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Effect of 711389-S, a New Antiarrhythmic Agent, on Myocardial Energy Metabolism in Guinea-Pigs and Rats

K. IWAKI, M. ISHII, T. SHINOSAKI, M. UEDA AND Y. YONETANI

Shionogi Research Laboratories, Shionogi & Co., Ltd, Fukushima-ku, Osaka, 553 Japan

Abstract—The effect of 711389-S, a new antiarrhythmic agent, on myocardial energy metabolism was investigated using anaesthetized guinea-pigs and rats. 711389-S elevated the adenylate energy charge and phosphorylation potential in normal guinea-pig myocardium. Large doses also increased the myocardial lactate content with ECG abnormalities. The close relationship between rate-pressure product and the myocardial energy state under 711389-S treatments showed the suppression of energy consumption due to a decrease of work output. In guinea-pigs with arrhythmic myocardia induced by intravenous infusion of ouabain, 711389-S prevented the loss of high-energy phosphate compounds and the acceleration of anaerobic glycolysis concomitant with the effective antiarrhythmic property. In ischaemic myocardium produced by ligation of the coronary artery in rats, 711389-S suppressed the decreases of creatine phosphate, NAD^+ and adenylate energy charge. Moreover, this agent effectively blocked the incidence of ventricular arrhythmias at an early stage following the ligation. In all of these actions, 711389-S was more effective than disopyramide, which is in the same class of antiarrhythmics. 711389-S was concluded to be a favourable antiarrhythmic agent offering beneficial action against arrhythmic and ischaemic metabolic changes in the myocardium.

711389-S, 1-{1-[2-(3-isopropylamino-2-hydroxypropoxy)-3,6-dichlorophenyl]vinyl}-1*H*-imidazole hydrochloride, is a new class 1a antiarrhythmic agent with low anticholinergic activity (Ogata et al 1984; Ninomiya et al 1984; Ueda 1985). Although its effects on antiarrhythmic and electrophysiological aspects have been investigated, there is no information of its effects on myocardial metabolism.

The present study was undertaken to evaluate the metabolic effects of this agent in several cardiac situations. First, we examined the effect of 711389-S on the contents of energy-related compounds in normal guinea-pig myocardium and the relationship between the biochemical and physiological parameters. Next, we studied the metabolic changes using the ouabain-induced arrhythmic myocardium in guinea-pigs under conditions where this agent could exhibit a major effect.

Acute myocardial infarction is a representative symptom of malignant arrhythmias and their prevention is the most fundamental medical treatment. Moreover, protection from ischaemic myocardial injury leads to a better prognosis. Thus, it is necessary to confirm that the drugs employed to prevent arrhythmias have no deleterious metabolic effects on the ischaemic myocardium. In the third part of this study, we examined the metabolic effect of 711389-S on ischaemic myocardium in addition to its antiarrhythmic action using rats with acute myocardial infarction induced by ligation of the left coronary artery.

The 711389-S effects were compared with those of disopyramide, which belongs to the same class of antiarrhythmics. Finally, the use of 711389-S as a practical drug was evaluated.

Correspondence to: K. Iwaki, Shionogi Research Laboratories, Shionogi & Co., Ltd, Fukushima-ku, Osaka 553, Japan.

Materials and Methods

Animals and drug treatments

Intact guinea-pigs. Experiments were performed with male guinea-pigs (300–400 g) of the Slc-Hartley strain, which had been anaesthetized with urethane (1·5 g kg^{-1} i.p.). Test agents were administered 30 min after anaesthesia, and the heart was excised 30 min later. For the intraduodenal administration, test agents were injected into duodenum after laparatomy, after which the abdominal wall was closed.

Ouabain-infused guinea-pigs. Ouabain was continuously infused from 10 min after the treatment with test agents as described above at a dose of 7 μg kg^{-1} min^{-1} via a cannula inserted into the left jugular vein. In the animals in which ventricular fibrillation was induced, the heart was excised immediately after the incidence, while in those not showing the fibrillation, the excision was at times corresponding to the above period.

Coronary artery-ligated rats. Experiments were performed using male rats (250–300 g) of the Slc-Wistar strain anaesthetized with urethane (1 g kg^{-1} i.p.). The animals were tracheotomized and connected to a small animal respirator to allow for positive-pressure ventilation upon thoracotomy. A midsternal thoracotomy was performed and the pericardium was incised. The left main coronary artery was ligated by means of needled suture (Akiyama Medical Mfg. Co., Ltd, Tokyo, Japan) 2–3 mm from its origin with part of the myocardial tissue. The heart was returned to the thoracic cavity, air was evacuated from the thorax, and the chest wall was rapidly closed with a suture. The respirator was then removed. Sham-operated rats were subjected to the same surgical procedures except that

the needled suture which was passed under the artery was not tied. The removal of heart or the collection of blood from the abdominal aorta was performed 2 h after the operation. Test agents were intraperitoneally given 10 min before and 1 h after coronary artery ligation.

Measurement of physiological parameters

Leads I and II of the electrocardiogram (ECG) were continuously recorded after anaesthesia. Cardiac rate and rhythm were measured from the ECG records. Blood pressure was measured from the catheter (PE50) inserted into the left carotid artery. The product of the heart rate and the systolic blood pressure was calculated and termed the rate-pressure product.

Sampling and analysis of myocardium

The animals remained anaesthetized throughout the experiment and the heart was removed under anaesthesia. After the thorax was opened, the heart was quickly frozen using an aluminium tong pre-cooled in liquid nitrogen. The frozen tissue was weighed, pulverized at liquid nitrogen temperature, and homogenized with 6% $HClO_4$. The resultant suspension was centrifuged in the cold, the supernatant was neutralized with 2 M K_2CO_3, and the $KClO_4$ formed was separated by centrifugation in the cold and discarded. The neutral extracts were subjected to chemical analysis.

Adenosine triphosphate (ATP) was determined fluorometrically using a hexokinase-glucose-6-phosphate dehydrogenase system (Greengard 1963). Creatine phosphate (CrP) was determined by the above procedure with creatine kinase according to Lamprecht et al (1974). Glucose was also assayed using the reaction system for ATP. Adenosine diphosphate (ADP) and adenosine-5'-monophosphate (AMP) were measured sequentially in the same cuvette using lactate dehydrogenase, pyruvate kinase and myokinase (Adam 1963). Inorganic phosphate (Pi) and oxidized nicotinamide adenine dinucleotide (NAD^+) were assayed by the method of Fawaz & Fawaz (1971) and Klingenberg (1974), respectively. Adenylate energy charge (AEC) and phosphorylation potential (PP), which represents the energy status of the cell (Atkinson 1968; Lehninger 1975), were calculated as follows:

$$AEC = ([ATP] + 0{\cdot}5[ADP])/([ATP] + [ADP] + [AMP])$$

$$PP = [ATP]/([ADP] \times [Pi])$$

Lactate and pyruvate were determined following the procedures described by Czok & Lamprecht (1974) and Gutman & Wahlefeld (1974), respectively. Based on these determinations, the redox potential (Eh: −mV) of the myocardium was calculated, using the following equation (Gudbjarnason et al 1962).

$$Eh = -204 - 30{\cdot}7 \log ([lactate]/[pyruvate])$$

Sampling and analysis of blood

The sampled blood was immediately cooled in ice water and centrifuged. The resultant plasma was mixed with 10 volumes of 0·007 M acetic acid, heated for 2 min in boiling water and then centrifuged. Glucose and lactate concentrations in deproteinized plasma were measured according to the procedure for myocardial extracts. Uric acid concentration in the above specimens was determined by the method described previously (Yonetani et al 1980).

Drugs used

The drugs used were 711389-S (Shionogi & Co., Ltd, Osaka, Japan), disopyramide and ouabain octahydrate (Sigma Chemical Co., St Louis, MO, USA). Disopyramide was extracted from the commercial preparation purchased from Nippon Roussel Co., Ltd, Tokyo, Japan. 711389-S was dissolved in saline. Disopyramide was dissolved in 1 M HCl, diluted with saline, then neutralized with 1 M $NaHCO_3$ before use. These drugs were administered at 2 mL kg^{-1} body weight. Ouabain was dissolved in a minimum volume of 95% ethanol and was diluted with saline. The solution was infused at a constant rate of 20 μL min^{-1} by means of a syringe pump. Saline was used in the controls. All other chemicals were of reagent grade.

Statistical analysis

The results are expressed as means ± s.e. For differences between group means, Student's *t*-test was used to determine the statistical significance and regression equations were determined by the method of least squares. A chi-square test was used to analyse the statistical significance of differences in the incidence of arrhythmias between groups.

Results

Effects on the normal myocardium in guinea-pigs

First, the effects of various intraperitoneal doses of 711389-S and disopyramide on myocardial energy metabolites, pyruvate and lactate, were examined in normal guinea-pigs (Table 1). Dose-dependent falls in heart rate

Table 1. Effects of intraperitoneal administration of 711389-S or disopyramide on heart rate and myocardial energy state and redox potential in guinea-pigs. Heart rate was measured at 30 min after the treatment with each drug, then the heart was freeze-clamped. Heart rate: beats min^{-1}. Metabolites: μmol g^{-1} wet weight.

	711389-S (mg kg^{-1} i.p.)			
	0	10	25	50
No. of animals	5	5	5	5
Heart rate	309 ± 6	284 ± 7*	247 ± 8**	147 ± 23**
CrP	5·84 ± 0·29	7·03 ± 0·38*	6·94 ± 0·38	8·00 ± 0·59*
ATP	3·62 ± 0·24	4·18 ± 0·11	4·53 ± 0·16*	4·28 ± 0·17
Pi	4·20 ± 0·25	3·92 ± 0·24	3·66 ± 0·29	2·54 ± 0·20**
AEC	0·907 ± 0·004	0·924 ± 0·002**	0·935 ± 0·002**	0·932 ± 0·002**
PP	1·324 ± 0·042	1·714 ± 0·118*	2·231 ± 0·196**	3·029 ± 0·217**
Lactate	0·84 ± 0·16	0·48 ± 0·03	0·54 ± 0·08	0·77 ± 0·06
Eh	233·1 ± 1·1	227·9 ± 2·5	234·0 ± 1·0	231·0 ± 0·7
	Disopyramide (mg min^{-1} i.p.)			
	0	10	50	100
No. of animals	4	5	5	5
Heart rate	307 ± 9	294 ± 12	230 ± 7**	173 ± 17**
CrP	4·22 ± 0·54	5·73 ± 0·47	6·05 ± 0·36*	5·85 ± 0·38*
ATP	4·88 ± 0·17	5·36 ± 0·04*	5·44 ± 0·20	5·03 ± 0·28
Pi	4·89 ± 0·69	3·93 ± 0·38	3·65 ± 0·19	3·34 ± 0·17*
AEC	0·925 ± 0·005	0·937 ± 0·002	0·945 ± 0·001**	0·945 ± 0·003*
PP	1·607 ± 0·295	2·286 ± 0·293	2·674 ± 0·183*	2·932 ± 0·310*
Lactate	0·69 ± 0·11	0·55 ± 0·06	0·33 ± 0·06*	0·93 ± 0·23
Eh	228·4 ± 1·5	225·2 ± 1·2	226·6 ± 3·7	240·9 ± 2·4**

*$P < 0{\cdot}05$ and **$P < 0{\cdot}01$: Significant difference from the control group.

were found with both drugs and, as for ECG changes, a deep drop of S-wave developed at 50 mg kg^{-1} of 711389-S and at 100 mg kg^{-1} of disopyramide (Fig. 1). Metabolically, 711389-S acted to increase the contents of energy-rich phosphate compounds and to decrease those of inorganic phosphate. As the result, the adenylate energy charge and phosphorylation potential, indexes of the energy state, were clearly elevated. On the other hand, no significant changes were observed in pyruvate and lactate contents and redox potential. A similar metabolic response of the energy state was found after disopyramide treatment at doses of 50 and 100 mg kg^{-1}. A slight elevation of the redox potential was observed after 100 mg kg^{-1} of disopyramide.

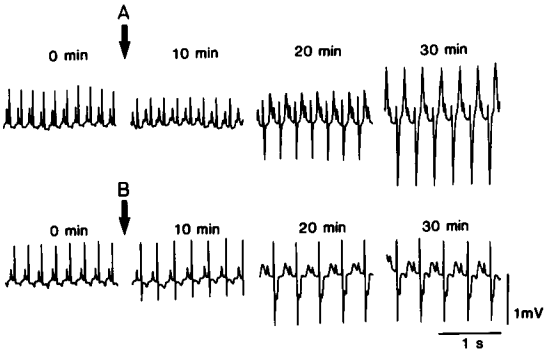

FIG. 1. Typical ECG changes produced by (A) 50 mg kg^{-1} i.p. of 711389-S and (B) 100 mg kg^{-1} i.p. of disopyramide in guinea-pigs.

To evaluate the relationship between the myocardial energy state and work output, effect of 711389-S on systolic blood pressure, heart rate and rate-pressure product with respect to the pre-administration are graphically expressed in Fig. 2. The blood pressure tended to rise with time at 0, 10 and 25 mg kg^{-1} doses, but declined at 50 mg kg^{-1}. The heart rate also markedly decreased with time at 50 mg kg^{-1}. As the result, the rate-pressure product was

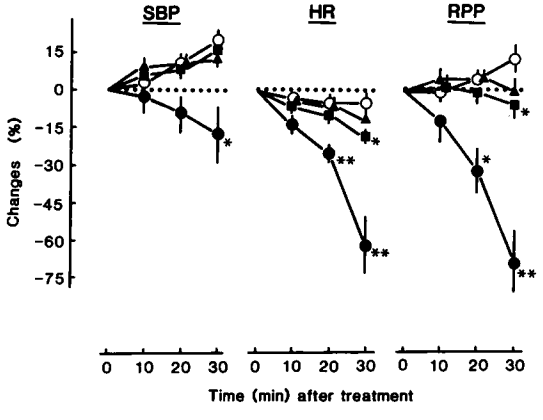

FIG. 2. Effect of 711389-S on systolic blood pressure, heart rate and rate-pressure product in guinea-pigs. 711389-S (○: 0 mg kg^{-1} n = 4, ▲: 10 mg kg^{-1} n = 4, ■: 25 mg kg^{-1} n = 4, ●: 50 mg kg^{-1} n = 4) was intraperitoneally administered. SBP, systolic blood pressure; HR, heart rate; RPP, rate-pressure product (SBP × HR). *P < 0·05 and **P < 0·01: Significant difference from the respective control value.

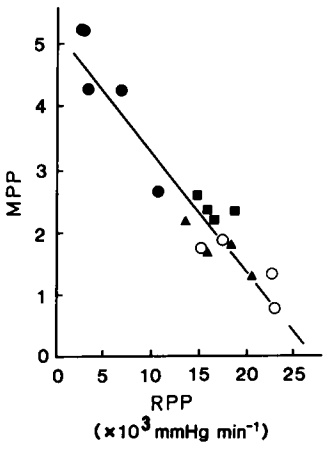

FIG. 3. Relationship between myocardial phosphorylation potential (MPP) and rate-pressure product (RPP) in guinea-pigs treated with 711389-S. The heart was freeze-clamped after the measurement of the rate-pressure product at 30 min as described in Fig. 2. r (correlation coefficient) = −0·944, n = 16, P < 0·01. Dose = ○ 0, ▲ 10, ■ 25 and ● 50 mg kg^{-1} i.p.

slightly and markedly decreased in 25 and 50 mg kg^{-1} doses, respectively, against the control.

As shown in Fig. 3, there was a good correlation of the rate-pressure product and the phosphorylation potential.

The changes of myocardial contents of the above metabolites were assayed after intraduodenal administration of 711389-S (Table 2). No appreciable effect was found on the contents of adenine nucleotides and the adenylate energy charge. The content of creatine phosphate increased at doses of 50 and 100 mg kg^{-1} and that of inorganic phosphate decreased at doses of more than 10 mg kg^{-1}. Phosphorylation potential was significantly elevated at 10 and 50 mg kg^{-1} doses. On the other hand, there was a significant increase of lactate content at doses of 50 and 100 mg kg^{-1}, which resulted in elevation of the redox potential. In these doses, 711389-S was arrhythmogenic. As shown in Fig. 4, marked increases of lactate content were found in the animals affected with atrioventricular block above 4 : 1 (one QRS-wave following more than 4P-waves).

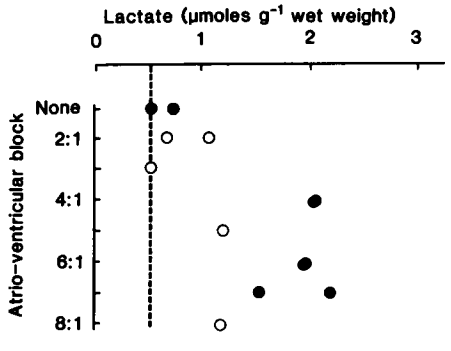

FIG. 4. Increase of myocardial lactate content in guinea-pigs with atrio-ventricular block after intraduodenal administration of 711389-S. See Table 2 for details. The lack of QRS was counted for 1 min immediately before the animal was killed. The mean value of the control group is indicated by the dotted line as a basis for comparison. Dose: ○ 50, ● 100 mg kg^{-1}.

Table 2. Effect of intraduodenal administration of 711389-S on myocardial energy state and redox potential in guinea-pigs. The heart was freeze-clamped at 30 min after the treatment with drug. Metabolites: μmol g^{-1} wet weight.

	711389-S (mg kg^{-1} i.d.)					
	0	5	10	25	50	100
No. of animals	8	4	4	6	5	6
CrP	6·45 ± 0·28	6·52 ± 0·69	7·53 ± 0·41	7·08 ± 0·60	7·86 ± 0·46*	7·49 ± 0·35*
ATP	4·84 ± 0·13	5·10 ± 0·13	5·06 ± 0·19	4·85 ± 0·25	5·18 ± 0·11	4·76 ± 0·12
Pi	3·73 ± 0·22	3·60 ± 0·25	2·84 ± 0·19*	2·88 ± 0·18*	2·43 ± 0·25*	3·04 ± 0·22*
AEC	0·934 ± 0·004	0·936 ± 0·003	0·941 ± 0·003	0·935 ± 0·003	0·942 ± 0·002	0·937 ± 0·002
PP	2·365 ± 0·251	2·415 ± 0·236	3·306 ± 0·335*	2·944 ± 0·312	3·899 ± 0·403**	3·068 ± 0·304
Lactate	0·56 ± 0·07	0·32 ± 0·08	0·37 ± 0·03	0·50 ± 0·08	0·93 ± 0·14*	1·50 ± 0·29**
Eh	231·7 ± 2·0	229·1 ± 7·4	236·4 ± 1·2	231·2 ± 1·1	245·2 ± 4·6*	245·4 ± 6·4*

*$P < 0.05$ and **$P < 0.01$: Significant difference from the control group.

Effects on ouabain-induced arrhythmic myocardium in guinea-pigs

The earliest observed rhythm abnormality by ouabain (7 μg kg^{-1} min^{-1} i.v.) was a variation in the interval between successive R-waves at about 10 min after the start of infusion. This was typically followed by ventricular extrasystoles about 17 min later, and lastly, ventricular fibrillation about 20 min later. The heart rate was not affected.

Slight changes of myocardial energy metabolism were found at the incidence of ventricular extrasystole. Increases of ADP and inorganic phosphate contents, but no other changes, were observed at this stage. Marked metabolic changes were observed at the stage of ventricular fibrillation. The creatine phosphate and ATP contents decreased with a concomitant increase in the ADP, AMP and inorganic phosphate contents. These changes caused drops in adenylate energy charge and phosphorylation potential. Moreover, the redox potential markedly rose with an increase of lactate.

Intraperitoneal administration of 711389-S (10 mg kg^{-1}) or disopyramide (50 mg kg^{-1}), at 10 min before ouabain infusion, offered protection against the incidence of ventricular arrhythmias. There were no animals showing ventricular extrasystole and fibrillation in each medicated group [711389-S (n = 5), disopyramide (n = 4)] within 21 min after the start of ouabain infusion, although these agents did not prevent the ouabain-induced sinus arrhythmias. As shown in Table 3, 711389-S and disopyramide effectively suppressed the deleterious myocardial metabolic changes with regard to the energy state and the redox potential induced by ouabain infusion. Disopyramide at 10 mg kg^{-1} was ineffective.

Table 3. Effects of 711389-S and disopyramide on ouabain-induced changes of myocardial energy state and redox potential in guinea-pigs. 711389-S (-A-, 10 mg kg^{-1} i.p.) and disoyramide (-B-, 50 mg kg^{-1} i.p.) were given 10 min before ouabain infusion (7 μg kg^{-1} min^{-1} i.v.). In the ouabain infusion, metabolic changes in the myocardium were measured when ventricular fibrillation was observed. No marked changes of high-energy phosphate compounds were observed at the stage of ventricular extrasystole by ouabain infusion. In the animals in which ventricular fibrillation was not induced, the heart was freeze-clamped at 21 min after the start of infusion, while in those in which it was, the heart was freeze-clamped immediately after the incidence. Metabolites: μmol g^{-1} wet weight.

-A-	Saline (n = 5)	711389-S (n = 5)	Ouabain (n = 5)	711389-S & Ouabain (n = 5)
CrP	6·51 ± 0·24	7·60 ± 0·27*	2·94 ± 0·40**	4·87 ± 0·41**,##
ATP	4·33 ± 0·07	4·59 ± 0·15	3·77 ± 0·20*	4·34 ± 0·16
Pi	3·70 ± 0·14	3·01 ± 0·18*	6·67 ± 0·48**	5·43 ± 0·28**
AEC	0·918 ± 0·002	0·931 ± 0·002**	0·867 ± 0·013**	0·905 ± 0·006#
PP	1·767 ± 0·081	2·535 ± 0·143**	0·637 ± 0·108**	1·067 ± 0·127**,#
Lactate	0·71 ± 0·21	0·87 ± 0·19	2·04 ± 0·12**	0·74 ± 0·14##
Eh	224·8 ± 3·4	230·2 ± 3·5	239·2 ± 0·8**	225·6 ± 2·9##
-B-	**Saline (n = 5)**	**Disopyramide (n = 4)**	**Ouabain (n = 4)**	**Disopyramide & Ouabain (n = 4)**
CrP	5·69 ± 0·30	6·69 ± 0·60	2·40 ± 0·34**	4·74 ± 0·50##
ATP	4·31 ± 0·20	4·49 ± 0·28	3·77 ± 0·17	3·96 ± 0·20
Pi	4·06 ± 0·33	3·12 ± 0·17*	6·67 ± 0·27**	5·05 ± 0·42#
AEC	0·919 ± 0·005	0·928 ± 0·003	0·870 ± 0·011**	0·907 ± 0·007#
PP	1·706 ± 0·191	2·451 ± 0·227*	0·605 ± 0·065**	1·183 ± 0·194#
Lactate	0·64 ± 0·12	0·46 ± 0·16	2·41 ± 0·44**	0·93 ± 0·17#
Eh	232·2 ± 1·4	222·6 ± 4·8	246·3 ± 2·0**	236·3 ± 3·1#

*$P < 0.05$ and **$P < 0.01$; Significant difference from the saline-treated group. #$P < 0.05$ and ##$P < 0.01$: Significant difference from the group treated only with ouabain.

Effects on coronary artery ligation-induced ischaemic myocardium in rats

Ligation of the left coronary artery resulted in the typical ECG signs of myocardial infarction, namely elevation of the ST-segment and different kinds of arrhythmias. The onset of ventricular arrhythmias such as extrasystole, tachycardia, flutter and fibrillation was generally 4 min post ligation and intermittently continued until 10 min after ligation. After that, there were no significant differences in heart rate between the sham-operated and coronary artery-ligated animals. Although 60% of the control animals displayed fibrillation, mortality was less than 10% because reversion to sinus rhythm was common with the help of artificial ventilation.

Fig. 5 summarizes the effects of 711389-S and disopyramide on the incidence of ventricular arrhythmias. 10 mg kg^{-1} of 711389-S and 25 mg kg^{-1} of disopyramide were intraperitoneally administered at 10 min before and 1 h after coronary artery ligation. Both drugs had an excellent antiarrhythmic effect; the number of animals that developed arrhythmias markedly decreased compared with the saline-treated control group. Neither drug produced changes in ST-segment elevation and R-wave height following the ligation throughout the experiment.

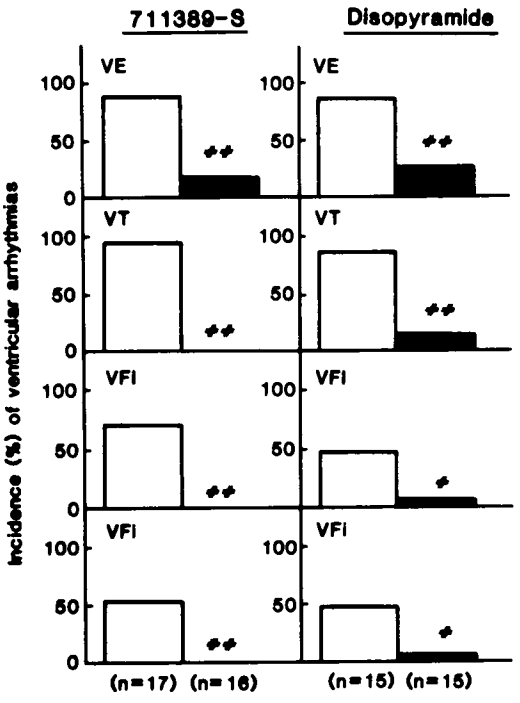

FIG. 5. Effects of 711389-S and disopyramide on the incidence of ventricular arrhythmias induced by coronary artery ligation in rats. Treatments (□: saline, ■: test agents) were done as described in Table 4. Data show the incidence of the arrhythmias, as the percentage of animals in the group. VE, ventricular extrasystole; VT, ventricular tachycardia; VF1, ventricular flutter; VFi, ventricular fibrillation. $\#P < 0.05$ and $\#\#P < 0.01$: Significant difference from the saline-treated group.

Table 4. Effects of 711389-S and disopyramide on myocardial metabolic changes induced by coronary artery ligation in rats. 711389-S (-A-, 10 mg kg^{-1} i.p.) and disopyramide (-B-, 25 mg kg^{-1} i.p.) were administered at 10 min before and 1 h after coronary artery ligation, and the heart was freeze-clamped at 2 h after the ligation. Metabolites: μmol g^{-1} wet weight.

	Sham-operation		Coronary artery ligation	
-A-	Saline (n = 7)	711389-S (n = 7)	Saline (n = 10)	711389-S (n = 8)
CrP	5·29 ± 0·33	5·88 ± 0·27	2·75 ± 0·15††	3·47 ± 0·15**,††
ATP	4·46 ± 0·19	4·32 ± 0·13	2·21 ± 0·09††	2·31 ± 0·11††
Pi	4·72 ± 0·17	3·03 ± 0·35**	4·78 ± 0·67	3·68 ± 0·45
AEC	0·890 ± 0·008	0·883 ± 0·005	0·807 ± 0·008††	0·833 ± 0·009*,††
PP	1·140 ± 0·095	1·768 ± 0·248*	0·878 ± 0·193††	1·195 ± 0·212
Lactate	0·86 ± 0·06	0·79 ± 0·08	4·81 ± 0·53††	3·46 ± 0·60††
Eh	235·1 ± 1·3	236·8 ± 1·4	260·0 ± 2·2††	258·7 ± 3·9††
Glucose	7·46 ± 0·56	4·07 ± 0·18**	9·77 ± 0·35††	6·04 ± 0·62**,†
NAD^+	0·77 ± 0·04	0·83 ± 0·03	0·48 ± 0·01††	0·53 ± 0·02*,††
	Sham-operation		Coronary artery ligation	
-B-	Saline (n = 6)	Disopyramide (n = 6)	Saline (n = 8)	Disopyramide (n = 8)
CrP	5·73 ± 0·33	5·51 ± 0·23	3·58 ± 0·33††	3·24 ± 0·18††
ATP	5·06 ± 0·11	4·77 ± 0·30	3·11 ± 0·16††	3·20 ± 0·20††
Pi	4·80 ± 0·45	4·97 ± 0·34	7·04 ± 0·49††	6·74 ± 0·40††
AEC	0·895 ± 0·007	0·896 ± 0·004	0·841 ± 0·010††	0·841 ± 0·013††
PP	1·151 ± 0·170	1·039 ± 0·077	0·580 ± 0·047††	0·605 ± 0·051††
Lactate	0·86 ± 0·10	0·89 ± 0·20	5·73 ± 0·82††	3·85 ± 0·57††
Eh	227·8 ± 1·8	231·5 ± 3·7	251·6 ± 2·2††	249·5 ± 2·4††
Glucose	5·88 ± 0·87	3·92 ± 0·63	8·03 ± 0·42†	3·94 ± 0·20**
NAD^+	0·81 ± 0·02	0·81 ± 0·02	0·57 ± 0·02††	0·58 ± 0·02††

*$P < 0.05$ and **$P < 0.01$: Significant difference from the corresponding saline-treated group. †$P < 0.05$ and ††$P < 0.01$: Significant difference from the corresponding sham-operated group.

Both 711389-S and disopyramide reduced the heart rate at 2 h after the operation by 85 and 83% in the sham-operated control group of 378 ± 9 and 373 ± 12 beats min^{-1}, respectively ($P < 0.01$), and by 88 and 87% in the coronary artery-ligated control group of 374 ± 7 and 379 ± 7 beats min^{-1}, respectively ($P < 0.01$).

As to the myocardial metabolism, coronary artery ligation led to marked losses of high-energy phosphate compounds and reduced the energy state, as shown by the adenylate energy charge and phosphorylation potential. Except for the energy-related components, the myocardial contents of glucose and lactate increased and that of NAD^+ decreased with the ligation (Table 4). There was no change in sham-operated animals in the above components compared with the intact ones under anaesthesia, except that the myocardial glucose content increased after sham-operation (intact level; 3·05 ± 0·17 μmol g^{-1} wet weight).

711389-S offered protection against coronary artery-ligated changes of creatine phosphate, glucose and NAD^+ contents but had no effect on those of lactate content and redox potential. This agent also suppressed the fall of the adenylate energy charge. In sham-operated animals, 711389-S decreased the inorganic phosphate and glucose contents, causing an elevated phosphorylation potential. On the other hand, disopyramide did not influence the high-energy phosphate compounds and the energy state, unlike as 711389-S. But it clearly suppressed the increase of glucose content.

Both thoracotomy and the following ligation of the coronary artery induced increases of various plasma metabolite levels, as shown in Fig. 6. In sham-operated and coronary artery-ligated animals, the plasma levels of

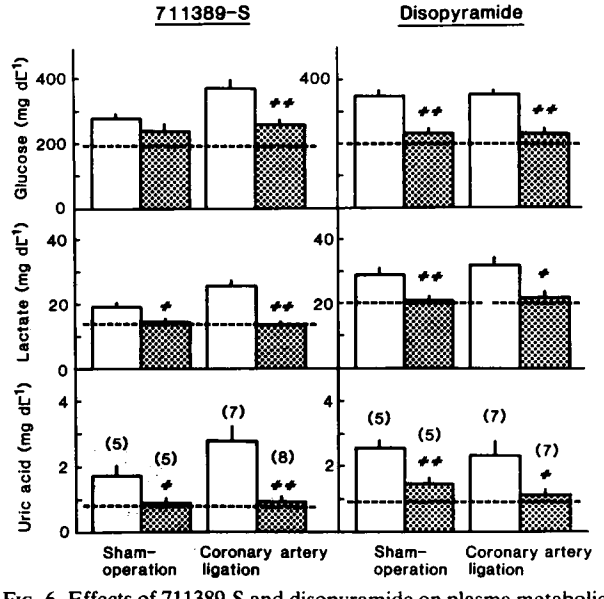

FIG. 6. Effects of 711389-S and disopyramide on plasma metabolic changes induced by coronary artery ligation in rats. Treatments (open column: saline, dotted column: test agents) were done as described in Table 4, and blood samples were collected from the abdominal aorta at 2 h after the ligation. The number of animals is given in parentheses. The mean values of the intact group (n = 5) after they had been left for the same period under anaesthesia are indicated by dotted lines for comparison. $\#P < 0.05$ and $\#\#P < 0.01$: Significant difference from the corresponding saline-treated group.

glucose, lactate and uric acid rose significantly compared with those in intact rats ($P < 0.01$). 711389-S and disopyramide suppressed the increases of those components.

Discussion

Class 1 antiarrhythmic agents are cardiovascular drugs having a low therapeutic index between antiarrhythmic and arrhythmogenic properties and also haemodynamic side effects (Gottdiener et al 1982; Block & Winkle 1983; Legrand & Collignon 1985). Moreover, severe cardiac failure is often latent in patients requiring antiarrhythmic therapy. For these reasons, it was necessary to investigate the influence of 711389-S, a new antiarrhythmic agent, on the myocardium from the view of energy metabolism, which is closely related to the cardiac work.

First, we determined the action of 711389-S on normal myocardium in guinea-pigs. 711389-S increased the adenylate energy charge and phosphorylation potential concomitantly with a decrease in the heart rate. Thus, the rise of the phosphorylation potential is closely correlated to the decrease of the rate-pressure product, which suggests that the rise of the myocardial energy state is the result of suppressed energy consumption due to the fall of work output. Such elevation of the energy state was also observed in the treatment with disopyramide but a higher dosage was required. These results using normal guinea-pig myocardium indicate that the elevation of the energy state caused by doses lower than 25 mg kg^{-1} of 711389-S is not a disadvantageous metabolic change because blood pressure and ECG were not influenced by the treatment. Instead, the energy-preserving capacity may antagonize the arrhythmic disorders with excessive energy consumption. On the other hand, the elevation of the energy state observed with doses of more than 50 mg kg^{-1} of 711389-S seems to be a harmful metabolic condition which is accompanied by a severe fall in the rate-pressure product and the accumulation of lactate in the myocardium, resulting in ECG abnormalities. The cause of the rise in lactate content of myocardium with severe atrio-ventricular block is not known in this study, but this arrhythmic condition did not induce the loss of energy-rich phosphate contents.

Next, we examined both the metabolic changes in the arrhythmic myocardium affected by ouabain infusion and the action of 711389-S and disopyramide under conditions where they could display their major effects. Ouabain-induced metabolic changes in the myocardium, such as increases of ADP and inorganic phosphate contents were first detected at the stage of ventricular extrasystole and severe decline of the energy state and accelerated anaerobic glycolysis were found at the stage of ventricular fibrillation. Toxic doses of ouabain reduce the myocardial contents of high-energy phosphate compounds with a concomitant increase in that of inorganic phosphate (Greiner 1952; Furchgott & DeGubareff 1958; Williams & Nayler 1977). Pretreatments with 711389-S (10 mg kg^{-1}) or disopyramide (50 mg kg^{-1}) prevented the occurrence of such deleterious metabolic changes and of ventricular arrhythmias induced by ouabain. Disopyramide, at 10 mg kg^{-1}, which has no energy level-elevating capacity, was ineffective. Although it is not clear whether myocardial energy conservation by these agents is necessary for the antagonism, these findings indicate that 711389-S is useful against arrhythmic disorders accompanied by metabolic aggravation in the myocardium, such as ouabain cardiotoxicity.

Medical reduction of lethal arrhythmic incidence is the major aim of any intervention in the acute myocardial infarction. Numerous clinical studies have shown that antiarrhythmic drugs greatly contribute to lowering mortality. Thus the agents should have no undesirable influence on the myocardial metabolic state. To obtain some insight into this subject, we used rats with ligated coronary arteries and investigated the effect of 711389-S on the incidence of arrhythmias and on ischaemic metabolic changes.

In this model, the most frequent arrhythmias were ventricular extrasystole and tachycardia, being aggravated to flutter and fibrillation in about half of the cases. The incidences of ventricular tachycardia, flutter and fibrillation could be completely prevented by 10 mg kg^{-1} of 711389-S. That treatment markedly reduced the number of animals with extrasystole. Disopyramide (25 mg kg^{-1}) also significantly reduced the number of arrhythmia-induced animals. At the doses of both drugs employed, the heart rate gradually declined and the drop became statistically significant from the non-medicated group 2 h later. 711389-S has been reported to have a potent and lasting antifibrillatory effect compared with other antiarrhythmic drugs using in-vitro and in-vivo guinea-pig preparations (Nakajima & Ueda 1985). Thus, 711389-S appears to be very effective for preventing sudden coronary death from ventricular fibrillation in acute myocardial infarction.

Metabolically, the contents of energy-rich phosphate compounds and the energy state in the myocardium markedly decreased 2 h after ligation of the coronary artery. Moreover, except for the energy-related compounds, the glucose and lactate contents increased while the NAD^+ content decreased. 711389-S suppressed the changes of creatine phosphate, adenylate energy charge glucose and NAD^+, while disopyramide only suppressed that of glucose content. From these results, we concluded that 711389-S had no further deleterious effect on the ischaemic myocardial metabolism and rather had a beneficial influence. 711389-S has a more pronounced local anaesthetic action than disopyramide and its potency is equal to that of lignocaine (lidocaine) (personal communication). Numerous studies have shown that lidocaine has protective effects on the energy state (Garamszegi et al 1979; Takats & Szekeres 1979), mitochondrial respiratory function (Baron et al 1983) and progress of infarction (Nasser et al 1980) in the ischaemic myocardium. Further studies are needed on the protective properties of 711389-S against ischaemic myocardial damage.

Our model of acute myocardial infarction resulted in marked elevations of plasma glucose, lactate and uric acid levels. However, the ligation of coronary artery is not necessary for the increases of plasma components because these changes could be also observed after the sham-operation. It is known that ligation of the coronary artery induces rapid activation of the sympathetic nervous system

(Bosnjak et al 1979; Karlsberg et al 1979; Bernauer 1983). In fact, the elevation of these plasma metabolites not only in coronary artery-ligated animals but also in sham-operated ones was nearly completely prevented by DL-propranolol hydrochloride (1 mg kg^{-1}) when administered in the same manner as the above agents (results not shown). Thus, we postulate that the cause for the changes of plasma components is the result of the activation of sympathetic nervous system and both 711389-S and disopyramide can inhibit this activation. Sympathetic stimulation has already been shown to increase the oxygen demand of the heart and thus can worsen myocardial damage during ischaemia. However, the result that only 711389-S has a suppressive effect on metabolic changes in ligated myocardium suggests that the energy-preserving effect of 711389-S on the myocardium is not dependent on the suppression of sympathetic overstimulation.

References

Adam, H. (1963) in: Bergmeyer, H. U. (ed.) Methods of Enzymatic Analysis. 1st edn, Academic Press, New York and London, pp 573–577

Atkinson, D. E. (1968) Biochemistry 7: 4030–4034

Baron, D. W., Sunamori, M., Harrison, C. E. (1983) Adv. Myocardiol. 4: 567–573

Bernauer, W. (1983) Eur. J. Pharmacol. 88: 161–168

Block, P. J., Winkle, R. A. (1983) Am. J. Cardiol. 52: 14C–23C

Bosnjak, Z. J., Zuperku, E. J., Coon, R. L., Kampine, J. P. (1979) Proc. Soc. Exp. Biol. Med. 161: 142–148

Czok, R., Lamprecht, W. (1974) in: Bergmeyer, H. U. (ed.) Methods of Enzymatic Analysis. 2nd edn, Academic Press, New York and London, pp 1446–1456

Fawaz, G., Fawaz, E. N. (1971) in: Schwartz, A. (eds) Methods in Pharmacology. Plenum Press, New York, Vol. 1, pp 515–551

Furchgott, R. F., DeGubareff, T. (1958) J. Pharmacol. Exp. Ther. 124: 203–218

Garamszegi, M., Takats, I., Szekeres, L. (1979) Acta Physiol. Acad. Sci. Hung. 53: 167

Gottdiener, J. S., DiBianco, R., Fletcher, R. D. (1982) Angiology 33: 228–238

Greengard, P. (1963) in: Bergmeyer, H. U. (ed.) Methods of Enzymatic Analysis. 1st edn, Academic Press, New York and London, pp 551–558

Greiner, T. (1952) J. Pharmacol. Exp. Ther. 105: 178–195

Gudbjarnason, S., Hayden, R. O., Wendt, V. E., Stock, T. B., Bing, R. J. (1962) Circulation 26: 937–945

Gutman, I., Wahleferd, A. W. (1974) in: Bergmeyer, H. U. (ed.) Methods of Enzymatic Analysis. 2nd edn, Academic Press, New York and London, pp 1464–1468

Karlsberg, R. P., Penkoske, P. A., Cryer, P. E., Corr, P., Roberts, R. (1979) Cardiovas. Res. 13: 523–531

Klingenberg, M. (1974) in: Bergmeyer, H. U. (ed.) Methods of Enzymatic Analysis. 2nd edn, Academic Press, New York and London, pp 2045–2072

Lamprecht, W., Stein, P., Heinz, F., Weisser, H. (1974) in: Bergmeyer, H. U. (ed.) Methods of Enzymatic Analysis. 2nd edn, Academic Press, New York and London, pp 1777–1781

Legrand V., Collignon, P. (1985) Drug 29 (suppl. 4): 54–57

Lehninger, A. L. (1975) in: Biochemistry, 2nd edn, Worth Publishers, New York, p. 539

Nakajima, S., Ueda, M. (1985) Jap. J. Pharmacol. 38: 436–438

Nasser, F. N., Walls, J. T., Edwards, W. D., Harrison, Jr., C. E. (1980) Am. J. Cardiol. 46: 967–975

Ninomiya, M., Toyama, I., Kodama, I., Yamada, K. (1984) J. Cardiovasc. Pharmacol. 6: 1222–1229

Ogata, M., Matsumoto, H., Takahashi, K., Kida, S., Ueda, M., Kimoto, S., Haruna, M. (1984) J. Med. Chem. 27: 1142–1149

Takats, I., Szekeres, L. (1979) Acta Physiol. Acad. Sci. Hung. 54: 191

Ueda, M. (1985) Drugs of the Future 10: 472–473

Williams, A., Nayler, W. G. (1977) J. Mol. Cell. Cardiol. 9 (suppl.): 64

Yonetani, Y., Ishii, M., Iwaki, K. (1980) Jap. J. Pharmacol. 30: 829–840