Review

Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 2: Cardiovascular system

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Summary. Urethane produces a level of surgical anesthesia characterized by preservation of a number of cardiovascular reflexes. When the proper route of administration is used, and the use of unnecessarily high doses is avoided, urethane anesthesia appears to be suitable for a number of investigations at cardiovascular level. However in certain types of studies involving pharmacological stimulation of peripheral adrenoceptors urethane affects markedly the magnitude of the response under study.
Key words. Urethane; anesthesia; physiology; pharmacology; in vivo experiments; reflexes.

In the first part of this review[35] we examined some general principles which should be taken into account when considering the suitability of urethane (as well as of any other anesthetic) for physiopharmacological experimentation. In this section we shall focus our attention on the suitability of urethane anesthesia for experiments at cardiovascular system level.

It is widely accepted that anesthetics influence, to varying degrees, the resting function of the cardiovascular system as well as its responsiveness to both exogenous and endogenous activation.

Urethane (as compared to other general anesthetics) produces a condition of surgical anesthesia characterized by a high degree of activity of the autonomic nervous system in controlling the cardiovascular function. This statement is substantiated by the observation that administration of autonomic blocking drugs (atropine, hexamethonium, propranolol or phentolamine) to urethane-anesthetized animals produces prompt and marked variations in heart rate, blood pressure and cardiac contractility[5, 19, 29, 30, 33, 46]. These observations indicate that, under urethane anesthesia, both parasympathetic and sympathetic divisions of the autonomic nervous system are tonically active in controlling resting cardiovascular parameters. It appears that urethane anesthesia is characterized (as compared to other anesthetics) by a fairly good preservation of various cardiovascular reflexes. Unfortunately many relevant studies on the effects of urethane on cardiovascular function have been performed using mixture(s) (in various proportions) of urethane and chloralose[25, 47, 54]. Our attention will be directed to studies in which data obtained under urethane anesthesia have been compared to those obtained in conscious, decerebrated or anesthetized (barbiturate, chloralose, ether, etc.) animals.

Effect of urethane on resting cardiovascular function

Urethane anesthesia, depending upon dose, route of administration and species can either reduce or have no effect on blood pressure; heart rate may be reduced, unaffected or even increased as compared to controls[6, 15, 17, 18, 20, 27, 29, 32, 43, 52]. These discrepancies have led to disparate conclusions about the suitability of urethane anesthesia for physiopharmacological investigation at cardiovascular level.

Giles et al.[18] reported, in urethane-anesthetized dogs (1.5–2.0 g/kg i.p.), a progressive decline of various cardiovascular parameters (stroke volume, blood pressure, etc.) during a 5-h observation period, along with an increase in heart rate.

Bell et al.[6] reported that, in rats, urethane (1.4 g/kg i.p.) does not affect either blood pressure or cardiac output but that renal, intestinal and hepatosplanchinic blood flow are reduced. It is well known that i.p. urethane produces toxic effects on mesenteric vasculature and intrabdominal organs[53, 56]. Taken together these findings contraindicate the i.p. route of administration for this anesthetic, unless specific reasons for its use are present[35].

Sapru and Krieger[52] reported that the i.v. administration of an anesthetic dose of urethane (0.75 g/kg) to decerebrated rats produces only a transient (5–15 min) reduction of blood pressure and heart rate.

Leenen and Provost[27] showed that administration of saralasin, a competitive antagonist of angiotensin II, decreased markedly blood pressure in urethane-anesthetized rats (1.25 g/kg i.p.) suggesting that an increase of plasma renin activity is essential for the maintenance of a normal blood pressure level in these animals. De Wildt et al.[45] reported that, in rats, urethane anesthesia (1 g/kg i.p.) induces a mild hypotension and reduction of cardiac output while various other hemodynamic parameters are unaffected.

In our hands i.p. urethane (1.5 g/kg) lowered significantly (as compared to unanesthetized animals) resting heart rate and systolic blood pressure[32]. These changes are much less evident or even absent in rats receiving s.c. urethane[29]. Taken together these findings demonstrate the importance of using, by the proper route of administration, the lowest dose necessary to obtain surgical anesthesia[35] to minimize the effects of the anesthetic on resting cardiovascular function.

Effect of urethane on microcirculation

Hodoval et al.[20] studied the effect of urethane (0.8 g/kg i.p.) on the s.c. microvasculature of the bat. In this species, urethane produced a 38% decrease in blood pressure and highly variable changes in heart rate. However, urethane induces only a moderate dilatation (about 15% increase above initial diameter) of small arteries while it had no effect on small veins[28]. The same authors reported

that the reflex response to carotid occlusion is well preserved, at microcirculatory level, in urethane-anesthetized bats[28].

The constrictor response of small arteries and veins of the rat cremaster muscle to topical noradrenaline was studied under urethane (1.2 g/kg i.p.) anesthesia[43]. This study revealed a lower sensitivity to topical noradrenaline in urethane – as compared to pentobarbital – or urethane plus chloralose-anesthetized animals[43].

Taken together these studies led to the conclusion that urethane might be the anesthetic of choice for experiments involving direct observation of the microcirculation as well as reflex changes in diameter of small vessels[28].

Effect of urethane on cardiovascular reflexes

As outlined in the first part of this review[35] one reason for the popularity of urethane as an anesthetic stems from the fact that, as compared to other anesthetics, it produces only little depression of various reflex responses[4, 5, 8, 9, 11, 12, 16, 17, 19, 22–24, 26, 30, 33, 44, 49, 50, 52].

Prochnik et al.[50] reported that the pressor response to carotid occlusion is greater in urethane – than pentobarbital – or ether-anesthetized dogs. Similar findings were obtained by us in urethane – as compared to sodium barbital – anesthetized rats[29].

Laporte and Montastruc[26] studied the pressor response to electrical stimulation of cutaneous sensory nerves in decerebrated as compared to urethane-anesthetized cats. Stimulation of delta fibers has a pressor effect in decerebrated animals but, under urethane anesthesia, a depressor response was observed[26]. The pressor response produced by low-frequency stimulation in decerebrated cats was at first reduced and then converted to a depressor episode by administration of low doses of urethane[26].

In cats urethane (1.0–1.8 g/kg) produces a dose-related depression of the hypertensive response to electrical stimulation of the superior laryngeal nerve, while a high dose (2 g/kg) of this anesthetic does not modify the pressor response to electrical stimulation of the splanchnic nerve[4]. Deepening of urethane anesthesia produced by increasing the dose from 1.0 to 1.9 g/kg does not modify the pressor response to carotid occlusion nor the depressor response to distension of the carotid sinus[4].

Barrett showed that, in urethane anesthetized rats (1.5 g/kg i.p.) consistent, hexamethonium-sensitive, cardiac chronotropic responses are elicited by application of painful stimuli[5]. In the same study it was observed that either bradycardic or tachycardic responses could be obtained following electrical stimulation of the appropriate hypothalamic nuclei in urethane- but not barbiturate-anesthetized rats[5].

Bunag and Mullenix[12] reported a marked reduction of intensity of the noradrenaline-induced reflex vagal bradycardia in urethane-anesthetized as compared to conscious rats. This effect was apparently due to the marked reduction of the pressor effect of noradrenaline and higher doses of the amine were not tested. Brezenoff[8] reported that, in urethane-anesthetized (1.3 g/kg i.p.) rats, the magnitude of the noradrenaline-induced reflex bradycardia was unchanged or strongly blunted in animals having high or low values of resting heart rate, respectively.

In rats receiving either i.p. or s.c. urethane (1.2–1.5 g/kg) we observed consistent bradycardic responses of vagal origin, providing that adequate pressor doses of adrenaline or noradrenaline were given[19, 30, 33].

In a recent study the bradycardic response to phenylephrine was studied in conscious rats as compared to urethane- (1.6 g/kg i.m.), chloralose- or barbiturate-anesthetized animals[17]. The main conclusion of this study seems to be that all anesthetics depress to some extent the baroreceptor reflex. However, only one dose of each anesthetic was used and no account is taken of the potential influence of different anesthetic depths. With regard to urethane it was found that this anesthetic induces a fivefold attenuation (as compared to conscious animals) of the bradycardic response. This effect is in part attributable to a reduced intensity of the pressor response to phenylephrine[17].

Taken together these findings indicate that a reflex bradycardia of vagal origin can be easily obtained in urethane-anesthetized rats providing that an adequate pressor stimulus is given.

In a study comparing the effects of various anesthetics on cardiovascular functions in decerebrate rats Sapru and Krieger[52] reported that an anesthetic dose of i.v. urethane (0.75 g/kg) depresses the cardiovascular response to 'tilt' and carotid occlusion. A similar degree of depression was observed following administration of subanesthetic doses of pentobarbital or ketamine.

Lalley[24] studied the effects of various anesthetics on both pressor and depressor cardiovascular responses produced by activating afferent fibers in the carotid sinus nerve (CSN) aortic nerve (AN) and vagus nerve (VN). Urethane induces a depression of both pressor and depressor responses to CSN stimulation and severely reduced the responses to AN and VN stimulation. However the depressant effect of urethane was unselective (i.e. both pressor and depressor responses were affected in a similar manner) while other anesthetics (thought to act by mimicking or enhancing the effect of GABA at CNS level) selectively abolish the depressor responses and converted them to pressor episodes[24].

In conclusion a wide range of evidence indicates that urethane anesthesia provides a suitable condition for acute studies concerned with the physiopharmacology of various reflex responses at cardiovascular level. The intensity of certain reflex responses may be depressed, as compared to conscious animals, by urethane. The relevance of factors such as total dose administered and/or route of administration on the quality and intensity of cardiovascular reflex responses in urethane-anesthetized animals has been poorly studied. Providing that unnecessarily high doses of this anesthetic are not administered, urethane interferes less than other commonly used anesthetics with various cardiovascular reflexes and, particularly, is devoid of certain GABA-mimetic properties[35] which could produce significant changes or even suppress reflex responses at cardiovascular level.

Effect of urethane on neurohormone-induced contractions of isolated blood vessels

It has been known for a long time that urethane anesthesia may produce hypotension. However the direct effect

of this anesthetic on spontaneous or stimulated contractility of isolated blood vessels has not been investigated until recently[1,2,32]. Previous findings indicate that, under urethane anaesthesia, vasoconstrictor responses to noradrenaline[8,12,43,48] tyramine[48] or angiotensin II[8,12,48,59] are depressed as compared to conscious animals[8,12,48] or preparations receiving other anesthetics[8,12,43,48,59].

Altura and Weinberg[2] reported that urethane reduces, at various degrees, neurohormone- (adrenaline, angiotensin II) or KCl-induced contractions of rat aorta and portal vein. Moreover urethane reduces the Ca^{++}-induced contractions of K^+-depolarized preparations[2]. This latter finding might suggest that urethane, at least in high concentrations (50–100 mM), interferes with transmembrane Ca^{++} fluxes through the potential-operated Ca^{++} channels (POCs)[7]. This hypothesis is suggested also by the observation that urethane reduces: a) the tonic component of the KCl-induced contractions of the isolated rabbit ear artery[32] and detrusor muscle[38]; b) Ca^{++}-induced contractions of the K^+-depolarized guinea pig trachealis[37] and c) KCl-induced rhythmic contractions of the guinea pig ureter[36]. These effects could be due to an 'unspecific' interference of urethane with Ca^{++} channel function rather than to a true Ca^{++} entry blocker-like mode of action of this anesthetic. This hypothesis is supported by the observation that depression of contractile responses sustained by a transmembrane Ca^{++} influx through POCs was observed at high (50–300 mM) concentrations of urethane[32,37,38] and depended at least in part, upon an osmotic effect[32]. In any case it appears unlikely that urethane interference with transmembrane Ca^{++} fluxes is involved in producing the hypotensive effect in the intact animal since it occurs only in concentrations higher than the anesthetic ones[32].

On the other hand urethane reduces, at concentrations lower than 50 mM, noradrenaline-induced contractions in a high K^+Ca^{++}-free medium containing EDTA of the rabbit isolated ear artery[32]. These experiments suggest that urethane affects mobilization of Ca^{++} from intracellular storage sites[31,40,41] at concentrations lower than those required to interfere with KCl- or neurohormone-induced transmembrane Ca^{++} fluxes[32].

Taken as a whole these studies provide evidence indicating that urethane has, at least at certain concentrations, a direct depressant action on vascular smooth muscle contractility.

Urethane anesthesia and the cardiac chronotropic response to isoprenaline

In the course of screening experiments searching for new beta adrenoceptor blockers we recognized that the intensity of the cardiac chronotropic response to a fixed, maximally effective, dose (0.15 µg/kg) of isoprenaline (ISO) depended, in urethane-anesthetized rats (1.5 g/kg i.p.), upon individual resting heart rate, i.e. the lower the resting heart rate, the greater was the chronotropic response to i.v. ISO[34]. This observation led to the conclusion that urethane anesthesia is unsuitable for testing the potential beta blocking properties of new molecules against a fixed dose of ISO because any substance capable of increasing the resting heart rate values 'is liable to reduce the magnitude of the ISO-induced tachycardia'[34].

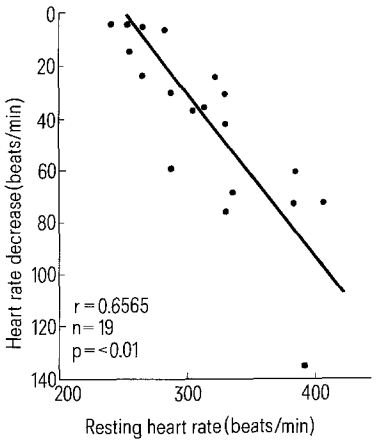
In a subsequent paper we compared the cardiac chronotropic effects of various doses of ISO in unanesthetized and urethane-anesthetized animals (1.5 g/kg i.p.)[32]. We found that the magnitude of the cardiac chronotropic response to ISO also depends upon resting heart rate at doses (1 ng/kg) which produce $\frac{1}{4}$–$\frac{1}{5}$ of the maximal chronotropic response[32]. Our findings were confirmed by Brunner and Poch[10] in rats receiving a mixture of i.p. (0.75 g/kg) and s.c. (0.5 g/kg) urethane. In addition Brunner and Poch[10] observed that, if a complete dose-response curve to ISO is obtained in each animal, the effectiveness of ISO (expressed as ED 50) in producing a chronotropic response is independent of resting heart rate. This observation suggests that the affinity of cardiac beta 1 adrenoceptors is unchanged by urethane[10] as indicated also by our in vitro experiments[32]. It appears that urethane-anesthetized animals could be used for testing potential beta blockers, providing that complete dose-response curves to ISO are obtained in each animal[10].

The reason why urethane makes chronotropic responses to ISO dependent on resting heart rate values is unclear. In vitro experiments indicate that urethane does not affect either chrono or inotropic effects of ISO on isolated cardiac preparations[32]. Brunner and Poch[10] argued that 'a direct dependence of Δ-values on pre-drug baseline values will be encountered for merely theoretical reasons in all cases where an absolute maximum biological response cannot be surpassed'. They proposed that such a mechanism might be involved in the genesis of the urethane-induced frequency dependence of cardiac chronotropic responses to ISO, possibly because of the large dispersion in resting heart rate values observed in urethane-anesthetized rats[10]. However the frequency dependence of cardiac chronotropic response was observed also in response to a submaximally effective dose of ISO[32].

We also observed that sodium barbital anesthesia (200 mg/kg i.p.) produces a dispersion of resting heart rate values similar to that produced by i.p. urethane[29] but no frequency dependence of the chronotropic responses to ISO was observed in barbiturate-anesthetized animals[34]. Two additional observations are that the ISO-induced chronotropic responses are independent of resting heart rate in a) reserpine-pretreated urethane-anesthetized animals[34] and b) rats anesthetized with s.c. urethane (1.2 g/kg)[29].

It is well known that i.p. urethane activates the sympathetic outflow from the CNS to the periphery[3,35,51]. It might be speculated that such effects are involved in the genesis of the frequency dependence of the cardiac chronotropic responses to ISO[10,32,34]. In rats anesthetized with i.p. urethane the resting heart rate is strongly influenced by the sympathetic tone, as indicated by the marked and frequency-dependent negative chronotropic effect of i.v. propranolol (fig.).

It should be noted that, in rat atria, activation of prejunctional beta adrenoceptors can increase the release of noradrenaline[39,42]. Therefore it is conceivable that, in vivo, the size of the cardiac chronotropic response to ISO may be augmented by the release of endogenous noradrenaline from sympathetic nerve endings. The facilitatory effect consequent to activation of prejunctional beta adrenoceptors on the release of noradrenaline are frequency-dependent, i.e. the greatest efflux of endoge-



Frequency dependence of the negative chronotropic effect of i.v. propranolol (0.2 mg/kg) in urethane-anesthetized (1.2 g/kg s.c.) rats. The magnitude of the negative chronotropic effect (measured at steady state, 5 min after injection) was inversely related to initial chronotropic values. The preparation was made as described by Maggi and Meli[34]. Mean values of resting heart rate and propranolol-induced bradycardia were 314 ± 42 and 41 ± 7 beats/min, respectively.

nous noradrenaline is produced at the lowest frequencies of stimulation[13, 39].

Since the negative chronotropic effect of propranolol in i.p. urethane-anesthetized rats was related to resting heart rate (fig.) we could assume that, in these animals, the preparations having the lowest resting heart rate represent those exhibiting the lower degree of activation of the sympathetic nervous system. If this were true then it might be expected that, in these animals, activation of prejunctional beta adrenoceptors by ISO produces the greatest release of endogenous noradrenaline thereby contributing to a greater extent to the overall chronotropic response. This hypothesis, which needs further evaluation, would account for the observation that, in reserpine-pretreated animals anesthetized with i.p. urethane the chronotropic responses to ISO does not depend upon resting heart rate values[34].

Urethane anesthesia and cardiovascular responses mediated by α_2 adrenoceptors

In the past few years evidence has been provided indicating that anesthesia induced by i.p. urethane (1.2–2.0 g/kg) reduces the bradycardic response consequent to administration of α_2-adrenoceptor agonists[3, 14, 45]. These studies were prompted by the observation that oxymetazoline, a selective α_2-adrenoceptor agonist, does not produce bradycardia in urethane-anesthetized rats[84]. Since oxymetazoline produces bradycardia in barbiturate-anesthetized animals[55], the hypothesis was advanced that urethane anesthesia interferes with the prejunctional α-adrenoceptors located on cardiac sympathetic nerves.

In two of these studies[3, 14] this adverse effect of urethane anesthesia was paralleled by increased plasma levels of adrenaline released from the adrenal medulla[3, 14]. This led to the proposal that, in urethane-anesthetized rats, the prejunctional α-adrenoceptors could be maximally activated because of the elevated plasma levels of adrenaline[14].

Armstrong et al.[3] investigated, in pithed rats, the influence of i.p. urethane (1.2 g/kg) on the pressor responses induced by a variety of α-adrenoceptor agonists, angiotensin II and serotonin. These authors reported that urethane has a somehow selective depressant action on responses induced by the activation of postjunctional α_2-adrenoceptors. Since in pithed rats pressor responses induced by α_2-adrenoceptor agonists are particularly sensitive to blockade by Ca^{++} entry blockers[57, 58] it was proposed that a verapamil-like mode of action accounts for the urethane-induced depression of pressor responses activated by neurohormones[3].

The mechanism(s) responsible for urethane interference with cardiovascular changes produced by α_2-adrenoceptor agonists are unclear. All studies on this topic have been performed in i.p. urethane-anesthetized animals so that it is not clear to what extent this adverse effect of urethane anesthesia depends upon the route of administration. The importance of using the proper route of administration to avoid certain adverse effects was outlined in the preceding section of this review[35].

Effect of urethane on isolated cardiac preparations

Only limited information is available concerning the direct effects of urethane on isolated cardiac preparations. In our hands urethane had little effect, unless at high concentrations (at least 30 mM) on frequency and contractile force of spontaneously beating guinea pig isolated right atria and ventricular strips[32]. These findings provide a basis for the notion that urethane anesthesia does not produce a significant depression of certain cardiovascular parameters such as heart rate and cardiac contractility[6, 15, 52].

Suitability of urethane anesthesia for physiopharmacological studies at cardiovascular level

Great caution is needed in interpreting data from urethane-anesthetized animals in relation to their physiological relevance. However, urethane anesthesia appears to be suitable for various types of physiopharmacological investigations at the cardiovascular level. For certain types of investigations, such as those concerned with GABAergic control of cardiovascular function, urethane anesthesia is probably the most suitable, as compared to other anesthetics, providing that unnecessarily high doses are not administered. For certain types of pharmacological investigation involving stimulation of α_2[3, 14, 45] or β[10, 32, 34] adrenoceptors, urethane anesthesia interferes with the magnitude of the response attainable. This adverse effect may result in misleading conclusion about the effects of drugs on the parameters under study. Although this could not be considered as an absolute contraindication to the use of urethane in this type of study, there is evidence that other types of anesthesia do not have these drawbacks.

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Short Communications

Protection by iloprost of the myocardial contractility and rhythmicity in frog ventricular strips[1]

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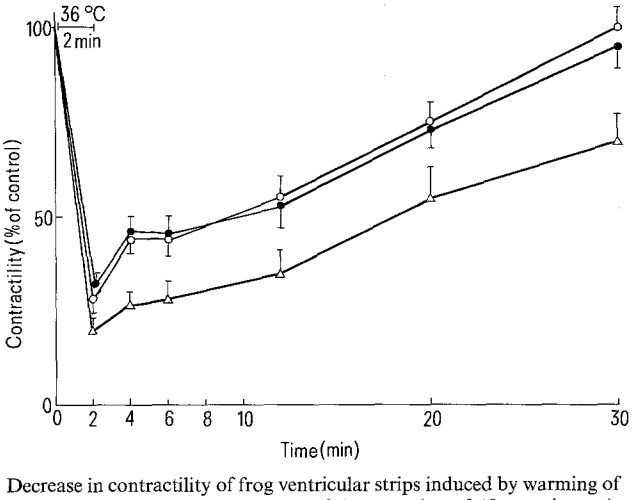
Summary. Incubation of frog ventricular strips with Ringer containing iloprost for 24 h at 4 °C can protect the contractility and rhythmicity from heat stimulation and aconitine when compared with strips incubated in Ringer alone under the same experimental conditions.
Key words. Iloprost; frog ventricular strips; contractility; tissue protection; aconitine; warming.

Iloprost has recently been synthetized as a new stable analog of prostacyclin (PGI_2). This new compound has been shown to have a profile of action similar to natural PGI_2 in various pharmacological preparations[2,3]. Natural PGI_2 has been described to protect myocardium from acute ischemia[4,5]. We have recently described an antiarrhythmic effect of iloprost against digoxin-induced ventricular extrasystoles in anesthetized guinea pigs and isolated Langendorff-perfused hearts from the same species[6]. In ongoing studies we have also shown the antidisrhythmic effect of iloprost in the isolated whole heart, spontaneously beating right atria from rabbit whole heart and spontaneously beating atrial and ventricular strips from frog[7]. The results reported in the present paper show that iloprost, when incubated with the tissue, protects for 24 h the contractility and rhythmicity of the isolated spontaneously frog ventricular strips.

Materials and methods. The experiments were carried out on ventricular strips from frog *(Rana ridibunda)* of either sex weighing 45–65 g, during the winter season (January–February). After decapitation the heart was quickly removed and placed in a frog Ringer solution at room temperature (21 °C). Atrium was carefully separated from ventricle and discarded. Ventricle was spirally cut through the atrioventricular margin to the apex with 4 mm width and 2 cm length. Both tips were carefully tied and suspended in a jacketed bath and superfused with oxygenated (5% CO_2 in O_2) Ringer solution (15 ml/min) at 21 °C. 1.0 g initial tension was applied and the isometric contractions were recorded on a Grass polygraph (Model 79 D) via a force-displacement transducer (Grass FT.03). Arrhythmias were followed from mechanical activity on the recorder and consisted of multifocal single or repetitive premature ventricular beats and the change in interval between contractions. The onset of ectopic beats and fibrillation were determined. Arrhythmias were produced by heating the medium from 21 °C to 36 °C for 2 min by a thermostatically controlled water circulating pump. In another series of experiments aconitine was used as an arrhythmogen. Intact freshly prepared strips were first bathed for 60 min and the contractions were recorded. Strips showing regular rhythmicity and stable contractility during this equilibration period were taken for further experiments. However, strips which were disrhythmic during the equilibration period were discarded. The strips which showed regular rhythmicity and stable contractility during control experiments were then carefully transferred into beakers containing Ringer's solution for cold storage in the refrigerator. Half of the strips were kept in Ringer alone, others in Ringer containing iloprost (10 ng/ml), without supplying exogenous oxygen, at a constant temperature of 4 °C for 24 h. In a preliminary experiment iloprost at the concentration of 1 ng/ml



Decrease in contractility of frog ventricular strips induced by warming of the bathing medium. ●—● control (mean value of 19 experiments). ○—○ preincubated with Ringer containing iloprost (10 ng/ml) (mean value of 10 experiments). △—△ preincubated with Ringer alone (mean value of 9 experiments). Vertical bars show SEM.