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Concerning the effect of the K^+ channel blocking agent glibenclamide on ischaemic and reperfusion arrhythmias

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Abstract

Reports on effects of ATP-dependent K^+ channel modulating drugs on ischaemia-induced cardiac arrhythmias have been scarce and contradictory. The channel blocking agent glibenclamide (glyburide) has been considered as an antiarrhythmic candidate, because it antagonizes the ischaemic K^+ efflux and the shortening of the refractory period. In the present investigation its effects were tested, therefore, in rat hearts with coronary occlusion and reperfusion. In untreated hearts, tachyarrhythmias occurred during the reperfusion, and less pronounced during the coronary occlusion itself. Large amounts of adenosine and its degradation products were released during the coronary reperfusion, particularly from hearts which developed ventricular fibrillation. Glibenclamide (0.1 and 1.0 μmol/l perfusion fluid) neither antagonized the ischaemic nor the reperfusion arrhythmias. Ischaemic arrhythmias were even intensified. Also in control hearts without coronary occlusion, pro-arrhythmic effects of glibenclamide were observed. Furthermore, the coronary flow was considerably decreased by the drug, and the release of adenosine and its metabolites was significantly increased. Sodium nitroprusside antagonized the glibenclamide-induced decrease in the coronary flow, but did not prevent the arrhythmias. The Ca^{2+} channel blocking agent gallopamil increased the coronary flow, decreased the adenosine release, and antagonized the arrhythmias in hearts with and without glibenclamide. In conclusion, the present findings do not favour the idea of an antiarrhythmic effect of glibenclamide. Rather, some propensity to the occurrence of arrhythmias can be produced by the drug.

Keywords: Glibenclamide (glyburide); K^+_{ATP} channel; Myocardial ischemia; Cardiac arrhythmia; Adenosine release

1. Introduction

K^+ channels, which are regulated by the intracellular concentration of ATP (ATP-sensitive K^+ channels, K^+_{ATP} channels), have first been discovered in cardiomyocytes (Noma, 1983; Trube and Hescheler, 1983, 1984), and subsequently also in other organs and tissues (see Lazdunski, 1994). Several substances are available which can modulate the open state of these channels. Whereas glibenclamide (glyburide) is a K^+_{ATP} blocking agent, cromakalim and pinacidil are K^+_{ATP} openers. (For a review see Edwards and Weston, 1993.) The sulfonylurea glibenclamide has gained great importance as an oral antidiabetic, because it increases the insulin secretion. Since activation of the ATP-sensitive K^+ channels in vascular smooth muscle is combined with hyperpolarization and consequent relaxation (Standen et al., 1989), K^+_{ATP} openers are of interest as new antihypertensives.

In myocardial ischaemia, the K^+_{ATP} channels are activated, and increased efflux of K^+ leads to reduction of the action potential duration (Cascio et al., 1990; Gasser and Vaughn-Jones, 1990; Wilde et al., 1990). By this way, a decrease in the Ca^{2+} influx into the cardiomyocytes is achieved. Since Ca^{2+} ions play a key role in the deleterious effects which ischaemia has on myocardial cells, the opening of the K^+_{ATP} channels is considered to be a natural protective mechanism (see Escande and Cavero, 1992; Gross and Auchampach, 1992). Possibly, also synthetic K^+ channel openers have a cardioprotective effect (Grover, 1994).

Rather unclear, however, is the effect of K^+_{ATP} channel modulators on the ischaemia-induced arrhythmias. In coronary ligated dogs pinacidil was found antiarrhythmic (Kerr et al., 1985) as well as pro-arrhythmic (Chi et al., 1990). Glibenclamide on the other hand, able to abolish the hypoxic shortening of the action potential (Fosset et al., 1988), antagonized ventricular fibrillation in globally ischaemic rat hearts (Kantor et al., 1987, 1990; Wolleben et

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al., 1989). However, in experiments with myocardial infarction in dogs, the findings with this drug were contradictory (Chi et al., 1989; Billman et al., 1993).

With the present investigation we want to contribute to the question whether the arrhythmias can be influenced by K^+_{ATP} channel modulating drugs. We were particularly interested in glibenclamide and tested it in rat hearts with coronary occlusion and reperfusion. Also the release of adenosine and its metabolites from the hearts was investigated. The amount of released adenosine is an indicator of the severity of the myocardial oxygen deficiency. And we expected that the K^+ channel blocker glibenclamide would perhaps decrease the coronary flow, competing with the coronary vasodilator adenosine, which is considered to be a K^+_{ATP} channel opener (Daut et al., 1990; Kirsch et al., 1990; Dart and Standen, 1993).

2. Materials and methods

2.1. Experimental procedure

Male Sprague-Dawley rats (body weight 538 ± 5 g), anaesthetized with pentobarbital sodium, 45 mg/kg intraperitoneally) were artificially respired via a tracheal cannula. The hearts were excised and perfused in a double-walled, water-heated chamber according to the Langendorff technique (37.5°C; constant pressure of 7.85 kPa). As perfusion fluid Tyrode's solution of the following composition (mmol/l) was used: NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6 (gassed with 95% O_2 and 5% CO_2, and filtered prior to use through a washed paper filter; pH value of the gassed solution 7.3). The electrocardiogram for the analysis of arrhythmias and the determination of the sinus rate was monitored continuously via two platinum blade electrodes attached to the surface of the left and right ventricle.

When the hearts had adapted to the situation of artificial perfusion (after 30 min), a 10 min sample of the coronary perfusate was collected under continuous stirring in an ice-chilled beaker. Then, the left coronary artery was occluded by ligation beneath the left auricular appendage (in the rat, the left coronary artery is a single descending trunk. There is no left circumflex artery; Johns and Olson, 1954).

The coronary ligature was released after 2, or 5, or 10 min, respectively, and coronary reperfusion was allowed for 20 min. During the whole period of coronary occlusion and reperfusion, the coronary perfusate was further collected in fractions. Then, the coronary ligature was tied again, and coronary perfusion was performed with a solution of chlorophyllin in normal saline (5 mg/ml). By this procedure, the well-perfused parts of the myocardium are stained green, whereas the non-perfused myocardium remains unstained and retains its bright red colour. Both parts of the myocardium, the 'infarct' area and the normally perfused part, were carefully separated by scissors, and the wet and dry weights of both parts were determined.

In experiments with glibenclamide, the drug was added to the perfusion fluid at the onset of the coronary perfusion. It was dissolved in 99.8% ethanol, and applied in final concentrations of 0.1 and 1 μmol/l (added volume always 0.5 ml/l perfusion fluid). Solvent controls received only ethanol (0.5 ml/l). In some experimental groups, gallopamil and sodium nitroprusside were tested. Gallopamil hydrochloride was added in final concentrations of 0.2 μmol/l to the perfusion fluid. Sodium nitroprusside was infused (under protection from light) into the fluid stream 10 cm before the heart (0.125 ml/min of a solution of 300 μg/ml 0.9% NaCl).

2.2. Determination of adenosine and its degradation products

For extraction of the nucleosides and oxypurines the chilled coronary perfusates were filtered and immediately shaken for 30 min with activated charcoal (0.2 mg/ml). After centrifugation, adenosine and its metabolites were eluted from the charcoal with 50% ethanol, adjusted to pH 10 with NaOH (shaking for 90 min). Thereafter, the charcoal was separated by centrifugation (2 times), and the supernatant was concentrated by evaporation at 60°C.

The determination of the nucleosides and oxypurines in the concentrated supernatants was performed enzymatically. For this purpose, we extended the method which was proposed by Heinz and Reckel (1985) for the determination of adenosine, to determine also its degradation products. In this assay, adenosine is degraded to uric acid by adenosine deaminase, nucleoside phosphorylase, and xanthine oxidase; and the uric acid is further converted into allantoin by uricase. Hydrogen peroxide, which is formed during the xanthine oxidase reaction, converts ethanol into acetaldehyde (with the aid of catalase), and the latter is converted into acetic acid by aldehyde dehydrogenase. Thereby, NAD(P) is reduced to NAD(P)H, which is measured photometrically at 334 nm. The individual metabolites in the perfusates can be determined, one after the other, directly in the photometer cuvette, by adding the adenosine degrading enzymes in the reverse order, i.e., uricase at first, and so on. It should be noted that there is no problem concerning NADH, possibly released in small amounts from the ischaemic myocardium into the perfusates. This starting value is set as zero value in the photometric determination (for all further details concerning the accuracy and specificity of the assay procedure, see Bernauer, 1991).

2.3. Drugs and chemicals

Glibenclamide was from Research Biochemicals International (Natick, MA, USA)(purchased from Bio Trend

Company, Cologne, Germany); gallopamil hydrochloride was from Knoll Company (Ludwigshafen, Germany), sodium nitroprusside from Sigma Chemicals (Deisenhofen, Germany) and ethanol (analytical grade) from Merck (Darmstadt, Germany). The enzymes and coenzymes for the determination of adenosine and its metabolites were from Boehringer-Mannheim (Mannheim, Germany).

2.4. Statistics

Mean values are arithmetic means ± standard errors of the means (S.E.M.). When multiple comparisons of data were performed, analysis of variance was applied, combined with the Bonferroni test (Wallenstein et al., 1980). When only two mean values had to be compared, at first the homogeneity of the variances was controlled with the *F*-test, and then the *t*-test for homogeneous, or heterogeneous variances, respectively, was used. When the incidence of an event in different groups was compared, the chi-square test was used.

The severity of ischaemic and reperfusion arrhythmias was evaluated with the aid of the arrhythmia severity index (ASI; Bernauer, 1986). The use of this index allows the statistical comparison and calculation of significances in experiments with tachyarrhythmias. The occurrence of up to 10 premature ectopic beats during the respective observation time is weighted with the value 1, the occurrence of more than 10 ectopic beats is weighted with the value 2. Ventricular tachycardia or ventricular flutter is weighted with the value 3, and ventricular fibrillation with the value 4. If more than one kind of arrhythmia appears, the respective values are added up to yield the ASI value, representing a numerical term for the severity of the dysrhythmia.

3. Results

3.1. Coronary flow, and appearance of arrhythmias

In experiments without any drug application, the occlusion of the left coronary artery reduced the flow through the heart to about the half. The extent of the flow decrease corresponded well to the extent of the non-perfused ('ischaemic') area, determined by the dye perfusion of the myocardium. Reopening of the coronary artery completely restored the coronary flow.

Glibenclamide (0.1 and 1 μmol/l) significantly decreased the coronary flow (Table 1). Occlusion of the left coronary artery further decreased the flow, by about 50%, like in the untreated hearts. Also in two control groups without coronary occlusion and reperfusion, glibenclamide itself (0.1 and 1.0 μmol/l; $n = 4$, $n = 7$) decreased the coronary flow (data not listed in Table 1). The solvent ethanol had no obvious effect.

During the coronary reperfusion, and less marked during the coronary occlusion itself, cardiac arrhythmias appeared. In the experiments with 2, or 5 min of coronary occlusion, only in one heart of each group extrasystolia, salvos, and ventricular tachycardia occurred during the myocardial 'ischaemia'. And in the group with 10 min of coronary ligation, 5 of the 8 hearts reacted with extrasystolia. Glibenclamide intensified the ischaemic arrhythmias, particularly at 1 μmol/l, where in all except one of the hearts with 2, or 5, or 10 min of coronary occlusion these arrhythmias were seen.

Very marked tachyarrhythmias occurred during the coronary reperfusion, usually beginning with premature ectopic beats and salvos, and sudden appearance of ventricular tachycardia or flutter, converting into ventricular

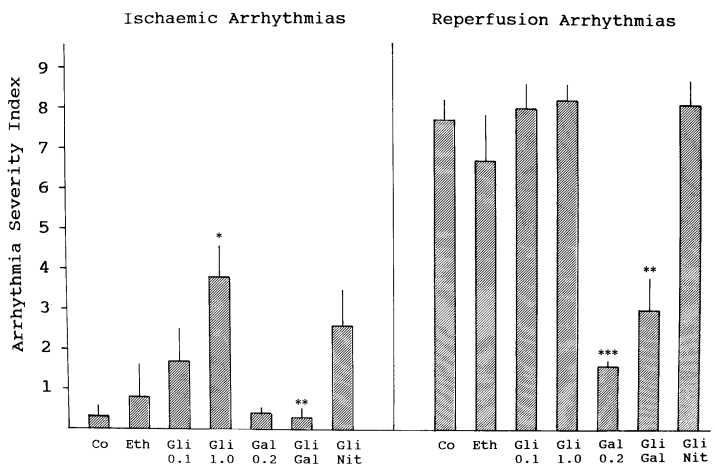

Fig. 1. The severity of the ischaemic and reperfusion arrhythmias is evaluated with the aid of the arrhythmia severity index (see Section 2), which allows statistical comparison of the arrhythmias in different experimental groups. The groups are those with 5 min of coronary ligation and 20 min of reperfusion, listed in Table 2, where also the numbers of the experiments are given. Co = untreated controls; Eth = ethanol solvent controls; Gli = glibenclamide (μmol/l); Gal = gallopamil (μmol/l); Gli/Gal = glibenclamide 1 μmol/l + gallopamil 0.2 μmol/l; Gli/Nit = glibenclamide 1 μmol/l + sodium nitroprusside (for final concentration in the perfusion fluid see text). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Significances refer to the experimental group without the respective drug (i.e., Gli 1.0 vs. Eth; Gli/Gal vs. Gli 1.0; Gal 0.2 vs. Co).

Table 1
Influence of the drugs on the coronary flow

Drug	Coronary flow (ml/min per g wet weight)			Ischaemic area (% of wet weight)	*n*
	Before coronary occlusion	During coronary occlusion	During coronary reperfusion		
–	14.4 ± 1.46	7.5 ± 0.61	14.7 ± 1.00	48.1 ± 1.45	22
Ethanol 8.57 mmol/l	14.7 ± 1.44	7.6 ± 0.61	14.8 ± 1.24	47.9 ± 2.71	6
Glibenclamide 0.1 μmol/l	8.1 ± 0.93 [a]	4.5 ± 0.65 [a]	9.8 ± 1.31 [a]	47.6 ± 2.93	6
Glibenclamide 1.0 μmol/l	6.0 ± 0.57 [b]	3.5 ± 0.37 [b]	7.2 ± 0.62 [b]	47.3 ± 1.74	16
Gallopamil 0.2 μmol/l	26.6 ± 1.01 [b]	16.1 ± 0.62 [b]	25.4 ± 1.27 [b]	48.6 ± 2.24	10
Glibenclamide 1.0 μmol/l + gallopamil 0.2 μmol/l	30.1 ± 1.51 [b]	15.9 ± 2.37 [b]	26.1 ± 0.96 [b]	48.6 ± 2.30	6
Glibenclamide 1.0 μmol/l + nitroprusside	16.2 ± 1.58 [b]	10.5 ± 0.69 [b]	17.3 ± 1.25 [b]	41.4 ± 1.79	7

Values are means ± S.E.M. In the group of untreated controls, and for comparison also in the group receiving only 1 μmol/l glibenclamide, all hearts with 2, 5, and 10 min of coronary occlusion are taken together. All further groups represent experiments with 5 min of coronary ligation. The drugs were present in the perfusion fluid from the outset of the coronary perfusion. Sodium nitroprusside, because of its instability, was infused into the fluid stream in amounts resulting in final concentrations of about 10 μmol/l, depending on the coronary flow (for exact concentration data see text). In the ethanol controls the same ethanol concentration was applied as was present as solvent in the glibenclamide experiments. [a] $P < 0.05$, [b] $P < 0.01$. Significances refer to the values in the group without the respective drug (i.e., comparison of glibenclamide vs. ethanol controls; gallopamil vs. untreated controls; glibenclamide + gallopamil vs. glibenclamide alone; glibenclamide + nitroprusside vs. glibenclamide alone).

fibrillation in 63, 86 and 100%, respectively, of the untreated hearts with 2, or 5, or 10 min of coronary occlusion. Glibenclamide rather had an unfavourable effect on the reperfusion arrhythmias. At 1 μmol/l, ventricular fibrillation occurred in 75, 100 and 100%, respectively, of the hearts with 2, or 5, or 10 min of coronary occlusion.

In Fig. 1 the occurrence of arrhythmias in the experiments with 5 min of coronary occlusion is evaluated with the aid of the arrhythmia severity index (ASI; see Section 2.4). Glibenclamide neither decreased the severity of the ischaemic, nor that of the reperfusion arrhythmias. Infusion of the vasodilating agent sodium nitroprusside into the perfusion fluid stream 10 cm before the heart, antagonized the glibenclamide-induced flow decrease (Table 1; final concentrations of nitroprusside in the perfusion fluid 8.2 ± 0.83 μmol/l before the coronary occlusion, 12.2 ± 0.80 during coronary occlusion, and 7.5 ± 0.59 during reperfusion, depending on the coronary flow). However, neither the ischaemic nor the reperfusion arrhythmias were prevented, in spite of the favourable effect on the coronary flow (Fig. 1). Ventricular fibrillation occurred in 6 out of 7 hearts during the coronary reperfusion. However, in 4 hearts the fibrillation ceased spontaneously already after 38 ± 10 s, whereas this was not the case in the experiments with glibenclamide alone (1 μmol/l; duration of fibrillation 868 ± 127 s), or in the untreated hearts (duration of fibrillation 1133 ± 51 s).

The Ca^{2+} channel blocking agent gallopamil (final concentration 0.2 μmol/l perfusion fluid) not only antagonized the flow-decreasing effect of glibenclamide, but considerably enhanced the coronary flow above the value found in untreated controls (Table 1). In addition, gallopamil had a marked antiarrhythmic effect, in the experiments with, and without glibenclamide.

Glibenclamide had also some arrhythmogenic effect on its own. When all hearts with 1 μmol/l were considered together, 20 out of 23 had premature ectopic beats, and 8 had periods of ventricular tachycardia in addition, already before the coronary occlusion was performed. At 0.1 μmol/l glibenclamide, 3 out of 6 hearts reacted with premature ectopic beats, and one with ventricular tachycardia. In a control group of 7 hearts without coronary occlusion and reperfusion, but with 1 μmol/l glibenclamide, ectopic beats were registered in all experiments. In 2 hearts, finally ventricular fibrillation occurred after a period of ventricular tachycardia. In a further control group with only 0.1 μmol/l glibenclamide, some few premature ectopic beats were seen in 3 out of 4 hearts.

3.2. Release of nucleosides and oxypurines

A release of large amounts of adenosine and its degradation products inosine, hypoxanthine, xanthine, and uric acid from the hearts was observed, especially during the coronary reperfusion. For instance, in the group of untreated control hearts with 5 min of coronary occlusion, 6.8 ± 0.84 nmol/g dry weight were released per minute before the coronary ligation, 15.1 ± 2.76 during the coronary occlusion, and 114.4 ± 18.12 during the reperfusion. The greater part of the released purine compounds was represented by inosine. As an example, in Fig. 2 the contribution of the individual metabolites to the total post-ischaemic release is given for the experiments with 10 min of coronary occlusion. In the group of untreated hearts, inosine made up 60.8% of the total amount, adenosine 27.5%, hypoxanthine + xanthine 9.4%, and uric acid 2.3%. As Table 2 shows, the occurrence of ventricular fibrillation was combined with a particularly high release of the nucleosides and oxypurines.

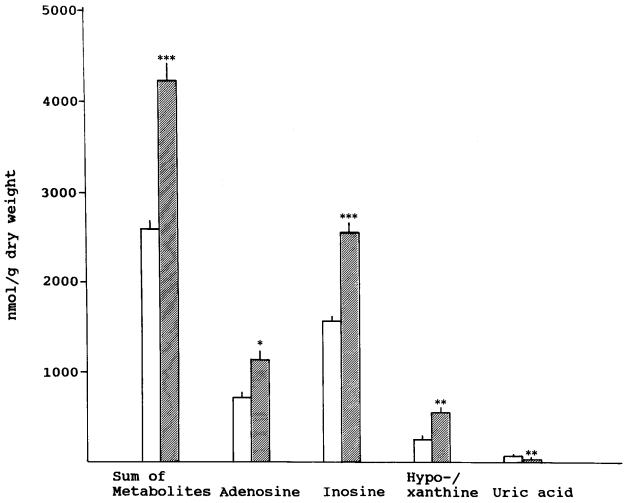

Fig. 2. Release of adenosine and its degradation products from hearts with coronary occlusion and reperfusion, in nmol/g dry weight of the hearts. The sum of all nucleosides and oxypurines is shown, released during 20 min of reperfusion after 10 min of coronary occlusion (Sum of Metabolites), as well as the release of the individual metabolites. The open columns represent hearts without drug treatment ($n = 7$), the hatched columns those with 1 μmol/l glibenclamide ($n = 6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Significances refer to the respective values in the hearts without drug treatment.

In experiments with 5, and 10 min of coronary occlusion, 1 μmol/l glibenclamide significantly increased the release of adenosine and its metabolites during the coronary reperfusion (Table 2). This was not simply due to a higher incidence of fibrillation in the glibenclamide-treated hearts, since it was also found if only the fibrillating hearts were compared. In the experiments with 2 min of coronary occlusion, the effect of glibenclamide did not reach statistical significance, due to the relatively small number of experiments in this group. The solvent ethanol did not significantly influence the release of adenosine and its degradation products (Table 2).

Gallopamil (0.2 μmol/l) very markedly decreased the release during the reperfusion in experiments with 5 min of coronary occlusion (Table 2). And this was also the case when gallopamil was given in addition to glibenclamide

Table 2
Release of nucleosides and oxypurines during postischaemic coronary reperfusion

Coronary occlusion/reperfusion	Drug	Total release (nmol/g dry weight during 20 min of reperfusion)					
		All hearts (n)		Non-fibrillating hearts (n)		Fibrillating hearts (n)	
−/−	−	134±16.5	(9)	134±16.5	(9)	–	
2 min/20 min	−	1162±276.3 [b]	(8)	508±48.8 [c]	(3)	1554±332.6	(5)
	Glibenclamide 1.0 μmol/l	2390±510.0	(4)	1385	(1)	2725±544.0	(3)
5 min/20 min	−	2288±362.5 [c]	(7)	711	(1)	2551±295.5	(6)
	Glibenclamide 0.1 μmol/l	1925±576.2	(6)	780	(1)	2154±647.5	(5)
	Glibenclamide 1.0 μmol/l	3801±292.5 [a]	(6)	–		3801±292.5 [a]	(6)
10 min/20 min	−	2587±103.2 [c]	(7)	–		2587±103.2	(7)
	Glibenclamide 1.0 μmol/l	4244±194.2 [c]	(6)	–		4244±194.2 [c]	(6)
5 min/20 min	Gallopamil 0.2 μmol/l	570±43.3 [b]	(10)	570±43.3	(10)	–	
	Glibenclamide 1.0 μmol/l+gallopamil 0.2 μmol/l	527±92.5 [c]	(6)	565±103.5	(5)	339	(1)
	Glibenclamide 1.0 μmol/l+nitroprusside	1288±342 [b]	(7)	592	(1)	1685±287 [b]	(6)
	Ethanol 8.57 mmol/l	1686±459.1	(6)	429±11.7	(2)	2315±362.5	(4)
−/−	Glibenclamide 0.1 μmol/l	156±40.7	(4)	156±40.7	(4)	–	
	Glibenclamide 1.0 μmol/l	1026±550.6	(7)	229±62.4	(5)	3019±876.9	(2)

Sum of adenosine, inosine, hypoxanthine, xanthine and uric acid. Values are means±S.E.M. For the contribution of the individual metabolites to the total release, see, e.g., Fig. 1. The drugs were present in the perfusion fluid from the outset of the coronary perfusion. Sodium nitroprusside, because of its instability, was infused into the fluid stream in amounts resulting in final concentrations of about 10 μmol/l, depending on the coronary flow (for exact concentration data see text). In the ethanol controls the same ethanol concentration was applied as was present as solvent in the glibenclamide experiments. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$. Significances in untreated coronary ligated hearts refer to the release data obtained in hearts without coronary ligation, during the corresponding time period of 20 min (first line). All other significances refer to the values in the groups with the same duration of coronary occlusion, but without the respective drug (for instance, gallopamil alone vs. the group with 5 min of coronary ligation without treatment; glibenclamide+gallopamil vs. glibenclamide alone, 1 μmol/l, 5 min of coronary ligation; glibenclamide 1 μmol/l, 5 min of coronary ligation, vs. the ethanol solvent controls; etc.).

(Table 2). Also sodium nitroprusside significantly decreased the release when applied in experiments with 1 μmol/l glibenclamide, though not to the same extent as did gallopamil (Table 2). It has to be noted that nitroprusside shortened the duration of fibrillation in 4 out of the 6 fibrillating hearts of this group, as already mentioned (38 ± 10 s of fibrillation, vs. 1123 ± 73 s in the 2 remaining hearts). In these 4 hearts, the total release of adenosine and its metabolites during the 20 min of coronary reperfusion was 814 ± 64.1 nmol/g dry weight, whereas it was 2586 ± 233.8 in the 2 hearts with long-lasting fibrillation. But also the latter value is still below that obtained in the fibrillating hearts with 1 μmol/l glibenclamide without nitroprusside (3801 ± 292.5 nmol/g dry weight).

3.3. Detection of a non-perfused subendocardial muscle layer

We became aware of a particular phenomenon in connection with the staining technique used for the determination of the extent of the ischaemic myocardial area. As could be expected, in hearts without coronary occlusion the whole myocardium was homogeneously stained green by the dye perfusion, and in the hearts with coronary ligation the myocardial area, which was excluded from the perfusion, remained unstained. This 'infarct' area was delimited very clearly from the surrounding well-perfused myocardium. However, there were also hearts with an additional non-stained muscle layer outside the infarcted area, beneath the endocardium, which comprised the inner part of the whole left ventricle, and which was particularly extensive in the apex region. The borderline of this muscle layer against the normally stained myocardium was always very sharp (Fig. 3).

This non-perfused inner muscle layer was only present in hearts with ventricular fibrillation. However, not all fibrillating hearts exhibited this phenomenon. In the untreated hearts with coronary ligation and reperfusion a total of 18 reacted with ventricular fibrillation, and in 7 of these hearts the non-perfused inner muscle layer was observed (= 38.9%; estimated thickness of the non-perfused muscle layer: 29 ± 2.5% of the left ventricular wall). In the presence of 1 μmol/l glibenclamide 15 hearts came to fibrillation, and 13 of them had a non-perfused inner muscle layer (= 86.7%; estimated thickness: 41 ± 3.4% of the left ventricular wall). This would mean a significantly higher incidence of the subendocardial perfusion stop ($P < 0.025$). However, also in the ethanol controls a high incidence of this phenomenon was obtained. All fibrillating hearts ($n = 4$) exhibited this subendocardial perfusion stop (estimated thickness: 31 ± 8.3% of the ventricular wall). In the non-fibrillating hearts ($n = 2$) it was lacking. It should be mentioned that in the glibenclamide control groups without coronary ligation/reperfusion, the non-perfused muscle layer was not observed, except in the 2 hearts which came to fibrillation, where it was very extensive (thickness 40 and 70%, respectively, of the ventricular wall).

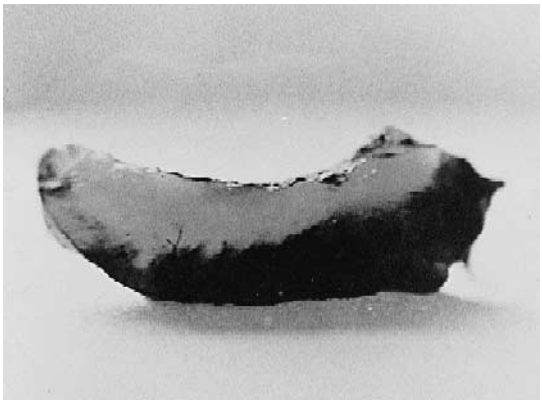

Fig. 3. Original photograph of a slice of the left ventricle of a rat heart, with ventricular fibrillation during the postischaemic coronary reperfusion. Dye perfusion of the coronary system at the end of the experiment, staining the well-perfused parts of the myocardium dark. The heart is cut longitudinally (apex region to the left), outside the infarct region. The upper concave side is the inner, endocardial surface of the ventricular myocardium. The convex side on the bottom is the outer, epicardial surface. Note the extensive non-perfused subendocardial muscle layer, which remained completely free of the dye.

In the group of coronary-ligated/reperfused hearts where sodium nitroprusside was applied in addition to glibenclamide, the non-perfused muscle layer was found only in 1 out of the 6 fibrillating hearts. In the experiments with gallopamil, with or without glibenclamide, the non-perfused muscle layer was missing; also in the heart which came to fibrillation.

4. Discussion

In the present experiments, glibenclamide did not prevent the appearance of premature ectopic beats and ventricular tachycardia during the myocardial ischaemia. Rather, the incidence of these arrhythmias was increased. Ventricular fibrillation did not play a role during the relatively short periods of coronary occlusion. However, it was the dominating arrhythmia during the coronary reperfusion, and it was not antagonized by glibenclamide. Also during the reperfusion there was rather a trend to an increased severity of the arrhythmias. This is in line with a report of Cole et al. (1991), concerning a pro-arrhythmic effect obtained in right ventricular walls of guinea pigs with 10 μmol/l glibenclamide, and to a lesser extent also with 1 μmol/l, during reperfusion after global ischaemia. In our experiments, glibenclamide was also pro-arrhythmic on its own, in control hearts without coronary occlusion.

What might have been the reason for this disadvantageous effect of glibenclamide? The decrease in the coronary flow has to be considered. Our observation that

glibenclamide decreases the coronary flow, is in good agreement with findings of Clayton et al. (1992) in isolated guinea-pig hearts, and Randall (1995) in isolated rat hearts. It appears possible that the decrease in the coronary flow worsened the ischaemic situation, and thus produced a propensity to the development of arrhythmias. In the above-mentioned experiments of Kantor et al. (1987, 1990) and Wolleben et al. (1989) with global ischaemia, the hearts were perfused at constant flow with the aid of perfusion pumps, whereas we perfused the hearts at constant pressure, where glibenclamide was able to fully exert its flow decreasing effect. This might explain why they missed the pro-arrhythmic effect observed by us.

Differences in the experimental model have also to be regarded when the antiarrhythmic effects are considered, obtained by these investigators. In the global ischaemia produced by restriction of the coronary flow to low values, the resulting homogeneous decrease in the refractoriness was overcome, perhaps, by glibenclamide. In our experiments with regional ischaemia, however, differences in the activation of the ATP-sensitive K^+ channels with the resulting dispersion of refractoriness between ischaemic, and non-ischaemic myocardial areas, might have been an important mechanism in the genesis of the arrhythmias, which was not easily antagonized by glibenclamide. And also in this respect, the situation could have been complicated by the glibenclamide-induced vasoconstriction. The experimental model of regional ischaemia by occlusion of a coronary artery is, on the other hand, of particular importance because of its similarity to the clinical situation of acute myocardial infarction. But Kantor et al. (1990) reported also some experiments with long-lasting regional ischaemia, where 1 μmol/l glibenclamide prevented almost completely the ventricular fibrillation. A drug-induced decrease in the coronary flow was not observed. However, also in their experiments, as well as in those of Wolleben et al. (1989), other kinds of arrhythmias, especially ventricular tachycardias, proved to be very resistant to an antiarrhythmic effect of glibenclamide. It should be emphasized that they used a concentration of glibenclamide which was also used in most of our experimental groups, namely 1 μmol/l. This concentration may appear relatively high. However, findings of Findlay (1993) in ventricular myocytes from rat hearts have shown that this concentration was necessary to inhibit the K^+ current activated by the K^+ channel opener bimakalim, or by dinitrophenol.

Counteracting the glibenclamide-induced coronary vasoconstriction by the vasodilator sodium nitroprusside reduced the duration of ventricular fibrillation in the greater part of the experiments. But a real antiarrhythmic effect was not observed. This fits well in the above-mentioned idea that the glibenclamide-induced decrease in the coronary flow aggravated to some degree the situation in the coronary ligated/reperfused hearts. It is easy to understand that the Ca^{2+} channel blocking agent gallopamil not only antagonized the decrease in the coronary flow produced by glibenclamide, but also markedly reduced the arrhythmias, because it is also an antiarrhythmic, per se, independent of its vascular effects.

High amounts of adenosine and its degradation products were released during the coronary reperfusion. The release was particularly pronounced in hearts with ventricular fibrillation, as also observed in previous investigations (Bernauer, 1991). Glibenclamide further increased the release. However, the coronary flow remained low. Obviously, even the high amounts of the coronary vasodilator adenosine detected in the coronary effluent, were not able to overcome the glibenclamide-induced coronary vasoconstriction. An observation of similar nature was made in isolated perfused guinea-pig hearts, where 2 μmol/l glibenclamide prevented the hypoxia-induced coronary dilation (Daut et al., 1990; Von Beckerath et al., 1991). The role of released adenosine, and that of the ATP decrease, in the opening of K^+ channels in hypoxia, or ischaemia respectively, has especially been discussed by Daut et al. (1990) and Nichols and Lederer (1991).

By what mechanism might glibenclamide have increased the adenosine release? Likely by restricting the coronary flow. This is suggested by the reduction in the adenosine release obtained with the coronary vasodilator sodium nitroprusside. The very marked decrease in the adenosine release produced by gallopamil can be explained by its vasodilating effect, and by the prevention of severe tachyarrhythmias with their increasing effect on the adenosine release. Moreover, as gallopamil is a cardiac depressant drug, it might have decreased the oxygen need of the hearts also by its negative inotropic effect, thus mitigating the myocardial hypoxia.

In the release of adenosine, also the phenomenon of a non-perfused subendocardial muscle layer, detected with our staining technique, could play a role. During fibrillation, where the cardiomyocytes are stimulated at an extremely high frequency, the oxygen consumption of isolated perfused rat hearts is increased, and the myocardial ATP concentrations are considerably decreased (Heuer and Bernauer, 1986; Latocha and Bernauer, 1991). It can be assumed that in our present experiments the oxygen deprivation was especially pronounced in the non-perfused subendocardial muscle layer. At the moment, we do not yet know the mechanisms which underlie this subendocardial stop of coronary perfusion. This is the first time we report about this phenomenon. It is clearly associated with fibrillation and, perhaps, also with long-lasting ventricular flutter, as suggested by experiments we are performing in connection with another investigation. The present experiments with sodium nitroprusside let assume that the non-perfused muscle layer is produced by a constriction in the coronary vascular bed. In still unpublished observations, we have seen a very mighty non-perfused subendocardial muscle layer also in isolated guinea-pig hearts, in which ventricular fibrillation was provoked by a toxic concentra-

tion of ouabain. As we know from previous investigations, glycoside intoxication also leads to massive release of adenosine and its metabolites (Bernauer, 1994).

We believe that the occurrence of the non-perfused subendocardial muscle layer can have serious consequences for the electrical situation in the heart. At the borderline between the non-perfused muscle layer and the adjacent normally perfused myocardium, the occurrence of reentry mechanisms might be facilitated, favouring the genesis and maintenance of fibrillation.

Finally, we want to emphasize that our observation of a pro- arrhythmic effect of glibenclamide in the isolated perfused rat heart should not be transferred uncritically to the situation in humans treated with glibenclamide because of diabetes. The general problems connected with the application of results obtained in animal experiments, to human beings, have to be regarded in an adequate manner. Nevertheless, it appears advisable to keep an eye on this possible undesirable effect, particularly in patients suffering also from coronary heart disease.

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