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Effect of antiarrhythmics on the release of adenosine in rat hearts with coronary occlusion and reperfusion

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Abstract

In isolated perfused rat hearts the left coronary artery was occluded for 5 min, with subsequent reperfusion for 20 min. During the reperfusion severe tachyarrhythmias were observed, with ventricular fibrillation occurring in all hearts. Simultaneously, large amounts of adenosine and its degradation products inosine, hypoxanthine, xanthine and uric acid were released into the coronary perfusate. The antiarrhythmics quinidine, lidocaine and gallopamil significantly decreased the release. The effect of quinidine and lidocaine was linked with the antifibrillatory action of these drugs. Also the interruption of fibrillation immediately after its appearance by potassium chloride decreased the release of adenosine and its metabolites in a highly significant way. The effect of gallopamil on the release was independent of an antifibrillatory action. The findings indicate that different kinds of antiarrhythmic drugs can affect the release of nucleosides and oxypurines in hearts with ischaemia and reperfusion.

Keywords: Coronary occlusion; Adenosine release; Reperfusion arrhythmia; Lidocaine; Quinidine; Gallopamil

1. Introduction

Release of adenosine is a characteristic event in myocardial ischaemia (see, for instance, Berne, 1980; Sparks and Bardenheuer, 1986). Depending on the duration of cardiac oxygen deficiency, considerable amounts of the nucleoside, and of its degradation products, appear during post-ischaemic reperfusion in the coronary perfusate. In a previous investigation in globally ischaemic rat hearts results were obtained, indicating that the antiarrhythmic lidocaine can affect the adenosine release (Bernauer, 1991). Because of the relevance that such an effect might have for the pharmacology of myocardial ischaemia, we believed it important to investigate this phenomenon on a broader basis. Adenosine not only is able to dilate coronary vessels, but, depending on the concentration, can stimulate, or inhibit ectopic impulse generation.

To exclude that the effect of lidocaine on the adenosine release is a particular property of this drug only, also other antiarrhythmics were tested. Quinidine and gallopamil (the methoxy derivative of verapamil) were used and compared with lidocaine in rat hearts with coronary ligation and reperfusion. As will be shown, special information about a connection between arrhythmias and adenosine release was obtained from experiments with interruption of ventricular fibrillation by application of potassium chloride.

2. Materials and methods

2.1. Experimental technique

Sprague-Dawley rats (male; body weight 538 ± 7 g; free access to water and food) were anaesthetized with pentobarbital sodium (45 mg/kg i.p.) and respired artificially with air through a tracheal cannula. The hearts were excised, mounted in a double-walled, water-heated chamber and perfused at 38°C and constant pressure of 7.85 kPa according to the Langendorff technique, with Tyrode's solution of the following composition (mmol · l^{-1}): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6. It was gassed with 95% O_2 and 5% CO_2 and filtered through a washed paper filter.

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Two platinum blade electrodes were attached to the surface of the ventricles and the electrocardiogram was monitored continuously. A run of at least four consecutive ventricular premature beats was defined as ventricular tachycardia. Two or three consecutive premature beats were classified as salvos. An electrocardiogram of absolute morphological instability in which individual QRS complexes could not be distinguished from one another was considered as ventricular fibrillation.

After the hearts had adapted to the artificial perfusion for 30 min, a 10 min sample of coronary perfusate was collected. Then, the left coronary artery was ligated beneath the left auricular appendage. (In the rat heart the left coronary artery is a single descending trunk. A true left circumflex coronary artery is lacking; Johns and Olson, 1954.) After 5 min the coronary ligature was released and coronary reperfusion was performed for 20 min. Afterwards, the coronary artery was ligated again, and the coronary system was perfused with chlorophyllin solution (5 mg/ml saline). By this procedure the normally perfused parts of the hearts were stained green, whereas the non-perfused areas remained unstained. Stained and unstained parts were separated carefully with scissors, and the wet and dry weights were determined.

The antiarrhythmics quinidine, lidocaine, or gallopamil respectively were added to the Tyrode solution already at the onset of the coronary perfusion. In further groups of experiments, antiarrhythmic drugs were applied only during the coronary reperfusion by infusion into the fluid stream before the heart, in order to prevent reperfusion-induced ventricular fibrillation. In another experimental group a solution of potassium chloride was infused immediately after reperfusion-induced ventricular fibrillation appeared, to interrupt the arrhythmia (0.5 ml/min of a solution of 1.1 mol $\cdot$ l^{-1}). The KCl infusion was stopped when the fibrillation ceased.

2.2. Determination of adenosine and its degradation products

The coronary effluent was collected in fractions before, during and after the coronary occlusion, until the end of the reperfusion. The perfusate dropped directly into 20 ml of distilled water of 92°C. Afterwards the perfusates were cooled and filtered, and shaken for 30 min with 0.2 mg activated charcoal per ml perfusate for adsorption of adenosine and its degradation products. The charcoal was separated by centrifugation, and for elution of the nucleosides and oxypurines shaken for 90 min with 6 ml 50% ethanol (adjusted to pH 10 with NaOH). Again, the charcoal was separated by centrifugation (2 times) and the supernatant was concentrated by the factor 3–10 by evaporation at 60°C.

The nucleosides and oxypurines were determined enzymatically as described in detail previously (Bernauer, 1991). Briefly, the method proposed by Heinz and Reckel (1985) for the determination of adenosine was modified for our purposes and extended to the simultaneous measurement of also inosine, hypoxanthine, xanthine and uric acid. In this assay, adenosine deaminase, nucleoside phosphorylase and xanthine oxidase degrade adenosine finally to uric acid. Hydrogen peroxide formed during the xanthine oxidase reaction, in the presence of catalase converts ethanol into acetaldehyde. The latter is converted by aldehyde dehydrogenase into acetic acid. Hereby, NAD(P) is reduced to NAD(P)H, which is measured photometrically at 334 nm. The values for hypoxanthine and xanthine in the Results section are given together as 'hypo-/xanthine', since both are converted simultaneously by xanthine oxidase into uric acid. (For all further details concerning the exact enzyme and substrate composition of the assay, the accuracy and specificity of the determination, see Bernauer, 1991.)

To test whether the antiarrhythmics impaired the extraction and determination procedure for adenosine and its degradation products, two different methods were used: The antiarrhythmics were added to coronary perfusates collected from isolated hearts; or Tyrode solution, with or without the addition of antiarrhythmics, was spiced with adenosine, inosine, hypoxanthine and uric acid. Samples of the perfusates, or the Tyrode solution respectively, were subjected to the whole exctraction and determination procedure. The most relevant drug concentrations applied in the experiments, were tested: quinidine 30 μmol $\cdot$ l^{-1}; lidocaine 30, and 70 μmol $\cdot$ l^{-1}, gallopamil 0.2 μmol $\cdot$ l^{-1}. In none of the tests the antiarrhythmics decreased the yield of the nucleosides and oxypurines.

2.3. Drugs and chemicals

Lidocaine and quinidine sulfate were from Sigma Chemicals (Deisenhofen, Germany), gallopamil hydrochloride from Knoll (Ludwigshafen, Germany). The enzymes and NAD were purchased from Boehringer (Mannheim, Germany). Ethanol, and the substances for the Tyrode solution were of analytical grade and purchased from Merck (Darmstadt, Germany). Chlorophyllin was from Serva (Heidelberg, Germany).

2.4. Statistics

All mean values are arithmetic means $\pm$ standard errors of the means. When multiple comparisons between different groups were performed, analysis of variance was combined with the Bonferroni test (Wallenstein et al., 1980). When only two values had to be compared, at first the homogeneity of the variances

was controlled with the F-test, and then calculation of significance was performed with the t-test for homogeneous or heterogeneous variances, respectively.

3. Results

3.1. Extent of the non-perfused ('ischaemic') areas

Ligation of the left coronary artery led to the exclusion of a considerable part of the myocardium from the tissue perfusion. For instance, in the control group without drug application the non-perfused ('ischaemic') area was 50.7 ± 3.9% of the wet weight of the hearts. In none of the other groups the extent of the non-perfused area was significantly different from that of the controls.

The coronary ligation led to a decrease in the coronary flow, which corresponded well with the extent of the non-perfused area. For instance, in the untreated controls the flow was decreased by 45.7 ± 3.7%. The release of the coronary ligature promptly restored the coronary flow.

3.2. Occurrence of arrhythmias

During the 5 min of coronary occlusion arrhythmias were of only minor importance. In the untreated control hearts premature ectopic beats were observed, and short periods of ventricular tachycardia. In four of the

Table 1
Release of adenine nucleotide metabolites (adenosine and its degradation products) in isolated perfused rat hearts before coronary occlusion (normoxia), during coronary occlusion, and during coronary reperfusion

Drug		Release of adenine nucleotide metabolites (nmol · min^{-1} · g dr wt^{-1})				
		Sum of metabolites	Individual metabolites			
			Adenosine	Inosine	Hypo-/xanth.	Uric acid
–	Normoxia	8.2 ± 1.7	0.9 ± 0.3 (11.0%)	5.3 ± 1.2 (64.6%)	1.3 ± 0.3 (15.9%)	0.7 ± 0.2 (8.5%)
(n = 10)	Coron. occlusion	18.8 ± 5.2	2.8 ± 0.5 (14.9%)	13.1 ± 3.9 (69.7%)	2.4 ± 0.8 (12.8%)	0.5 ± 0.4 (2.7%)
	Reperfusion	162.9 ± 12.3	40.0 ± 3.4 (24.6%)	111.6 ± 8.3 (68.5%)	10.5 ± 1.3 (6.5%)	0.8 ± 0.2 (0.5%)
Quinidine	Normoxia	6.3 ± 0.1	0.8 ± 0.2 (12.7%)	3.5 ± 0.7 (55.6%)	1.2 ± 0.2 (19.1%)	0.8 ± 0.3 (12.7%)
3 μmol · l^{-1}	Coron. occlusion	11.3 ± 2.5	2.3 ± 0.8 (20.4%)	6.6 ± 1.5 (58.4%)	1.8 ± 0.4 (15.9%)	0.6 ± 0.2 (5.3%)
(n = 9)	Reperfusion	88.7 ± 23.9 [a]	22.6 ± 7.4 (25.5%)	59.1 ± 15.3 [a] (66.6%)	6.2 ± 1.5 (7.0%)	0.8 ± 0.2 (0.9%)
30 μmol · l^{-1}	Normoxia	15.0 ± 3.3	3.3 ± 0.8 (22.0%)	8.9 ± 1.9 (59.3%)	1.7 ± 0.5 (11.3%)	1.1 ± 0.3 (7.3%)
(n = 7)	Coron. occlusion	19.2 ± 3.6	4.1 ± 0.6 (21.4%)	11.7 ± 2.5 (60.9%)	2.1 ± 0.6 (10.9%)	1.3 ± 0.6 (6.8%)
	Reperfusion	48.0 ± 6.2 [b]	12.5 ± 2.1 [b] (26.0%)	31.8 ± 4.2 [b] (66.3%)	3.0 ± 0.4 [b] (6.3%)	0.7 ± 0.2 (1.5%)
Lidocaine	Normoxia	9.3 ± 1.4	1.3 ± 0.4 (14.0%)	6.1 ± 1.0 (65.6%)	1.5 ± 0.3 (16.1%)	0.4 ± 0.2 (4.3%)
3 μmol · l^{-1}	Coron. occlusion	18.0 ± 3.9	3.6 ± 1.0 (20.0%)	12.0 ± 2.8 (66.7%)	2.2 ± 0.4 (12.2%)	0.2 ± 0.2 (1.1%)
(n = 5)	Reperfusion	189.8 ± 6.1	53.9 ± 2.4 [a] (28.4%)	122.1 ± 4.6 (64.3%)	13.0 ± 0.8 (6.9%)	0.8 ± 0.4 (0.4%)
30 μmol · l^{-1}	Normoxia	12.6 ± 3.1	2.3 ± 0.5 (18.3%)	7.7 ± 2.0 (61.1%)	2.2 ± 0.5 (17.5%)	0.4 ± 0.3 (3.2%)
(n = 6)	Coron. occlusion	18.9 ± 4.0	3.4 ± 0.7 (18.0%)	11.2 ± 2.6 (59.3%)	3.1 ± 0.5 (16.4%)	1.2 ± 0.5 (6.4%)
	Reperfusion	60.7 ± 12.1 [b]	14.9 ± 3.1 [b] (24.6%)	40.0 ± 8.2 [b] (65.9%)	4.9 ± 0.8 [b] (8.1%)	0.9 ± 0.4 (1.5%)
Gallopamil	Normoxia	13.1 ± 1.6	3.4 ± 0.8 [a] (26.0%)	6.0 ± 1.0 (46.0%)	2.2 ± 0.9 (16.8%)	1.5 ± 0.5 (11.5%)
0.2 μmol · l^{-1}	Coron. occlusion	15.3 ± 2.2	3.5 ± 0.7 (22.9%)	8.4 ± 1.3 (54.9%)	1.3 ± 0.1 (8.5%)	2.1 ± 0.6 (13.7%)
(n = 6)	Reperfusion	31.8 ± 2.4 [b]	6.9 ± 0.7 [b] (21.7%)	20.3 ± 1.8 [b] (63.8%)	2.3 ± 0.2 [b] (7.2%)	2.3 ± 0.4 (7.2%)

Values are means ± S.E.M. The sum of adenosine and its degradation products is given ('Sum of metabolites'), as well as the individual metabolites, and their percent contribution (in brackets). Hypo-/xanth. = hypoxanthine + xanthine. g dr wt = gram dry weight of the hearts. n = number of experiments. [a] $P < 0.05$, [b] $P < 0.01$. Significances refer to the respective values in the group without drug application.

experiments ischaemic arrhythmias were completely lacking. Coronary reperfusion, however, provoked a severe state of tachyarrhythmia, usually beginning with extrasystolia shortly after releasing the coronary ligature, and followed by ventricular tachycardia, which converted in all experiments into ventricular fibrillation (Fig. 1).

A concentration of 30 μmol · l^{-1} quinidine completely prevented the reperfusion-induced fibrillation. In two out of seven experiments some premature ectopic beats were still observed. When only 3 μmol · l^{-1} was applied, however, four out of nine hearts fibrillated during the coronary reperfusion. Arrhythmias during the coronary occlusion were practically prevented by both concentrations of quinidine.

Lidocaine was somewhat less effective. Even in the presence of 30 μmol · l^{-1} most of the hearts reacted with ventricular tachycardia during reperfusion, and one out of six hearts with ventricular fibrillation. When only 3 μmol · l^{-1} was applied, arrhythmias were as severe as in untreated hearts. And both concentrations of lidocaine did not completely prevent the arrhythmias during the coronary occlusion.

Gallopamil was very effective as an antiarrhythmic. Already 0.2 μmol · l^{-1} completely prevented reperfusion-induced ventricular tachycardia and fibrillation. Premature ectopic beats and salvos, however, were still observed. During the coronary occlusion, in three experiments some ectopic beats were seen.

3.3. Release of adenosine and its degradation products

Considerable amounts of adenosine and its degradation products were released in the untreated hearts with coronary occlusion and reperfusion. As long as the left coronary artery was occluded, only minor quantities appeared in the perfusates. High amounts of the nucleosides and oxypurines were released, however, shortly after reopening the coronary artery.

In the normoxic state of the myocardium before coronary occlusion, as well as during coronary occlusion and reperfusion, inosine represented the greater part of the released metabolites. Adenosine made up 11.0% before the coronary occlusion. It increased to 24.6% during the reperfusion, whereas the percentage of hypoxanthine, xanthine, and uric acid was decreased (Table 1).

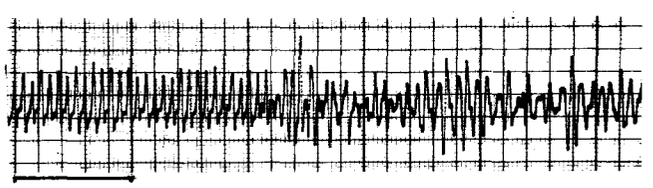

Fig. 1. Electrocardiogram of an isolated perfused rat heart during coronary reperfusion. The point is shown where high-frequent, but still regular ventricular tachycardia converts into ventricular fibrillation. Line at the bottom = 0.5 s.

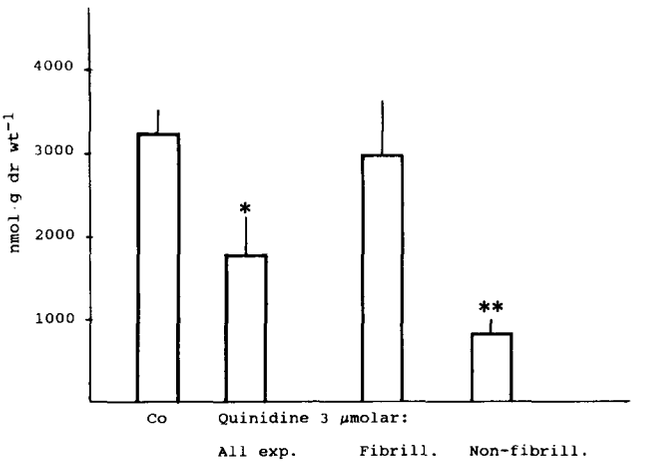

Fig. 2. The total amount of adenosine and its degradation products released during 20 min of coronary reperfusion is shown. For clarity, the percent contribution of the individual metabolites is omitted. With quinidine 3 μM, a moderate decrease of the release was obtained when all experiments were considered together (second column: 'All exp.'; $n = 9$). Subdividing the hearts into those with, and without reperfusion-induced ventricular fibrillation ($n = 4$; $n = 5$) revealed that in hearts in which fibrillation was prevented by the antiarrhythmic, the release was decreased in a highly significant way; but not in the fibrillating hearts. Co = untreated control hearts with coronary ligation and reperfusion ($n = 10$; all hearts fibrillating during the reperfusion). g dr wt = gram dry weight of the hearts $^{*}P < 0.05$, $^{**}P < 0.01$.

All three antiarrhythmics decreased the release during the coronary reperfusion. As Table 1 shows, with 30 μmol · l^{-1} quinidine and lidocaine a highly significant effect was obtained. At 3 μmol · l^{-1} quinidine a less pronounced, but still statistically significant decrease was observed, whereas lidocaine was ineffective at this concentration.

In the group with 3 μmol · l^{-1} quinidine, the release of nucleosides and oxypurines was reduced only in hearts in which the fibrillation was prevented (Fig. 2). The importance of the antifibrillatory effect for the decrease of the release is particularly evident if the course of the release is compared in fibrillating and non-fibrillating hearts (Fig. 3).

Gallopamil (0.2 μmol · l^{-1}) very effectively reduced the release of the nucleosides and oxypurines during the coronary reperfusion (Table 1). Adding up the release during the 20 min of reperfusion yielded a total of only 636.3 ± 47.8 nmol/g dry weight of the hearts, instead of 3257.9 ± 246.4 in the controls ($p < 0.001$).

In further groups of experiments antiarrhythmics were applied only during the coronary reperfusion. Lidocaine was infused into the fluid stream about 5 cm before the heart, immediately after re-opening the coronary ligature, in amounts of 0.2 mg · min^{-1}, result-

ing in a final concentration of 68 ± 9.8 μmol · 1^{-1} in the coronary perfusate. Ventricular fibrillation was prevented in three out of six experiments. The release of adenosine and its degradation products was decreased in a highly significant way in the non-fibrillating hearts, but not in the fibrillating hearts (Fig. 4).

In 23 experiments gallopamil was infused immediately after starting the coronary reperfusion in amounts resulting in a final concentration of 4.2 ± 0.4 μmol · 1^{-1} in the coronary perfusate. In five hearts fibrillation was prevented. Other arrhythmias appeared, however, namely ventricular tachycardia, and extrasystolia and salvos. Also in the 18 fibrillating hearts, other kinds of severe arrhythmias were observed in addition. Surprisingly, the release of adenosine and its degradation products was decreased in a highly significant way, irrespective of whether the non-fibrillating hearts were considered, or the fibrillating hearts, or all hearts together (Fig. 4).

When an infusion of potassium chloride was started immediately after the appearance of reperfusion-induced ventricular fibrillation (final concentration in the perfusion fluid 33.2 ± 3.9 mmol · 1^{-1}, including the 2.7 mmol · 1^{-1} already present in the Tyrode solution), the arrhythmia was interrupted in all six experiments. It lasted only 27 ± 6.5 s, on average. The release of adenosine and its degradation products during the 20 min of coronary reperfusion was decreased in a highly significant way, compared with controls in which the fibrillation was not stopped. It amounted only to 1176.2 $\pm$ 172.9 nmol/g dry weight, instead of 3257.9 $\pm$ 246.4 nmol/g dry weight obtained in the controls ($p < 0.001$).

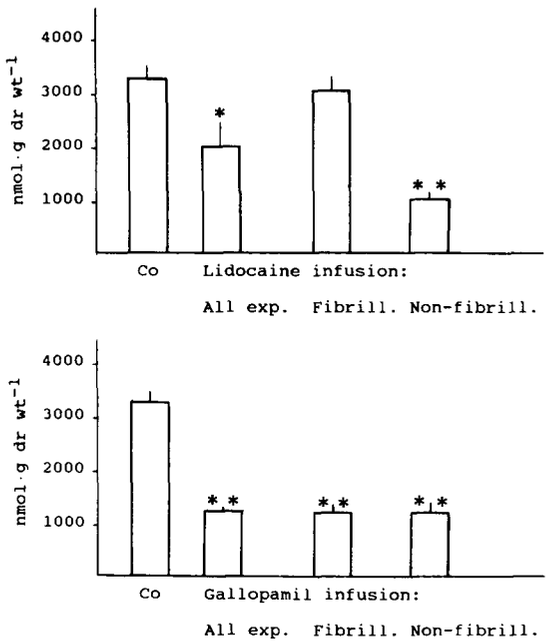

Fig. 4. Total amount of adenosine and its degradation products released during 20 min of coronary reperfusion. Upper part: Infusion of lidocaine during the coronary reperfusion led to minor reduction of the release when all experiments were considered together ('All exp.'; $n = 6$). Subdividing the hearts into those with, and without reperfusion-induced fibrillation ($n = 3$, each) shows that in non-fibrillating hearts the release was decreased in a highly significant way, but not at all in the fibrillating hearts. Lower part: Infusion of gallopamil during coronary reperfusion decreased the release of adenosine and its degradation products in a highly significant way, irrespective of whether all hearts were considered together ($n = 23$), or the fibrillating hearts ($n = 18$), or the non-fibrillating hearts ($n = 5$). Co = untreated control hearts with coronary occlusion and reperfusion ($n = 10$; all hearts fibrillating during the reperfusion). g dr wt = gram dry weight of the hearts. $^{*}P < 0.05$, $^{**}P < 0.01$.

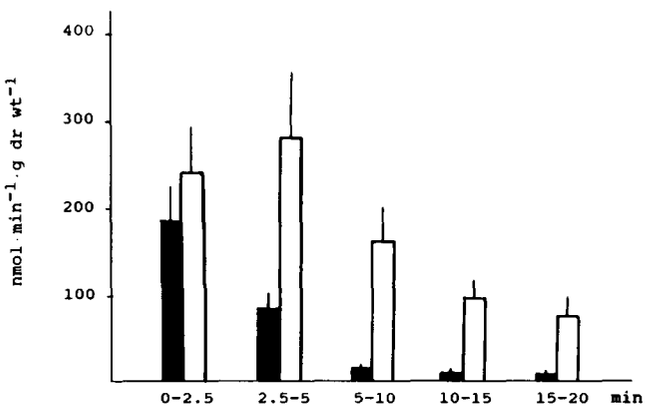

Fig. 3. The time course of the release of adenosine and its degradation products during the coronary reperfusion in the quinidine-treated hearts of Fig. 2 is shown. Open columns represent the fibrillating hearts, filled columns the non-fibrillating hearts. Whereas in non-fibrillating hearts the release goes back to very low values after some minutes, in fibrillating hearts there is a long-lasting high release of adenosine and its degradation products. g dr wt = gram dry weight of the hearts.

3.4. Coronary flow

The flow during the coronary reperfusion, determined by the timed collection of the perfusates, equalled that before the coronary ligation, or even exceeded it. For instance, in the untreated controls the flow was 10.6 ± 1.5 ml/min/g wet weight before coronary ligation, 5.5 ± 0.5 ml/min/g wet weight during the coronary occlusion, and 13.3 ± 1.0 ml/min/g wet weight during the reperfusion.

When quinidine or lidocaine was present from the outset of the experiments, the coronary flow was essentially the same as in the controls. In the presence of 0.2 μmol · 1^{-1} gallopamil, the coronary flow was considerably increased (27.2 ± 1.3 ml/min/g wet weight). It decreased to $59.5 \pm 2.8\%$ of this value after coronary ligation (extent of the 'ischaemic' area $50.2 \pm 1.0\%$ of the heart). During coronary reperfusion it reached again the pre-occlusion value.

Application of gallopamil by infusion during the coronary reperfusion increased the coronary flow to 138.8 ± 10.8% of the pre-ligation value. There was no difference between fibrillating and non-fibrillating hearts. Also infusion of lidocaine during the reperfusion increased the coronary flow (to 132.2 ± 16.7% of the pre-ligation value). When potassium chloride was infused, however, the flow during the reperfusion amounted only to 76.8 ± 8.4% of the value determined before the coronary occlusion.

3.5. Sinus rate

In the control hearts the sinus rate immediately before coronary occlusion was 258 ± 29/min. Application of 30 $\mu mol \cdot l^{-1}$ quinidine already at the outset of the experiments decreased the rate to 129 ± 14/min ($P < 0.01$), whereas 3 $\mu mol \cdot l^{-1}$ had no effect. Neither 3 nor 30 $\mu mol \cdot l^{-1}$ lidocaine decreased the sinus rate significantly. There was only a trend to a decrease with the higher concentration (203 ± 16/min). And also with 0.2 $\mu mol \cdot l^{-1}$ gallopamil only a trend to a decreased sinus rate was observed (200 ± 11/min).

4. Discussion

In the present experiments different kinds of antiarrhythmics markedly decreased the release of adenosine and its degradation products during post-ischaemic coronary reperfusion. The extent of the release was about the same when the antiarrhythmics were applied only during the reperfusion, as it was with application before the coronary occlusion. This indicates that in the experiments without an antiarrhythmic there was a portion of adenosine which was formed during the reperfusion, and which appeared in the coronary perfusate in addition to the amounts which were liberated during coronary occlusion by the ischaemic breakdown of ATP.

In the experiments with quinidine and lidocaine, a decrease in the adenosine release was only seen when the antiarrhythmics prevented the reperfusion-induced ventricular fibrillation. This suggests that fibrillation triggers the release of adenosine. This is further supported by the experiments with infusion of potassium chloride during the coronary reperfusion. Interruption of the fibrillation immediately after its appearance decreased the release to a level found otherwise in non-fibrillating hearts.

The effect of the class IV antiarrhythmic gallopamil was somewhat different from that of the class I antiarrhythmics quinidine and lidocaine. Indeed, the release of adenosine and its degradation products was antagonized very effectively, at a much lower concentration than with quinidine and lidocaine. In the experiments with application of gallopamil before the coronary occlusion, this is not surprising. One would expect, of course, that in the presence of gallopamil with its Ca^{2+} channel blocking activity, the breakdown of ATP within the ischaemic area is reduced, and hence also the release of adenosine. Indeed, several investigations in isolated heart preparations have shown a preservation of myocardial ATP when the Ca^{2+} channel blocking drugs verapamil, D 600 (i.e. gallopamil), nifedipine or diltiazem were applied before or during a period of ischaemia or anoxic perfusion, respectively (Nayler et al., 1976; Watts et al., 1980, 1986; Bush et al., 1981; Ferrari et al., 1989). A possible role of an antiarrhythmic effect of the drugs was not discussed in connection with these observations. In our experiments, however, the release of adenosine and its degradation products was decreased in a highly significant way also when gallopamil was applied only during the coronary reperfusion. Since the release was decreased also in fibrillating hearts, Ca^{2+} entry into the cardiomyocytes seems to be an important link between the electrical phenomenon of fibrillation and the release of adenosine.

Several years ago, it was seen in isolated perfused rat hearts that nifedipine and diltiazem decreased the release of the nucleosides and oxypurines, when they were applied before or during myocardial ischaemia (De Jong et al., 1982, 1984; Harmsen et al., 1983). A model of low-flow ischaemia was used, in which the major part of the metabolites appeared in the coronary perfusate already during the ischaemic period itself. The release went down during the subsequent reperfusion. Obviously, reperfusion arrhythmias did not appear in this experimental model. It seems that the findings concerned that part of purine release which stems from the ischaemic breakdown of ATP. Our experiments mainly deal with the additional, arrhythmia-induced release of nucleosides and oxypurines, which occurs during reperfusion after complete interruption of the coronary flow.

It is unlikely that the decrease in the purine release was due to nucleoside transport inhibition by the antiarrhythmics. In the rat, the nucleoside transporter is very insensitive to pharmacological inhibition (Hopkins and Goldie, 1971; Kolassa et al., 1971; Clanachan et al., 1987; Plagemann et al., 1988). Furthermore, in the presence of a nucleoside transport inhibitor the release of adenosine is increased, whereas the release of its degradation products is decreased, resulting in a marked increase in the adenosine/inosine ratio (Van Belle et al., 1987; Hugtenburg et al., 1991). In our experiments, the adenosine/inosine ratio during the coronary reperfusion was always the same, irrespective of whether antiarrhythmics were present, or not (Table 1). Moreover, it would be very difficult to understand why lidocaine and quinidine did not affect the metabolite release in fibrillating hearts, but very extensively in

non-fibrillating hearts. And it is known of the Ca^{2+} channel blocking agents of the phenylalkylamine type (to which belongs gallopamil) that they are very ineffective inhibitors of the nucleoside transporter (Plagemann et al., 1988).

There was no parallelism between the coronary flow during the reperfusion, and the height of the release. Indeed, in the experiments with KCl stop of fibrillation the coronary flow was relatively low, as was also the release of adenosine and its metabolites. However, in the gallopamil groups a high coronary flow was combined with a small release. And also the effects of quinidine and lidocaine cannot be explained by alterations in the coronary flow, and hence differences in the washout of adenosine and its metabolites from the cardiac tissue.

Is the effect of antiarrhythmics on the adenosine release of any importance? We want to be cautious with our speculations. It may be allowed, however, to point to some possible implications of this phenomenon in the situation of myocardial ischaemia. Besides its well-known vasodilatory potency, adenosine can also affect impulse generation and conduction in the heart. For instance, exogenously applied adenosine was shown to antagonize ventricular automaticity in rat and guinea-pig hearts (Szentmiklosi et al., 1980; Heller and Olsson, 1985), and also the generation of slow action potentials in rat and guinea-pig atria (Schrader et al., 1975; Knabb et al., 1984). Very small concentrations of adenosine (0.1–10 $nmol \cdot l^{-1}$) were found to stimulate the automaticity in isolated right ventricles of rats (and this effect depended on the presence of endogenous catecholamines), whereas higher concentrations (10–100 $\mu mol \cdot l^{-1}$) had an inhibitory action (Laorden et al., 1986; Hernandez et al., 1989). Furthermore, adenosine inhibits atrioventricular conduction (Drury and Szent-Györgyi, 1929), and therefore released adenosine seems to be partly responsible for the well-known impairment of impulse transmission in cardiac oxygen deficiency (Belardinelli et al., 1980; Clemo and Belardinelli, 1986). As in coronary-ligated rats and dogs the application of adenosine antagonized ischaemic and reperfusion arrhythmias, the theory was put forward that also endogenously released adenosine may function as an antiarrhythmic agent (Fagbemi and Parratt, 1984; Wainwright and Parratt, 1988). It seems possible, therefore, that the fibrillation-induced release of adenosine which is suggested by our findings, aims at the interruption of the arrhythmia. And antiarrhythmic drugs seem to influence arrhythmias in a more complicated way than is usually believed, namely, in addition to their direct electrophysiological effects, by interfering with the process of adenosine release.

Finally, a special aspect should be briefly mentioned. In hearts with ischaemia and reperfusion a loss of large amounts of nucleosides may be unfavourable for the post-ischaemic recovery, as part of them are normally used for the resynthesis of energy-rich phosphates. In a previous investigation, the occurrence of fibrillation impeded the replenishment of the myocardial ATP stores, or even led to further loss of energy-rich phosphates. Application of antiarrhythmic drugs clearly improved the post-ischaemic metabolic recovery (Latocha and Bernauer, 1991). Our present findings indicate that antiarrhythmic drugs indeed are able to reduce the loss of nucleosides from hearts with ischaemia and reperfusion.

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