCs were shifted to the right in a non-parallel manner in the presence of urethane, and the adrenaline- and angiotensin-induced maximum tensions were more affected than those induced by potassium. It is reasonable to suggest that urethane also exerts an intracellular effect in addition to its extracellular action discussed above, since the two former agonists are known, at high concentrations, to utilize intracellular Ca^{2+} for their contractile responses (Bohr, 1973; Bilek, Laven, Peiper & Regnat, 1974). Urethane is thought to penetrate rapidly into vascular smooth muscle cells (Johansson, 1969) and also to be capable of sequestering calcium from blood plasma (Peng, Cooper & Munson, 1972) in a non-defined manner of interaction. An interaction of urethane with intracellularly-bound calcium could prevent this divalent cation from being released from its intracellular storage sites, thus inhibiting one of

Table 2 Effects of urethane on adrenaline-, angiotensin-, potassium-, and calcium-induced maximal contractile tensions in rat aortae and portal veins

	Aortic maximum developed tension (mg)			*Portal venous maximum average developed tension (mg)*		
Agonist	*Control*	*Urethane*		*Control*	*Urethane*	
		(5.0×10^{-2} M)	(1.0×10^{-1} M)		(5.0×10^{-2} M)	(1.0×10^{-2} M)
Adrenaline	1700.0 ± 187.4 (4)*	1363.0 ± 134.5† (4)	575 ± 110.9† (4)	1720.8 ± 180.6 (6)	1670.8 ± 213.2 (6)	1025.0 ± 166.7† (6)
Angiotensin	794.0 ± 25.8 (4)	206.0 ± 6.3† (4)	88.0 ± 12.5† (4)	1070.8 ± 267.3 (6)	858.3 ± 175.9 (6)	483.3 ± 85.1† (6)
KCl	1119.0 ± 77.3 (6)	756.0 ± 47.2† (6)	500.0 ± 35.4† (6)	1512.5 ± 87.3 (6)	1358.3 ± 141.8 (6)	850.0 ± 129.9† (6)
$CaCl_2$	1343.0 ± 58.8 (10)	1235.0 ± 101.1† (5)	930.0 ± 40.6† (5)	1967.0 ± 55.0 (9)	1494.0 ± 76.6 (5)	975.0 ± 80.6† (5)

* Number of different values; values are mean ± s.e. mean.
† Significantly different from paired controls ($P < 0.05$).

the components responsible for vascular muscle contractions elicited by adrenaline or angiotensin (Van Breemen, Farinas, Gerba & McNaughton, 1972). This would result in a more pronounced inhibition of maximum contractions for adrenaline and angiotensin, exactly as observed on the DRCs for the aortae in the present study (Figures 5, 6 and Table 2).

However, the same relative inhibition was not observed for the portal veins. Here, in the presence of high concentrations of urethane (1.0×10^{-1} M), the maximum responses for all agonists (including $CaCl_2$) showed the same degree of inhibition (Figures 5, 6, 7, and Table 2). This would suggest that urethane causes in portal veins the same degree, and probably the same kind, of inhibition towards all agonists used. Considering that drug-induced contractions in portal veins are more dependent on extracellular calcium (Golenhofen, Hermstein & Lammel, 1973; Bilek, Laven, Peiper & Regnat, 1974; Turlapaty, Altura & Altura, 1978), the aforesaid results could be an additional indication that urethane causes its inhibition mainly at the membrane level in portal veins.

It is unlikely that the effects of urethane on contractions induced by the different vasoactive agents could be solely a reflection of inhibition of cellular metabolism. If urethane were acting via such a mechanism, then the equi-potent, sub-maximal contractions induced by the three agonists on both types of vessels (Figures 2 and 3) should have been inhibited to the same extent by each concentration of urethane, rather than exhibit a relative order of sensitivity to the anaesthetic agent, where KCl > angiotensin > adrenaline.

It is noteworthy that other classes of anaesthetic molecules that are also capable of inducing peripheral vasodilatation and hypotension, barbiturates, steroids, ketamine, ether and ethanol, have been reported, in anaesthetic concentrations, to exert on vascular smooth muscle actions similar to those reported here (Altura & Altura, 1975; 1978b; Edgarian & Altura, 1976; Altura, Edgarian & Altura, 1976; Altura, 1978). In view of the latter, one should consider the possibility that these general anaesthetics, including urethane, may compromise cardiovascular function by virtue of their direct vasodepressant actions on vascular smooth muscle. Lastly, it becomes evident that caution must be exercised in the choice and use of anaesthetic agent when cardiovascular parameters and the actions of vasoactive drugs are studied in intact animals.

This research was supported by National Heart and Lung Institute Grants HLB-18002 and 18015 and National Institute of Mental Health Grant MH-26236. J.W. is post-doctoral fellow on leave from the Universidade Federal de Minas Gerais, Brazil, partially supported by Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior. Requests for reprints should be sent to B.M.A.

References

ALTURA, B.M. (1972). Sex as a factor influencing the responsiveness of arterioles to catecholamines. *Eur. J. Pharmac.* **20**, 261–265.

ALTURA, B.M. (1978). Pharmacology of venular smooth muscle: New insights. *Microvasc. Res.*, **16**, 91–117.

ALTURA, B.M. & ALTURA, B.T. (1970). Differential effects of substrate depletion on drug-induced contractions of rabbit aorta. *Am. J. Physiol.*, **219**, 1698–1705.

ALTURA, B.M. & ALTIRA, B.T. (1974). Peripheral vascular actions of glucocorticoids and their relationship to protection in circulatory shock. *J. Pharmac. exp. Ther.*, **190**, 300–315.

ALTURA, B.T. & ALTURA, B.M. (1975). Barbiturates and aortic and venous smooth-muscle function. *Anesthesiology*, **43**, 432–444.

ALTURA, B.T. & ALTURA, B.M. (1978a). Factors affecting vascular responsiveness. In *Microcirculation*, Vol. 2, ed. Kaley, G. & Altura, B.M. pp. 547–615. Baltimore: Univ. Park Press.

ALTURA, B.T. & ALTURA, B.M. (1978b). Intravenous anesthetic agents and vascular smooth muscle function. In *Mechanisms of Vasodilatation*. ed. Vanhoutte, P.M. & Leusen, I. pp. 165–172. Basel: Karger.

ALTURA, B.M., EDGARIAN, H., & ALTURA, B.T. (1976). Differential effects of ethanol and mannitol on contraction of arterial smooth muscle. *J. Pharmac. exp. Ther.*, **197**, 352–361.

BAILEY, H.S. & CHRISTIAN, J.E. (1952). The distribution of N^{15} in rat tissues following the intraperitoneal administration of nitrogen-labeled urethane. *J. Am. pharm. Ass.* **41**, 517–521.

BEICKERT, A. (1951). Tierexperimentelle Untersuchungen über das Schicksal des Urethans in Organismus. *Z. Ges. exp. Med.*, **117**, 10–16.

BILEK, I., LAVEN, R., PEIPER, U., & REGNAT, K. (1974). The effect of verapamil on the response to noradrenaline or to potassium-depolarization in isolated vascular strips. *Microvasc. Res.*, **7**, 181–189.

BOHR, D.F. (1973). Vascular smooth muscle updated. *Circulation Res.*, **32**, 665–672.

BOYLAND, E. & RHODEN, E. (1949). The distribution of urethane in animal tissues, as determined by a microdiffusion method, and the effects of urethane treatment on enzymes. *Biochem. J.*, **44**, 528–531.

BREZENOFF, H.E. (1973). Cardiovascular responses to noradrenaline in the rat before and after administration of various anesthetics. *Br. J. Pharmac.*, **49**, 565–572.

BUÑAG, R. & MULLENIX, P. (1972). Augmentation of drug-induced blood pressure increases in rats by amobarbital. *Br. J. Pharmac.* **46**, 511–513.

EDGARIAN, H. & ALTURA, B.M. (1976). Ethanol and contraction of venous smooth muscle. *Anesthesiology*, **44**, 311–317.

GILES, T.D., QUIROZ, A.C., & BURCH, G.E. (1969). Hemo-

dynamic alterations produced by prolonged urethan anesthesia in the intact dog. *Am. Heart J.*, **78**, 281–282.

GODFRAIND, G. (1976). Calcium exchange in vascular smooth muscle, action of noradrenaline and lanthanum. *J. Physiol.*, **260**, 21–35.

GODFRAIND, T., & KABA, A. (1972) The role of calcium in the action of drugs on vascular smooth muscle. *Archs int. Pharmacodyn. Thér.*, **196**, (Suppl.), 35–49.

GOLENHOFEN, K., HERMSTEIN, N. & LAMMEL, E. (1973). Membrane potential and contraction of vascular smooth muscle (portal vein) during application of noradrenaline and high potassium, and selective inhibitory effects of iproveratril (verapamil). *Microvasc. Res.*, **5**, 72–80.

HILLEBRAND, A., VAN DER MEER, C. & ARIËNS, A.T. (1971). The effect of anesthetics on the occurrence of kidney lesions caused by hypotension. *Eur. J. Pharmac.* **14**, 217–237.

HUCHARD, H. (1886). Action hypnotique de l'urethane (ou carbonate d'éthile). *Bull. gen Ther.*, **110**, 103–110.

JOHANSSON, B. (1969). Permeability characteristics of vascular smooth muscle cells as revealed by their osmotic responses to non-electrolytes. *Acta physiol. scand.* **77**, 282–297.

JOHANSSON, B. & JONSSON, O. (1968). Cell volume as a factor influencing electrical and mechanical activity of vascular smooth muscle. *Acta physiol. scand.*, **72**, 456–468.

MILLER, F.N. & WIEGMAN, D.L. (1977). Anesthesia-induced alteration of small vessel responses to norepinephrine. *Eur. J. Pharmac.*, **44**, 331–337.

OWEN, D.A.A. (1971). Responses to pressor substances in conscious and anaesthetized cats. *Br. J. Pharmac.*, **43**, 668–670.

PENG, T.C., COOPER, C.W., & MUNSON, P.L. (1972). The hypocalcemic effect of urethane in rats. *J. Pharmac. exp. Ther.*, **182**, 522–527.

SCHMIEDEBERG, O. (1885). Ueber die Pharmakologischen Wirkungen und die Therapeutische anwendung einiger Carbaminsäure-ester. *Naunyn Schmiedebergs Arch. exp. Path. Pharmak.*, **20**, 203–216.

TURLAPATY, P.D.M.V., ALTURA, B.T. & ALTURA, B.M. (1978). Influence of Tris on contractile responses of isolated rat aorta and portal vein. *Am. J. Physiol.*, **235**, H208–H213.

VAN BREEMEN, C., FARINAS, B.R., GERBA, P. & MCNAUGHTON, E.D. (1972). Excitation-contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. *Circulation Res.*, **30**, 44–54.

VAN DER MEER, C., VERSLUYS-BROERS,, J.A.M., TUYNMAN, H.A.R.E. & BURR, V.A.J. (1975). The effect of ethylurethane on hematocrit, blood pressure and plasma-glucose. *Archs int. Pharmacodyn.*, **217**, 257–275.

VOLICER, L. & LOEW, C.G. (1971). The effect of urethane anesthesia on the cardiovascular action of angiotensin II. *Pharmacology*, **6**, 193–201.

(Received December 13, 1978
Revised February 28, 1979.)