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Full Papers

An analysis of the effects of urethane on cardiovascular responsiveness to catecholamines in terms of its interference with Ca^{++} mobilization from both intra and extracellular pools

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Summary. Urethane (1×10^{-2}-1×10^{-1} M) reduced, in a concentration-dependent manner, both intra and extracellular Ca^{++} dependent noradrenaline-induced contractions of perfused rabbit ear artery as well as the tonic contractions produced by perfusion with high K^+ solution. However, a quantitative analysis of the data indicated that for urethane concentrations similar to those found in plasma during anesthesia urethane antagonism is confined to noradrenaline-induced contractions which depend upon the mobilization of Ca^{++} from intracellular storage sites. In KCl-contracted arteries, urethane enhanced the relaxant effects of isoprenaline. - Urethane reduced the amplitude of contractions of spontaneously beating guinea-pig right atrium at concentrations which have only a limited effect on frequency. In addition, it decreased in a concentration-dependent manner the amplitude of isoprenaline-activated electrically driven, and K^+ depolarized guinea-pig right ventricular strips. Urethane had no effect on the chrono and inotropic actions of isoprenaline on cardiac preparations. In in vivo experiments the chronotropic response to low doses of isoprenaline was significantly higher in urethane-treated as compared to unanesthetized rats. The higher dose of isoprenaline tested produced a significant fall in systolic blood pressure in urethane-anesthetized rats. A significant correlation exists between the chronotropic response to isoprenaline and resting heart rate values in urethane-anesthetized rats.
These results indicate that urethane, at concentrations similar to those found in plasma during anesthesia selectively interferes with mobilization of Ca^{++} from intracellular storage sites. In addition, the interference of urethane anesthesia with the isoprenaline chronotropic effect 'in vivo' cannot be explained by a direct interference of urethane with β-adrenoceptors at cardiac level.

Introduction

Urethane is a widely used anesthetic in animal experimentation mainly because of its long duration of action and skeletal muscle relaxant properties[35,38]. Its use is widespread although it is known to lower blood pressure in intact animals[11,16] and to decrease the contractile response of vascular smooth muscle to noradrenaline[10,11,29,30] to angiotensin[11,40] and in a somehow specific manner to α_2-adrenoreceptor agonists[3,4].

Both the hypotensive action of urethane and its antagonism toward vasoactive agents 'in vivo' have

been attributed to a direct depressant action on vascular smooth muscle through interference with Ca^{++} availability for contraction[1,2]. A similar mechanism could be responsible for urethane inhibition of histamine-induced contractions of tracheal smooth muscle[24]. In addition, urethane anesthesia appears to interfere with catecholamine-induced chronotropic responses mediated by: a) postjunctional β_1-adrenoreceptors[23] b) prejunctional α_2-adrenoreceptors[4,13] and c) reflex activation of the vagus nerve[10].

In view of the above it appeared worthwhile to investigate the potential interference of urethane on Ca^{++} mobilization by using two experimental models suitable for testing the effect of Ca^{++} entry blockers in cardiac[25] and vascular smooth muscle[26]. In addition we investigated the potential interference of urethane on isoprenaline-induced chronotropic effects 'in vitro' and in unanesthetized animals.

Methods

In vitro experiments

New Zealand male albino rabbits weighing 2.5–3 kg and male albino guinea-pigs weighing 200–300 g were used. Rabbits received i.v. heparin (1000 IU) and were killed by a blow on the head. Guinea-pigs were killed by a blow on the head and exsanguinated. Solutions used throughout the study (gassed with 95% O_2 and 5% CO_2) had the following composition (mM): Normal Krebs solution: NaCl 119; $NaHCO_3$ 25; Glucose 11; KCl 4.7; $MgSO_4$ 1.5; KH_2PO_4 1.2; $CaCl_2$ 2.5. High K^+ Krebs solution: NaCl 69; $NaHCO_3$ 25; Glucose 11; KCl 54.7; $MgSO_4$ 1.5; KH_2PO_4 1.2; $CaCl_2$ 2.5. High K^+ Ca^{++} free Krebs solution: NaCl 69; $NaHCO_3$ 25; Glucose 11; KCl 54.7; $MgSO_4$ 1.5; KH_2PO_4 1.2 EDTA 0.77. Tyrode's solution: NaCl 115; $NaHCO_3$ 25; Glucose 10; KCl 4.7, $MgSO_4$ 1.2; KH_2PO_4 1.2; $CaCl_2$ 3.6. In order to assess whether or not the effect of high concentrations of urethane (1×10^{-1}–3×10^{-1} M) could be ascribed to an osmotic effect, control experiments were performed with high concentrations of glucose (1×10^{-1}–3×10^{-1} M).

Rabbit ear artery

A 3-cm segment of central ear artery was dissected free from adhering tissues, cannulated at both ends with polyethylene cannulae and transferred to a 7-ml organ bath at 37 °C whose volume was mantained constant by means of an overflow. The arterial segment was perfused intraluminally by means of De Saga 131900 six-channel peristaltic pump at a rate of 5 ml/min while extraluminal perfusion at a rate of 8 ml/min was obtained by means of a Mariotte bottle. Changes in the intraluminal perfusion system were taken as an indirect measure of increase in arterial contraction above the resting tone.

After a 1-h stabilization period the extraluminal and intraluminal normal solution was replaced by a high-K^+ Ca^{++}-free solution which produced a rapid contraction with gradual return to resting values. 5 min later intraluminal perfusion fluid was replaced for 3 min by high-K^+ Ca^{++}-free solution containing noradrenaline (5×10^{-6} M), which produced a contraction followed by a return to resting values[27]. Subsequent intra- and extraluminal perfusion with normal Krebs solution resulted in a further rapid contraction, which depended upon the presence of extracellular Ca^{++} and was related to the concentration of the agonist previously added to the inner perfusion fluid[26].

Peak amplitude and duration of each contraction were estimated. Preliminary experiments showed that a 25-min perfusion period with Krebs solution provided reproducible responses to subsequent challenge with noradrenaline. Modification of the noradrenaline responses was studied by the addition of urethane in the inner perfusion fluid for 3 min before and during noradrenaline challenge as well as for 5 min during the reperfusion with normal Krebs solution.

Under these experimental conditions, no contraction on reperfusion with normal Krebs solution could be observed unless intraluminal noradrenaline had been previously added to the medium[26]. This suggests that the contractile response in segments exposed to noradrenaline on reperfusion with normal Krebs solution is likely to be ascribable to the agonist-induced enhancement in Ca^{++} conductance across the sarcolemma without any participation of Ca^{++} fluxes through Ca^{++} channels activated by K^+ depolarization[28].

In a second series of experiments, after a 1-h equilibration period normal Krebs solution was replaced both intra- and extraluminally by a high K^+ (54 mM) Krebs solution. This produced a phasic followed by a steady tonic contraction. Since in these experimental conditions intraluminal phentolamine (1×10^{-6} M) has no significant inhibitory effect on the tonic component of contraction, this is likely to be entirely sustained by Ca^{++} fluxes through Ca^{++} channels activated by K^+ depolarization[8].

Increasing concentrations of urethane were added in the inner perfusion fluid, the next concentration being added when the effects of the preceding one had reached its maximal relaxant effects.

In some experiments the potential interaction between urethane (5×10^{-2} M) and isoprenaline (1×10^{-6} M) on KCl-induced tonic contractions have been investigated by comparing the relaxant effect of each drug alone and in combination (see fig. 3).

Guinea-pig right atrium

The hearts were rapidly removed and placed in Tyrode's solution at 37 °C. The right atrium was

dissected out and mounted in a 10-ml organ bath maintained at 37 °C under a resting tension of 1 g. The contractions were recorded by means of a MARB F isometric transducer connected to a MARB 776 DC preamplifier and displayed on a Hewlett Packard 7402 A polygraph.

In a first series of experiments, after a 1-h stabilization period the potential effects of urethane on frequency and amplitude of spontaneous contractions were assessed. A cumulative concentration response curve (CRC) to urethane was obtained, the next concentration being added when the effects of the preceding one had reached a steady state. In a second series of experiments the potential interference of urethane (1×10^{-1} M) with the chronotropic effects of isoprenaline have been investigated. After a 1-h equilibration period a cumulative CRC to isoprenaline (3 min for each concentration) was obtained before and after the addition of urethane (incubation period was 10 min).

Guinea-pig right ventricular strips

The hearts were rapidly removed and placed in Tyrode's solution at 37 °C. Right ventricular strips (about 10 mm long and 2 mm wide) were obtained and mounted in a 10-ml organ bath maintained at 37 °C under a resting tension of 1 g. The recording apparatus is described in the preceding paragraph. Strips were point-simulated with square wave pulses of threshold voltage (2 Hz) by means of a stimulator.

In a first series of experiments, after a 1-h equilibration period, the potential Ca^{++} entry blocker properties of urethane were evaluated as described by Mantelli et al.[25]. Normal Tyrode's solution was replaced by a high K^+ (22 mM) Tyrode's solution obtained by an equimolar reduction in NaCl. Under these conditions the preparation rapidly becomes inexcitable due to the inactivation of the fast Na^+ channels[31,39].

As soon as the preparation became quiescent, the rate of stimulation was reduced to 0.2 Hz and the voltage quintuplicated while pulse width remained unchanged. After 5 min of perfusion with high K^+ Tyrode's solution the addition of isoprenaline (1×10^{-6} M) restored the contractility in a few min. It has been proposed that contractions elicited in these experimental conditions are directly proportional to Ca^{++} entry from extracellular space[31,39] and are particularly sensitive to Ca^{++} entry blockers[25]. After a 30-min contact with isoprenaline a cumulative CRC to urethane was obtained, the next concentration being added after a 10-min interval. In a second series of experiments, after a 1-h equilibration period in normal Tyrode's solution the potential interference of urethane with the inotropic effects of isoprenaline was investigated as described for guinea-pig right atria.

In vivo experiments

Male albino rats (Wistar-Morini strain) weighing 180–220 g were used throughout the study. Basal systolic blood pressure (SBP) was measured, at room temperature (22–23 °C) by the tail cuff method[12] in unanesthetized control or anesthetized rats (urethane i.p., 1.2 g/kg, 20 min before). Mean pulse rate (MPR) was derived from the microphone output of the blood pressure recorder (W & W Electronics BP recorder 8005). A 25-gauge 0.5 mm diameter butterfly needle connected by polyethylene tubing to a 1-ml syringe (containing saline plus heparine 10 IU/ml) was inserted into the tail vein for isoprenaline injection. A 20-min stabilization period was required in conscious rats to obtain steady SBP and MPR values. At this point isoprenaline 1 ng/kg in 0.1 ml of warm (37 °C) saline was injected and followed by a rapid washout of the tubing with 0.2 ml of saline. Peak SBP and MPR variations were recorded usually within 30–60 sec after isoprenaline injection.

Thereafter SBP and MPR peak variations to i.v. isoprenaline (10 and 100 ng/kg) were recorded at 5-min intervals. This was done because of differences in time required by the animals to regain pre-drug values. For this reason only the effects of the first dose of isoprenaline (peak variations in SBP and MPR) were used for correlation analysis.

Statistical analysis

Each value in the text is expressed as mean±SE. Statistical analysis of the data was performed by means of Student's t-test for paired or unpaired data, whenever indicated. Regression analysis was performed by means of the least squares method and ED_{50} and relative 95% confidence limits calculated according to Litchfield & Wilcoxon[21].

Drugs

Drugs used were: urethane (Merck), isoprenaline HCl (Serva) and noradrenaline HCl (Fluka). Care was taken to avoid exposure to the light of isoprenaline and noradrenaline solutions (which contained ascorbic acid (0.5 mg/ml)) to prevent catecholamine oxidation.

Results

In vitro experiments

Effect of urethane on noradrenaline induced contractions of rabbit ear artery in high K^+ Ca^{++} free medium and after reperfusion with normal Krebs solution. Exposure to noradrenaline (5×10^{-6} M) in high K^+ Ca^{++} free medium and reperfusion with normal Krebs solution produced typical phasic contractions

(see fig. 1) whose amplitude was 63.1±4.8 and 171.9±4.1 mm Hg (n = 6) respectively. For the sake of convenience these will hereafter be referred to as cellular and extracellular Ca^{++} dependent noradrenaline-induced contractions. Urethane (1×10^{-2}–3×10^{-1} M) produced a concentration-related inhibition of noradrenaline-induced contractions (fig. 2), its ED_{50} and relative 95% confidence limits being 3.01×10^{-2} (1.2–7.4×10^{-2} M) and 1.34×10^{-1} (9.5×10^{-2}–1.9×10^{-1} M) for the cellular and extracellular Ca^{++} dependent contractions respectively. Control experiments with glucose (1×10^{-1}–3×10^{-1} M) indicated that osmotic effects are not involved in the inhibitory action of urethane on cellular Ca^{++} dependent responses. On the other hand a significant inhibitory effect of glucose was observed at 300 mM on extracellular Ca^{++} dependent noradrenaline-induced contractions.

Effect of urethane on KCl induced contractions of rabbit ear artery and its interaction with isoprenaline. Exposure to KCl (54 mM) produced a steady tonic contraction whose amplitude was 129.2±5.2 mm Hg (n = 8). Urethane (1×10^{-2}–3×10^{-1} M) produced a concentration-related inhibition of KCl-induced tonic contraction (n = 4) (fig. 2) its ED_{50} and 95% confidence limits being 1.15×10^{-1} (6.5×10^{-2}–2.1×10^{-1} M). The relaxant effects of urethane disappeared upon its removal from the perfusion medium. Control experiments with glucose indicated that only at high concentrations (3×10^{-1} M) could urethane exert its action through an osmotic effect.

Isoprenaline (1×10^{-6} M) produced a 15.1±1.9% inhibition of KCl-induced tonic contraction (n = 4) which in presence of urethane (5×10^{-2} M) increased to 56.6±3.6% (fig. 3). This value is significantly higher ($p < 0.01$) than the effect of both isoprenaline and urethane (5×10^{-2} M) (13.1±2.1%) when administered alone.

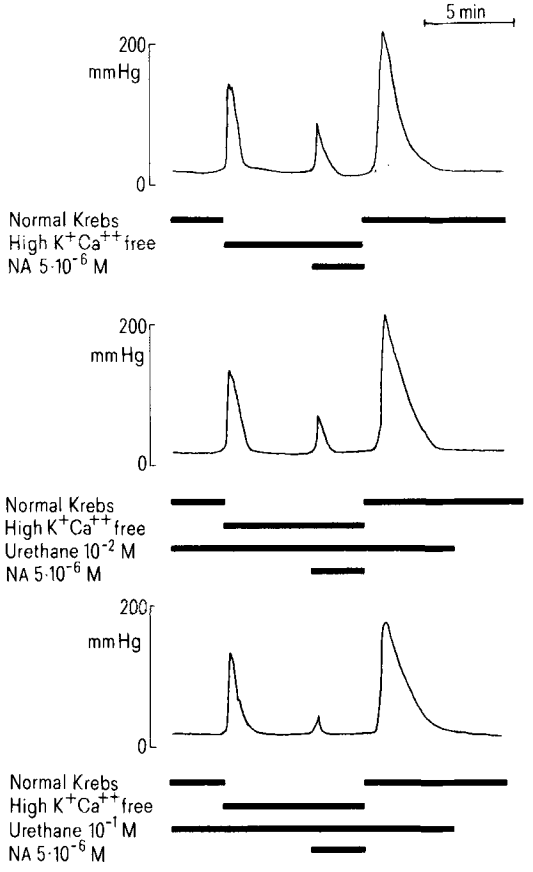

Figure 1. Typical tracing showing the effect of urethane ($1\cdot10^{-2}$–$1\cdot10^{-1}$ M) on cellular and extracellular Ca^{++} dependent NA induced contractions of intraluminally perfused rabbit ear artery. Both urethane and NA were dissolved in high K^+ Ca^{++} free medium.

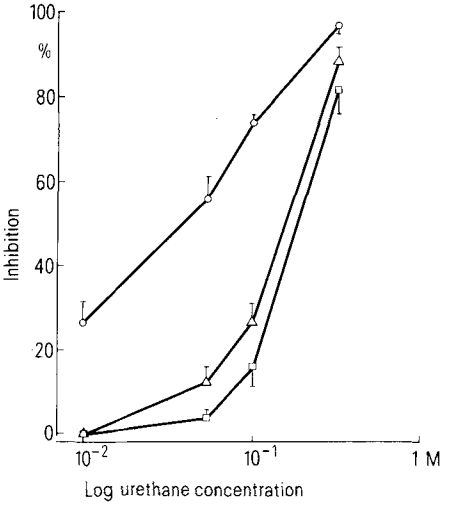

Figure 2. The effect of urethane on the amplitude of cellular (○) and extracellular (□) Ca^{++} dependent NA ($5\cdot10^{-6}$M) and on high K^+ (54 mM) (△) induced contractions of rabbit ear artery. Each point is the mean ± SE of at least 6 experiments.

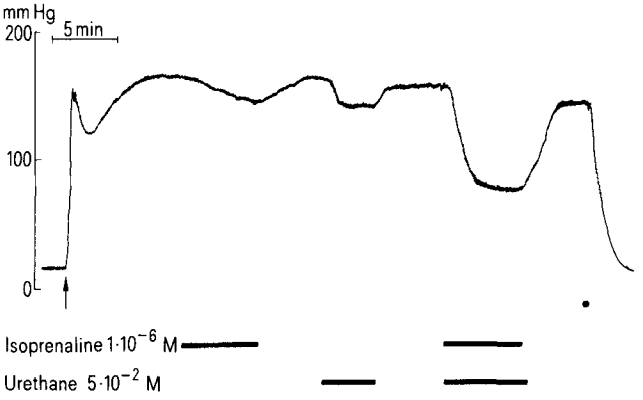

Figure 3. Typical tracing showing the effect on tonic component of high K^+ (54 mM)-induced contraction of rabbit ear artery of isoprenaline 10^{-6}M and urethane $5\cdot10^{-2}$M alone and in combination. Horizontal bars indicated time during which the drugs were present in the intraluminal perfusion solution. Intra- and extraluminal perfusion with high-K^+ solution started at the arrow and finished at the dot.

Effect of urethane on frequency and amplitude of contractions of spontaneously beating guinea-pig right atria. Urethane (1×10^{-2}–1×10^{-1} M) produced a concentration-related inhibition of both frequency and amplitude of contractions in spontaneously beating guinea-pig right atrium (fig. 4), its maximal inhibitory effect on amplitude (71.2±2.7% at 10^{-1} M, n = 6) being significantly ($p < 0.001$) higher than that on frequency (18.7±2.1%). Urethane 3×10^{-1} M suppressed cardiac beat. This effect disappeared promptly upon removal of urethane. Urethane ED_{50} and 95% confidence limits on amplitude of contractions resulted to be 4.93×10^{-2} (4.5–5.4×10^{-2}). Control experiments indicated that glucose (3×10^{-1} M) had only minor effects on frequency and amplitude of contraction of guinea-pig right atria.

Effect of urethane on isoprenaline-induced contractions of guinea-pig right ventricle strips in high K^+ medium. Urethane (1×10^{-2}–3×10^{-1} M) produced a concentration-related inhibition of isoprenaline (1×10^{-6} M) induced contractions in a high K^+ (22 mM) medium of electrically driven strips of guinea-pig right ventricle (n = 6, fig. 4). Its ED_{50} and 95% confidence limits resulted to be 1.25×10^{-1} M (9.96×10^{-2}–1.57×10^{-1} M). Control experiments with glucose (1×10^{-1}–3×10^{-1} M) indicated that osmotic effects cannot be held responsible for urethane antagonism toward isoprenaline induced contractions of guinea-pig right ventricular strips in high K^+ medium.

Effect of urethane on chrono and inotropic effects of isoprenaline CRCs on guinea-pig right atrium and right ventricle strips. Urethane (1×10^{-1} M) had no significant effect on isoprenaline-induced chronotropic (n = 5, fig. 5A) and inotropic (n = 5, fig. 5B) effects on either guinea-pig right atrium or ventricular strips.

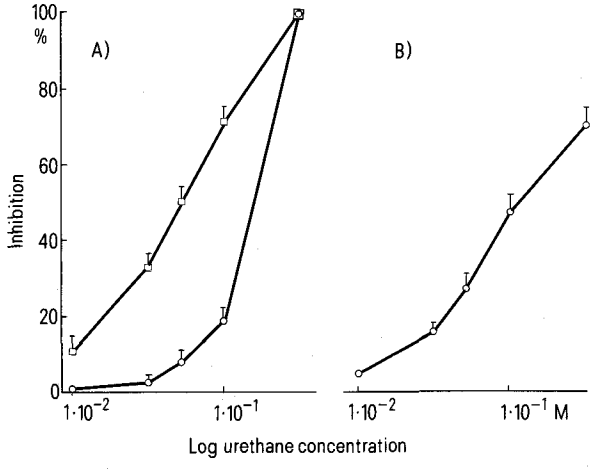

Figure 4. *A* Concentration dependent inhibition by urethane of frequency (circles) and amplitude (squares) of spontaneously beating guinea-pig right atria. *B* Concentration dependent inhibition by urethane of amplitude of contractions elicited by isoprenaline ($1\cdot10^{-6}$M) in depolarized guinea-pig ventricular strips. Each point is the mean ± SE of at least 6 experiments.

In vivo experiments

Resting SBP and MPR values in unanesthetized rats were 133.3±1.6 mm Hg and 370.6±4.5 beats/min respectively (n = 38). Urethane-anesthetized rats (n = 32) had significantly lower ($p < 0.001$) SBP (96.0±3.1 mm Hg) and MPR (318.7±5.2 beats/min) values as compared to controls (fig. 6).

Isoprenaline (i.v., 1–100 ng/kg) produced a significant dose-related increase in MPR in both groups but significantly lowered SBP only in urethane-pretreated animals at the higher dose tested (fig. 6). MPR increase produced by the lower and the intermediate dose of isoprenaline were significantly higher in ure-

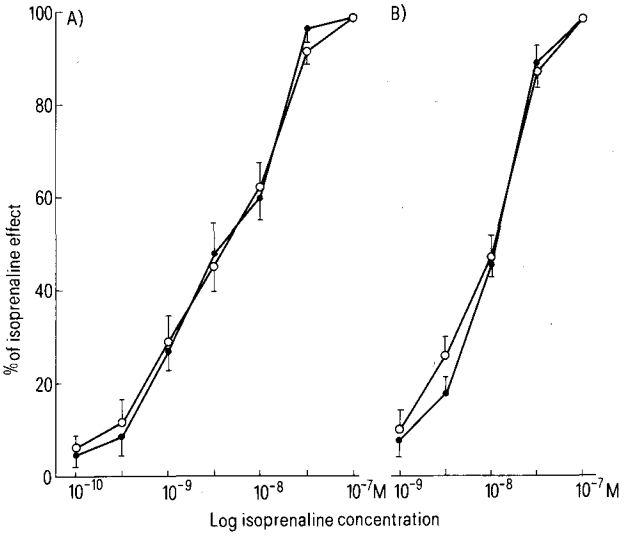

Figure 5. Effect of urethane on chrono- (A) and inotropic (B) activity of isoprenaline on guinea-pig right atrium and ventricular strips respectively. ○, Control; ●, after urethane ($1\cdot10^{-1}$M). Each point is the mean ± SE of at least 6 experiments.

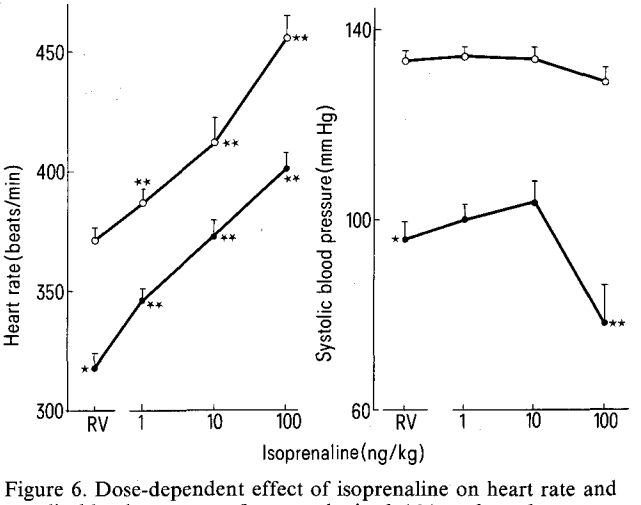

Figure 6. Dose-dependent effect of isoprenaline on heart rate and systolic blood pressure of unanesthetized (○) and urethane-pretreated (●) rats. RV; resting value. Each point is the mean ± SE of 38 and 32 animals respectively. * Significantly different ($p < 0.05$) from the corresponding resting values of unanesthetized rats; ** significantly different ($p < 0.05$) from the respective resting values.

thane as compared to unanesthetized rats (fig. 7). A significant correlation exists between resting MPR values and the chronotropic effects of isoprenaline (1 ng/kg) in urethane-treated but not in unanesthetized rats (fig. 8).

Discussion

Urethane has been reported to antagonize Ca^{++}-induced contractions of K^+ depolarized vascular[1] and tracheal[24] smooth muscle. Interference with Ca^{++} availability for contraction has been proposed as the cellular mechanism(s) underlying its vasodepressant and hypotensive actions in the intact animal[1,2].

We have observed that urethane, in concentrations lower than 5×10^{-2} M, abolished noradrenaline-induced contractions in high K^+ Ca^{++} free medium, thus revealing its ability to inhibit mobilization of intracellular Ca^{++} pool(s) from tightly bound storage sites[26,27]. Since this inhibitory action of urethane (presumably related to its ability to cross the plasma membrane[17]), occurs in concentrations quite similar to those ($1.5\text{–}2.5 \times 10^{-2}$ M) found in plasma during urethane anesthesia[6,7,9], it could be hypothesized that

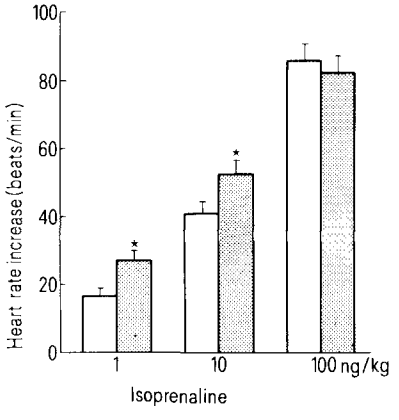

Figure 7. Dose-dependent increase of heart rate by isoprenaline in unanesthetized (open columns) and urethane pretreated (dashed columns) rats. Each value is the mean ± SE of 38 and 32 animals respectively. * $p < 0.05$.

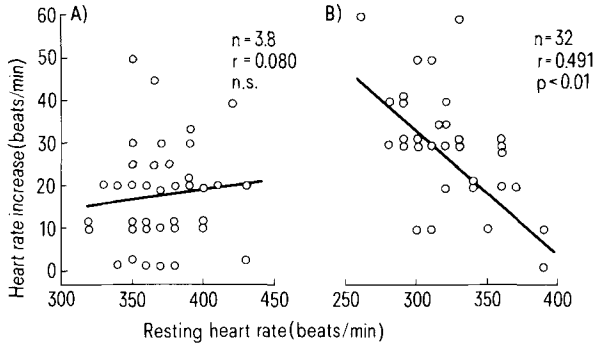

Figure 8. Correlation between resting heart rate and heart rate increase in unanesthetized (A) and urethane-pretreated (B) rats. Regression line for B: $y = 443{,}8713 - 71{,}7407\,x$.

urethane inhibition of intracellular Ca^{++} mobilization is responsible for its in vivo vasodepressant and hypotensive effect. Interestingly enough, Altura and Weinberg[1] observed that urethane even in concentrations lower than 5×10^{-2} M antagonized angiotensin II-induced contractions of rat aorta and portal vein more effectively than those produced by adrenaline or KCl. This is in line with the observation that angiotensin II-induced contractions of vascular smooth muscle are highly dependent upon the mobilization of intracellular Ca^{++} pools[15,18,42].

Urethane exerted a negative inotropic effect on spontaneously beating guinea-pig right atria at concentrations that did not significantly affect their frequency, and at higher concentrations suppressed cardiac beat. An inhibitory effect on mobilization of Ca^{++} from intracellular storage sites by urethane at concentrations lower than 5×10^{-2} M could explain our observation on guinea-pig right atrium.

This hypothesis agrees well with the multicompartmental model of cardiac excitation contraction coupling which postulates that transmembrane Ca^{++} influx (slow inward current) does not play a major role in the activation of the contractile proteins, but triggers the release of Ca^{++} from the sarcoplasmic reticulum which, in turn, is the bulk of the activator Ca^{++} for the development of the contractile strength[19,43]. Our results indicate that in concentrations higher than 5×10^{-2} M urethane is almost equieffective in antagonizing contractions sustained by Ca^{++} influx from the extracellular space at both vascular and cardiac level.

The inhibitory action of urethane on isoprenaline-induced contractions of K^+ depolarized guinea-pig right ventricular strips could be ascribed to a 'Ca^{++} entry blocker' - like mode of action.

On the other hand, an osmotic effect appears to be responsible for the action exerted by urethane in high concentrations on rabbit ear artery. Therefore, since, at least for the higher concentrations, an osmotic effect appears to be involved, no firm conclusion can be drawn as to whether or not a 'Ca^{++} entry blocker' mode of action plays a role in the actions of this anesthetic at vascular level.

Barrett[5] observed that higher doses of i.v. administered isoprenaline were required to produce the same cardiac chronotropic response in urethane- than in pentobarbitone-anesthetized rats. The observation that the chronotropic response to isoprenaline is depressed in urethane- as compared to barbiturate-anesthetized rats was confirmed and extended by our own findings[23].

However, in vitro experiments on guinea-pig cardiac preparations indicate that urethane, in a concentration (1×10^{-1} M) 4–6 times higher than those observed in plasma during anesthesia, does not inhibit either chrono or inotropic effects of isoprenaline. In

addition in vivo experiments indicate that the chronotropic response to isoprenaline is enhanced in urethane-anesthetized as compared to unanesthetized rats and confirm our previous findings[23] indicating that, in urethane-anesthetized animals, the magnitude of the chronotropic responses to isoprenaline is inversely related to resting heart rate values.

Urethane anesthesia is known to produce a variety of metabolic and/or endocrine effects such as increase in corticosterone levels[36], catecholamine release from adrenal medulla[37], increased adrenaline plasma levels[4,34,37] hypocalcaemia[32], hemoconcentration and hyperglycaemia[41], increase in plasma renin activity[20,33], and increase in plasma prolactin levels[14]. These, alone or in combination might have profound and unpredictable effects on cardiovascular responsiveness to exogenous agents both directly and through an alteration of homeostatic cardiovacular reflexes.

In view of the above the lack of any direct effect of urethane on both spontaneous and stimulated cardiac chronotropism in vitro does not allow any interpretation of the effects of urethane anesthesia in terms of simple interrelationship between the anesthetic and isoprenaline at sinoatrial node level. By analogy, the observation that urethane-anesthetized rats have lower resting heart rate values than either barbital-anesthetized[23] or unanesthetized rats suggests an indirect effect of urethane on cardiac function in vivo.

This is supported by the observation that under urethane anesthesia there is a high level of autonomic nervous system control on heart function as illustrated by the marked chronotropic effects of β-blockade[5], atropine or vagotomy[5,22]. In addition, reserpine pretreatment has been reported to abolish the dependence of isoprenaline chronotropic effects from resting heart rate in urethanized rats[23].

Finally our in vivo results indicate that the vasodepressor effect of isoprenaline is somehow potentiated by urethane anesthesia as compared to unanesthetized rats. The fall in systolic blood pressure could not be simply interpreted in terms of an increased vasodilator effect of isoprenaline under urethane anesthesia. However in vitro experiments are suggestive that some sort of positive interaction might exist in the relaxant properties of isoprenaline and urethane at vascular level.

In conclusion our findings indicate that: a) urethane at concentrations similar to those observed during anesthesia interferes with the mobilization of Ca^{++} from intracellular storage sites in vascular smooth muscle; b) only at higher concentrations does urethane inhibit contractions sustained by a influx of Ca^{++} from the extracellular space; c) urethane possesses a direct depressant action on cardiac contractility which could be related to its effects on Ca^{++} mobilization; d) urethane does not interfere directly with either chrono or inotropic effects of isoprenaline in vitro and e), an indirect effect of urethane anesthesia makes the chronotropic response to isoprenaline dependent upon resting heart rate values.

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Electrophoretic patterns of hemoglobin in different *Xenopus* species, subspecies and interspecies hybrids[1]

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Summary. Hemoglobins of 12 diploid and polyploid species, 6 subspecies and 15 different inter-species hybrids of the genus *Xenopus* were compared by electrophoresis on polyacrylamide gels or by isoelectrofocusing. Multiple hemoglobin bands were detected in all taxa. - Each species can be identified by its specific hemoglobin pattern, in spite of the intraspecific polymorphism observed within 5 of the 12 species. In contrast to the observed interspecific variation, the hemoglobin pattern of the six subspecies of *Xenopus laevis* is almost invariable and does not allow an unequivocal identification of these taxa. - Hemoglobin patterns of all interspecies hybrids represent the sum of those of their parental species. In spite of this codominant expression of all parental hemoglobins, no hybrid molecules containing globins of both parents can be detected.

Introduction

The different species and subspecies of the African clawed toad, genus *Xenopus,* represent from an evolutionary point of view a most interesting group of lower vertebrates. *Xenopus tropicalis,* considered to be the most ancient extant species of the genus[2], has a mitotic chromosome number of $2n = 20$[24]. *X. epitropicalis,* a newly described species[7], has a mitotic chromosome number of $2n \mp 40$ and is, therefore, tetraploid and, moreover, closely related to *X. tropicalis* with respect to its morphology and karyotype[28]. Numerous species, amongst them *X. laevis,* which is