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Hoe 694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischaemia

[1]W. Scholz, U. Albus, H.J. Lang, W. Linz, P.A. Martorana, H.C. Englert & B.A. Schölkens

Hoechst AG, Postfach 800 320, 6230 Frankfurt/M. 80, Germany

1 The benzoylguanidine derivative Hoe 694 ((3-methylsulphonyl-4-piperidino-benzoyl) guanidine methanesulphonate) was characterized as an inhibitor of Na^+/H^+ exchange in rabbit erythrocytes, rat platelets and bovine endothelial cells. The potency of the compound was slightly lower or comparable to ethylisopropyl amiloride (EIPA).

2 To investigate a possible cardioprotective role of the Na^+/H^+ exchange inhibitor Hoe 694, rat isolated working hearts were subjected to ischaemia and reperfusion. In these experiments all untreated hearts suffered ventricular fibrillation on reperfusion. Addition of 10^{-7} M Hoe 694 to the perfusate almost abolished reperfusion arrhythmias in the rat isolated working hearts.

3 Hoe 694 reduced the release of lactate dehydrogenase (LDH) and creatine kinase (CK), which are indicators of cellular damage during ischaemia, into the venous effluent of the hearts by 60% and 54%, respectively.

4 The tissue content of glycogen at the end of the experiments was increased by 60% and the high energy phosphates ATP and creatine phosphate were increased by 240% and 270% respectively in the treated hearts as compared to control hearts.

5 Antiischaemic effects of the Na^+/H^+ exchange inhibitor, Hoe 694, were investigated in a second experiment in anaesthetized rats undergoing coronary artery ligation. In these animals, pretreatment with Hoe 694 caused a dose-dependent reduction of ventricular premature beats and ventricular tachycardia as well as a complete suppression of ventricular fibrillation down to doses of 0.1 mg kg^{-1}, i.v. Blood pressure and heart rate remained unchanged.

6 We conclude that the new Na^+/H^+ exchange inhibitor, Hoe 694, shows cardioprotective and antiarrhythmic effects in ischaemia and reperfusion in rat isolated hearts and in anaesthetized rats. In view of the role which Na^+/H^+ exchange seems to play in the pathophysiology of cardiac ischaemia these effects could probably be attributed to Na^+/H^+ exchange inhibition.

Keywords: Hoe 694; Na^+/H^+ exchange, ischaemia; arrhythmia, cardiac protection

Introduction

The plasma membrane Na^+/H^+ exchanger is an ubiquitous pH regulating cellular ion transport system. It is driven by the Na^+ gradient and extrudes protons from the cytosol in exchange for extracellular Na^+ ions (Aronson 1985; Frelin *et al.*, 1988). In cardiac tissue the exchanger seems to have a major role in the control of intracellular pH. At the onset of cardiac ischaemia and in reperfusion, Na^+/H^+ exchange is excessively activated by low intracellular pH (Lazdunski *et al.*, 1985; Frelin *et al.*, 1985). Since the deleterious Na^+ influx in this condition was found to originate mainly from Na^+/H^+ exchange (Frelin *et al.*, 1984; Schömig *et al.*, 1988), the exchanger seems responsible for an increase in cytosolic sodium in ischaemic cells. The accumulation of intracellular Na^+ causes an activation of Na^+/K^+ ATPase (Frelin *et al.*, 1984; Rassmussen *et al.*, 1989) which in turn increases ATP consumption. During ischaemia the anaerobic metabolism of glucose terminates in lactic acid. A vicious circle leads to a further decrease of intracellular pH and to a further activation of Na^+/H^+ exchange, resulting in energy depletion, cellular Na^+ overload and finally due to the coupling of Na^+ and Ca^{2+} transport via Na^+/Ca^{2+} exchange, cellular Ca^{2+} overload (Frelin *et al.*, 1984; Lazdunski *et al.*, 1985; Tani & Neely, 1990). Especially in ischaemic cardiac tissue where Na^+/H^+ exchange is the dominant pH regulating ion transport system (Frelin *et al.*, 1985; Weissenberg *et al.*, 1989) these pathophysiological events could lead to increased excitability and precipitation of cellular death.

Therefore it seemed desirable to find potent and well tolerated inhibitors of Na^+/H^+ exchange which should be able to interrupt this vicious cycle, to conserve cellular energy stores and to diminish excitability and necrosis in cardiac ischaemia. In previous experiments we have demonstrated such effects with the relatively weak or toxic standard inhibitors of Na^+/H^+ exchange, amiloride and ethylisopropyl amiloride (Scholz *et al.*, 1991; 1992).

In the present study a new compound, the benzoyl guanidine derivative, Hoe 694 (Figure 1) was characterized as a Na^+/H^+ exchange inhibitor in erythrocytes, platelets, and endothelial cells. Possible anti-ischaemic effects of Hoe 694 were investigated in rat isolated working hearts with ischaemia-reperfusion injuries. Furthermore, the *in vivo* activity

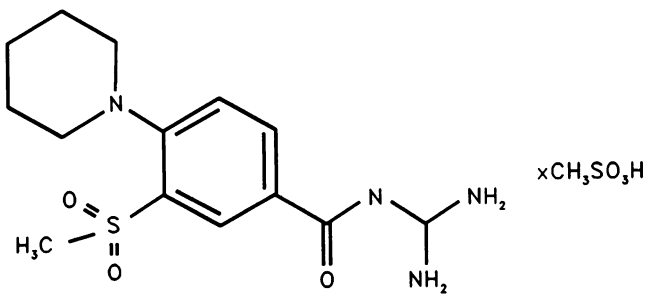

Figure 1 Hoe 694 (3-methylsulphonyl-4-piperidinobenzoyl) guanidine methanesulphonate.

[1] Author for correspondence.

of Hoe 694 in myocardial ischaemia was examined in anaesthetized rats with ligation of a coronary artery.

Methods

Sodium influx into erythrocytes

New Zealand white rabbits (Ivanovas) were fed a rabbit standard chow with 2% cholesterol for 6 weeks to increase the Na^+/H^+ exchange (Scholz *et al.*, 1990) and to make the erythrocytes suitable for measurement of sodium influx via Na^+/H^+ exchange by flame photometry. Blood was drawn from the ear artery of the rabbits and coagulation prevented with 25 i.u. ml^{-1} potassium heparin. The haematocrit of the sample was determined in duplicate by centrifugation. Aliquots of 100 µl were taken to measure the initial sodium content of the erythrocytes.

To determine the amiloride sensitive sodium influx into erythrocytes, 100 µl of each blood sample was added to 5 ml of buffer made hyperosmolar by sucrose (in mM: NaCl 140, KCl 3, sucrose 150, ouabain 0.1, tris-hydroxy-methylaminomethane 20, pH 7.4) and incubated for 60 min at 37°C. Subsequently, the erythrocytes were washed three times in ice-cold $MgCl_2$-ouabain-solution (in mM: $MgCl_2$ 112, ouabain 0.1) with centrifugation.

For determination of intracellular Na^+ content, the cells were haemolysed in distilled water, the cell membranes were centrifuged and the sodium concentration of the haemolysate was measured by flame-photometry.

Net influx of sodium into the erythrocytes was calculated from the difference between the initial sodium content and the sodium content after the incubation. Amiloride-sensitive Na^+ influx was calculated from the difference between Na^+ content of erythrocytes incubated with and without amiloride 3×10^{-4} M. Each experiment was done with the erythrocytes of 6 different animals. In each case the comparison of Na^+ content was made between erythrocytes from the same animal.

Statistical analysis of the data obtained was performed with Student's *t* test for paired groups. Differences were considered significant if $P < 0.05$. Results are given as mean values ± s.e.mean.

Inhibition of Na^+/H^+ exchange in platelets

Wistar rats (Möllegard, 250 g–350 g) were used for the experiments. Blood (approx. 5 ml) was withdrawn from the abdominal aorta under ethylether anaesthesia. Coagulation was inhibited by 0.8 ml acid citrate dextrose (in mM: citric acid 65, glucose 11, and trisodium citrate 85). The blood was immediately centrifuged at 90 *g* for 10 min and a platelet count was done from the platelet rich plasma (PRP) with a Casey 1 multichannelyser (Schärfe System, D7410 Reutlingen). Each of the experiments was performed with 10–50 µl PRP containing 20×10^6 platelets which was made up to a volume of 100 µl with saline. To activate Na^+/H^+ exchange in the platelets by intracellular acidification, 500 µl propionate buffer (mM: Na-propionate 135, HCl 1, $CaCl_2$ 1, $MgCl_2$ 1, glucose 10, HEPES 20, pH 6.7, 22°C) was added to the PRP/NaCl solution. Decrease of the optical density of the PRP solution as a result of the platelet swelling was measured with a Turbitimer (Behringwerke, Marburg, Germany). The device was activated photometrically by the addition of propionate buffer to the cuvette. To identify Na^+/H^+ exchange-induced change of extinction the experiments were performed with and without the Na^+/H^+ exchange inhibitor 5-(N-ethyl-N-isopropyl)amiloride (EIPA). During the experiments all solutions were kept at 22°C in a temperature controlled water bath.

Results are given as means ± s.d. Student's *t* test was employed for statistical evaluation. A *P*-value of < 0.05 was considered significant.

Inhibition of Na^+/H^+ exchange in bovine aortic endothelial cells

Bovine aortic endothelial cells (BAEC) were isolated by dispase digestion from bovine aorta obtained from animals killed at the local slaughter house. The cells were cultured on 35 mm dishes and grown to confluency in Dulbecco's Minimum Essential Medium (DMEM, Gibco) under an atmosphere containing 10% CO_2. Three days after confluency the cells were used for measurement of $^{22}Na^+$ influx.

The cells were washed twice with Krebs-Ringer-solution buffered with Hepes/Tris (KRB) in which sodium chloride had been replaced by choline chloride (in mM: choline chloride 130, $CaCl_2$ 1.5, KCl 5, $MgCl_2$ 1, HEPES 20, pH 7.0 with Tris) and then incubated for 20 min at 37°C in the same buffer which also contained 0.1% bovine serum albumin (BSA) and glucose 10 mM. To stimulate Na^+/H^+ exchange the culture dishes were incubated for another 10 min with 500 µl/dish KRB in which all sodium chloride had been replaced by 65 mM each of choline chloride and Na^+-propionate or with KRB in which 50% of the sodium chloride had been replaced by choline chloride for unstimulated controls. In addition the buffer contained 2 µCi ml^{-1} $^{22}Na^+$ and the test compounds EIPA or Hoe 694. After the stimulation period, sodium influx was terminated by washing the cells twice with ice-cold stop solution (in mM: $MgCl_2$ 0.1, Tris 10, pH 7.0). Subsequently, the cells were lysed with 250 µl trichloric acid and scraped from the dishes. Radioactivity was determined in a Packard gamma counter.

Results are given as mean ± s.d. Statistical analysis of the data obtained was performed with Student's *t* test for independent groups. Differences were considered significant if $P < 0.05$.

Rat isolated working heart preparation

Isolated working hearts prepared from Wistar rats of either sex weighing 280–300 g were used in all experiments as described before (Linz *et al.*, 1986). Animals were injected intraperitoneally with sodium heparin (500 u kg^{-1}) 1 h before they were killed.

The hearts were excised and placed in ice-cold perfusion medium until contraction had ceased (after approximately 5 s). Isolated hearts were then perfused via the aorta at a constant perfusion pressure equivalent to 65 mmHg (Neely *et al.*, 1967) with a modified Krebs-Henseleit buffer (mM: NaCl 113.8, $NaHCO_3$ 22, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.1, $CaCl_2$ 2.5, glucose 11.0, Na pyruvate 2.0). The medium was gassed with 95% O_2 plus 5% CO_2 at 37°C and pH 7.4. The perfusate was not recirculated. Thereafter, a silicone balloon was introduced into the left ventricle through the mitral valve. Preload was held at 7.4 mmHg and afterload as well as coronary perfusion was kept constant at 65 mmHg. Hoe 694 10^{-7} M was present in the respective experiments throughout the perfusion period. This concentration was chosen because the IC_{50} of Hoe 694 on Na^+/H^+ exchange was close to 10^{-7} M in several tissues (see results). The compound was dissolved in the perfusion medium without the use of any solvent.

After the perfusion had started the hearts immediately began to pump into the artificial systemic circulation and to perform pressure volume work (for detailed description see Linz *et al.*, 1986). Hearts were perfused for an initial 20 min period (control perfusion period). Acute regional myocardial ischaemia was then produced by clamping the left coronary artery close to its origin for 15 min (ischaemic period). Thereafter, the clip was reopened and changes during reperfusion were monitored for 30 min (reperfusion period).

The following parameters were measured: left ventricular pressure (LVP) via a Statham pressure transducer P23 Db, which on differentiation yielded $LV\ dP/dt_{max}$ and heart rate (HR). Coronary flow (CF) was determined by an electromagnetic flow probe in the aortic cannula. An epicardial

electrocardiogram recording was obtained via two silver electrodes attached to the heart. All parameters were recorded on a Brush 2600 recorder.

For the determination of lactate release, lactate dehydrogenase (LDH) and creatine kinase (CK) activities in the perfusate, samples were taken from the coronary effluent for spectrophotometrical measurements (Linz *et al.*, 1986). All heart weights are given in g of wet tissue. Wet weight of the hearts (approximately 1.2 g) was determined immediately after excision from the rats and before they were mounted in the perfusion chamber. Dry tissue weight (approximately 240 mg) was measured after drying the hearts for 12 h at 110°C.

After the experiments, hearts were rapidly frozen in liquid nitrogen and stored at minus 80°C. For tissue analysis of lactate, glycogen and high energy phosphates 500 mg of the left ventricle were taken, put into 5 ml ice-cold $HClO_4$ (0.6 M), and homogenized with an Ultra-Turrax (Junke & Kunkel, Ika-Werk, Model TP).

Glycogen was hydrolized to glucose with amyloglycosidase (pH 4.8) (Linz *et al.*, 1986). Lactate was assayed spectrophotometrically according to Noll (1984). ATP and creatine phosphate (CP) were measured according to Trautschold *et al.* (1985) and Heinz & Weisser (1985).

Results are given as mean values ± s.e.mean. Statistical analysis of the data obtained was performed with Student's *t* test for independent groups. Differences were considered significant if $P < 0.05$.

Anaesthetized rats

Male Sprague-Dawley rats (280–440 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). A tube was tied into the trachea and the animals were ventilated with room air 10–13 ml kg^{-1} at 54 breaths min^{-1} by a Rhema respirator (Frankfurt, Germany).

A catheter was inserted into the left carotid artery and connected to a pressure transducer (Type P23 Dd, Statham, Hato Ray, Puerto Rico) to record blood pressure with a Brush Mark 220 polygraph.

The ECG was recorded from lead II and arrhythmias were evaluated according to the guidelines of the Lambeth conventions (Walker *et al.*, 1988) where: VPB's (ventricular premature beats) were defined as discrete and identifiable premature QRS complexes (premature in relation to the P wave). All the VPB's occurring in the ischaemic period (15 min) were counted except runs of VPB's which had to be defined as VT. VT (ventricular tachycardia) was defined as a run of four or more consecutive ventricular premature beats. The duration of VT, in seconds, was measured. VF (ventricular fibrillation) was defined as a signal for which individual QRS deflections could no longer be distinguished from one another. The duration of VF, in seconds, was measured. The heart rate was counted from the ECG.

Thoracotomy for coronary artery ligation was performed at the fifth intercostal space. During an equilibration period of 5 min rats with spontaneous arrhythmias or a systolic blood pressure below 70 mmHg were rejected. The left coronary artery was then ligated for 15 min. For intravenous applications a catheter was placed into the right jugular vein.

Hoe 694 (0.001 to 3 mg kg^{-1} body weight) was given intravenously as a bolus 5 min before coronary artery occlusion.

The results are presented as mean ± s.e.mean. Differences between mean values were analysed with Mann-Whitneys U-test for independent groups. Differences were considered significant if $P < 0.05$. Differences of the incidences of arrhythmias between treated groups and controls were analysed with the Chi-square test and were considered significant at a level of 95%.

Results

Sodium influx into rabbit erythrocytes

As shown in Figure 2, Hoe 694 caused a concentration-dependent inhibition of Na^+ influx into the rabbit erythrocytes. This effect was limited to amiloride-inhibitable Na^+ influx. There was no additional effect of Hoe 694 on Na^+ influx when the erythrocytes were pretreated with 3×10^{-4} M amiloride. The IC_{50} for the inhibition of amiloride inhibitable Na^+ influx by Hoe 694 was 0.8×10^{-6} M.

Na^+/H^+ exchange in rat platelets

Intracellular acidification with Na propionate caused a decrease of the optical density of the platelet solution. The time course of the rate of this decrease of extinction is shown in Figure 3 as well as its inhibition by the Na^+/H^+ exchange inhibitor EIPA at 10^{-5} M or by Hoe 694 at 10^{-5} M. Figure 4 demonstrates the concentration-dependency of the inhibition of platelet swelling by EIPA or by Hoe 694 20 s after the beginning of the reaction. The IC_{50} values were close to 1×10^{-7} M for both compounds.

Inhibition of Na^+/H^+ exchange in bovine aortic endothelial cells

In bovine aortic endothelial cells, acidification with propionate caused a marked increase of $^{22}Na^+$ influx which was totally inhibited by 10^{-5} M EIPA. The Na^+/H^+ exchange

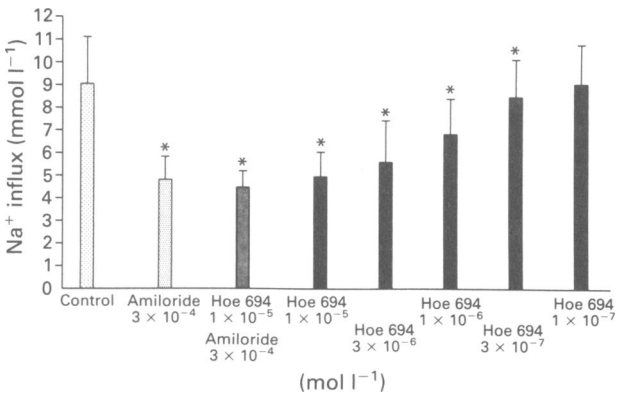

Figure 2 Concentration-dependent inhibition of amiloride-sensitive Na^+ influx in rabbit erythrocytes by Hoe 694. Mean with s.e.mean, $n = 6$ for each data point. $^*P < 0.05$ vs control.

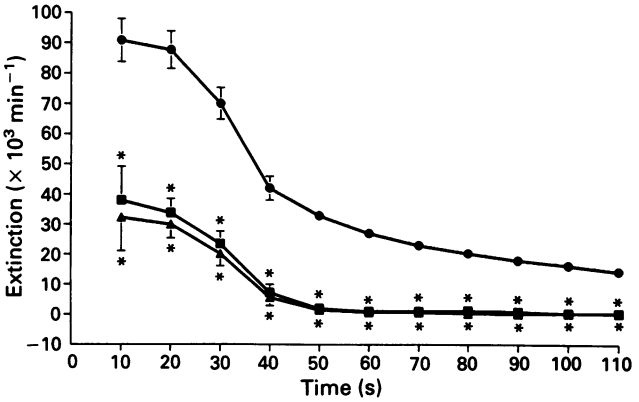

Figure 3 Rates of decrease of extinction of rat platelet solutions after intracellular acidification by propionate. Control (●), inhibition by 5-(N-ethyl-*N*-isopropyl) amiloride (▲) and by Hoe 694 (■) at 10^{-5} mol l^{-1}. Mean with s.d., $n = 6$ for each data point. $^*P < 0.05$ vs control.

inhibitor had no effect in non-acidified controls (Figure 5). Hoe 694 caused a concentration-dependent inhibition of EIPA-sensitive $^{22}Na^+$ influx induced by propionate (Figure 6). When given together with EIPA into the incubation medium, Hoe 694 had no additional effect on $^{22}Na^+$ influx. The IC_{50} of Hoe 694 was estimated to be in the range of 10^{-7} M which is comparable to the IC_{50} of EIPA in the same experimental setup (Figures 5,6).

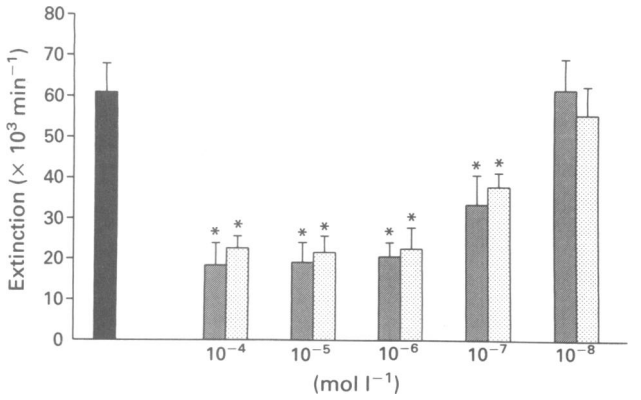

Figure 4 Rate of decrease of extinction of acidified rat platelet solutions 20 s after the beginning of the reaction. Concentration-dependent inhibition by 5-(N-ethyl-*N*-isopropyl) amiloride (hatched columns) and Hoe 694 (stippled columns). Means, s.d., $n = 4$ for each data point. $^*P < 0.05$ vs control.

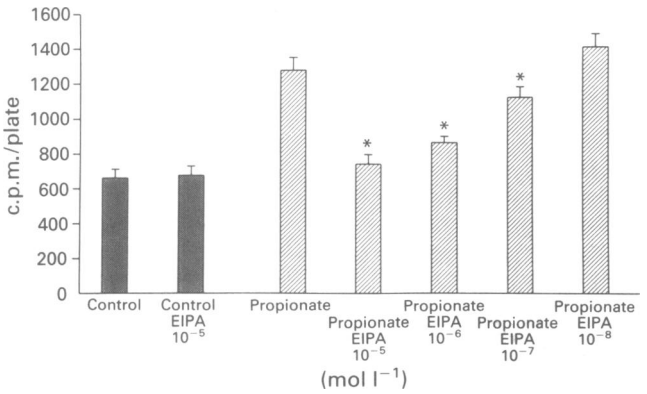

Figure 5 $^{22}Na^+$ influx measured as counts per min per plate (c.p.m./plate) into bovine aortic endothelial cells with and without intracellular acidification by propionate. Concentration-dependent inhibition by 5-(N-ethyl-*N*-isopropyl) amiloride (EIPA). Mean, with s.d., $n = 6$ for each data point. $^*P < 0.05$ vs control.

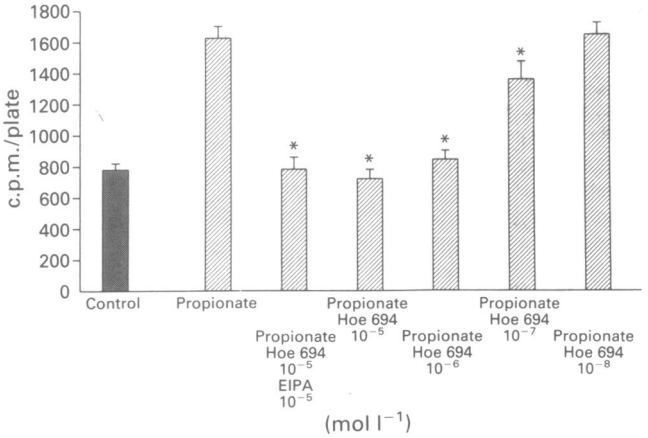

Figure 6 $^{22}Na^+$ influx measured as counts per min per plate (c.p.m./plate) into bovine aortic endothelial cells with and without intracellular acidification by propionate. Concentration-dependent inhibition by Hoe 694. Mean, with s.d., $n = 6$ for each data point. $^*P < 0.05$ vs control.

Rat isolated working heart preparation

To investigate a possible protective role of Hoe 694 under ischaemic conditions rat isolated hearts were subjected to ischaemia and reperfusion. In these experiments all untreated hearts suffered ventricular fibrillation with a mean duration of 15.4 ± 3.2 min (mean ± s.e.mean) during the reperfusion period of 30 min. However, when 10^{-7} M Hoe 694 was added to the perfusion solution, ventricular fibrillation during reperfusion was reduced to 1.3 ± 0.6 min in the working rat hearts (Figure 7).

During ischaemia LV *dP/dt* significantly increased in hearts treated with 10^{-7} M Hoe 694 as compared to control hearts while coronary flow (CF) and heart rate were not affected by the compound (Figure 7).

In addition, the release of LDH, CK and lactate into the venous effluent from the hearts treated with Hoe 694 was markedly reduced during ischaemia (Figure 7).

Analysis of the energy stores of the treated hearts at the end of the experiments showed marked differences in the levels of glycogen, ATP and CP as compared to control hearts. Tissue contents of glycogen, ATP and CP (μM g^{-1} dry wt ± s.d.) were increased from 73.5 ± 3.7, 5.5 ± 0.4 and 4.9 ± 0.3 to 119.3 ± 2.5, 18.4 ± 1.0, and 18.3 ± 0.9, respectively, while lactate (μM g^{-1} dry wt ± s.d.) was decreased from 15.3 ± 1.8 to 10.4 ± 1.2 in Hoe 694-treated hearts as compared to control hearts (Figure 7).

Anaesthetized rats

The effects of Hoe 694 in cardiac ischaemia were investigated under *in vivo* conditions, in which the compound was given intravenously at doses (mg kg^{-1} body wt) of 0.001, 0.01, 0.3, 1.0 or 3.0 to anaesthetized rats 5 min before they underwent coronary artery ligation.

Approximately 5 min after the occlusion of the coronary artery, control animals which received the vehicle only, showed marked ventricular ectopic activity which lasted for about 10 min. All control rats exhibited VPB and 9 out of 10 controls showed VT. In addition 5 out of 10 animals showed VF. However, pretreatment of the animals with Hoe 694 caused a dose-dependent reduction of VPB, VT and VF (Figures 8–10). In particular, VPB were significantly reduced at doses of Hoe 694 ranging from 0.1–3 mg kg^{-1} (Figure 8).

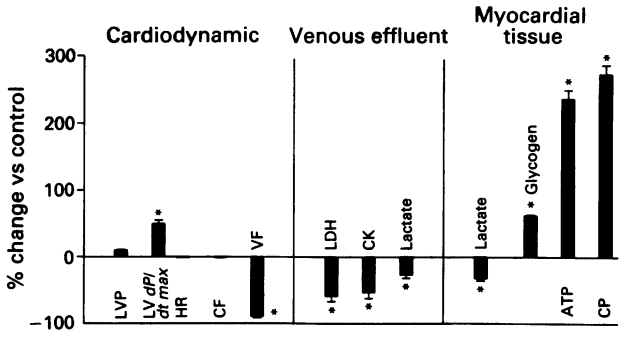

Figure 7 Changes in cardiodynamics, enzyme release and high energy phosphates tissue content in rat isolated working hearts caused by Hoe 694 10^{-7} M. Cardiodynamics: LVP, LV *dP/dt max.*, heart rate (HR) and coronary flow (CF) during ischaemia, ventricular fibrillation (VF) during reperfusion. Venous effluent: release of lactate dehydrogenase (LDH), creatine kinase (CK) and lactate during ischaemia. Myocardial tissue: glycogen, high energy phosphates and lactate after ischaemia and reperfusion. Mean, with s.e.mean, $n = 6$. $^*P < 0.05$ vs control.

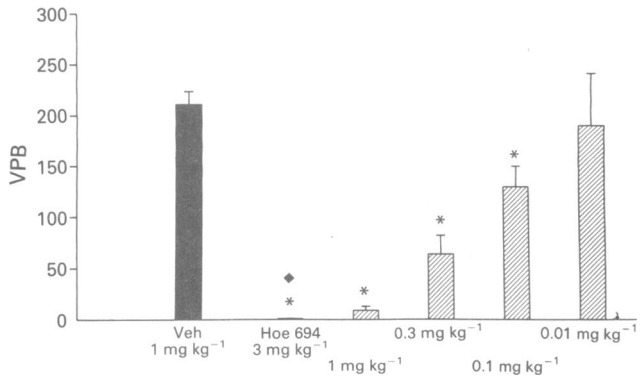

Figure 8 Dose-dependent effect of intravenous Hoe 694 on ventricular premature beats (VPB) in anaesthetized rats during cardiac ischaemia, $n = 10$ for each data point, mean, with s.e.mean. Veh = vehicle. $^*P < 0.05$ vs control for number or duration of arrhythmias; ◆Chi-Square >95% vs control for incidence of arrhythmias.

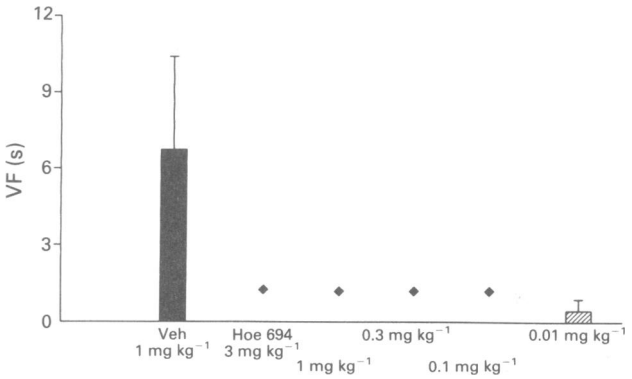

Figure 10 Dose-dependent effect of Hoe 694 on ventricular fibrillation (VF) in anaesthetized rats during cardiac ischaemia, $n = 10$ for each data point, mean, with s.e.mean. Veh = vehicle. $^*P < 0.05$ vs control for number or duration of arrhythmias; ◆ Chi-square >95% vs control for incidence of arrhythmias.

VT was abolished at doses of 1 and 3 mg kg^{-1} (Figure 9) and significantly reduced at 0.1 and 0.3 mg kg^{-1}. VF was completely prevented at doses ranging from 0.1 to 3 mg kg^{-1} Hoe 694 (Figure 10). Incidences (out of 10) of VPB were 10, 10, 8, 6, 3, incidences of VT were 9, 2, 1, 0, 0 and of VF were 1, 0, 0, 0, 0 at doses of Hoe 694 of 0.01, 0.1, 0.3, 1 and 3 mg kg^{-1}. As compared to controls the incidence of VPB was significantly reduced at 3 mg kg^{-1} (Figure 8) and for VT and VF at doses ranging from 0.1 mg kg^{-1} to 3 mg kg^{-1} (Figures 9, 10). The compound was well tolerated by the rats and had no effect on blood pressure or heart rate.

Discussion

In previous experiments using amiloride and EIPA, well characterized inhibitors of Na^+/H^+ exchange (Aickin & Thomas, 1977; Rindler *et al.*, 1979; Vigne *et al.*, 1983), it has been shown that erythrocytes from rabbits fed a high cholesterol diet exhibit enhanced Na^+/H^+ exchange activity (Scholz *et al.*, 1990). In the present study this erythrocyte model was used to investigate possible effects of the benzoyl guanidine compound, Hoe 694, on Na^+/H^+ exchange. Hoe 694 was found to inhibit amiloride-sensitive Na^+ influx into rabbit erythrocytes with an IC_{50} of 0.8×10^{-6} M. When the erythrocytes were exposed to amiloride and Hoe 694 at the same time no additional effect of Hoe 694 on Na^+ influx could be observed. Since particularly the amiloride-sensitive fraction of Na^+ influx was affected by the compound it is likely that Hoe 694 selectively inhibited Na^+ influx via Na^+/H^+ exchange in the erythrocytes.

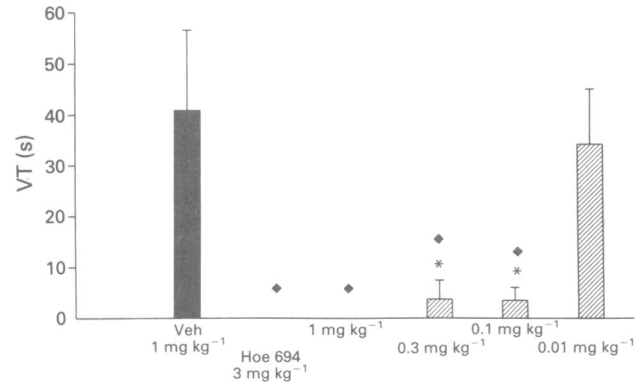

Figure 9 Dose-dependent effect of Hoe 694 on ventricular tachyarrhythmias (VT) in anaesthetized rats during cardiac ischaemia, $n = 10$ for each data point, mean, with s.e.mean. Veh = vehicle. $^*P < 0.05$ vs control for number or duration of arrhythmias; ◆ Chi-square >95% vs control for incidence of arrhythmias.

Some years ago it was found by Livne *et al.* (1987a) that intracellular acidification of platelets led to a pronounced swelling of these blood cells. This effect was caused by an activation of Na^+/H^+ exchange and could be prevented by Na^+/H^+ exchange inhibitors (Livne *et al.*, 1987a). Other investigators had shown that the volume regulatory properties of platelets can be measured photometrically (Fantl, 1968). In recent experiments it was demonstrated that the light transmission of a platelet solution was increased when the acidified platelets were swelling and that the rate of the increase was correlated to Na^+/H^+ exchange activity (Rosskopf *et al.*, 1991). In the present study both EIPA and Hoe 694 inhibited the decrease of extinction which occurs in rat platelet solutions after intracellular acidification with propionate. The IC_{50} was about 10^{-7} M. Although the data do not prove that the effects of Hoe 694 on platelet swelling are definitely due to Na^+/H^+ exchange inhibition, it can be stated that the compound showed the same actions and the same potency as the Na^+/H^+ exchange inhibitor, EIPA, in this experiment.

Furthermore, the effect of Hoe 694 on EIPA-sensitive $^{22}Na^+$ influx was investigated in bovine endothelial cells. While EIPA had no effect under normal conditions, the increase of Na^+ influx caused by acidification was almost totally inhibited by EIPA and by Hoe 694 in concentrations of 10^{-6} M. For both compounds the IC_{50} of this effect was close to 10^{-7} M. These observations suggest a marked activation of Na^+/H^+ exchange in acidified bovine endothelial cells which could be blocked by EIPA or Hoe 694. Since Hoe 694 had no additional effect on $^{22}Na^+$ influx in the presence of EIPA it seems that, as in the erythrocyte experiment, the compound is a specific inhibitor of Na^+ influx by inhibiting Na^+/H^+ exchange.

Altogether these results indicate that Hoe 694 is a potent and specific inhibitor of Na^+/H^+ exchange in different tissues. In addition, the compound has been evaluated in porcine cerebral endothelial cells using the ammonium chloride prepulse technique (Schmid *et al.*, 1991; 1992). In these studies Hoe 694 qualified as Na^+/H^+ exchange inhibitor by preventing pH recovery after intracellular acidification with the same profile and the same potency as EIPA.

In our experiments with rat isolated working hearts Hoe 694 ameliorated cardiac performance and reduced the release of intracellular enzymes during ischaemia indicating a reduction of cell injury and cell death. It also prevented reperfusion arrhythmias and caused a very marked preservation of

cellular energy stores during ischaemia and reperfusion. The tissue levels of high energy phosphates and glycogen in Hoe 694-treated hearts were comparable to those found in non-ischaemic working control hearts of a previous study which was also performed in our lab (Linz *et al.*, 1986; glycogen 114.3 ± 4.3, ATP 16.4 ± 1.6, CP 17.3 ± 2.1 $\mu M\ g^{-1}$ dry weight).

Furthermore, Hoe 694 showed marked antiarrhythmic activity in an animal model of cardiac ischaemia. In a large range of dosages the compound effectively reduced arrhythmias and prevented ventricular fibrillation during ischaemia in coronary artery ligated rats.

We have observed cardioprotective effects of a similar character in previous experiments with amiloride and EIPA (Scholz *et al.*, 1991; 1992) which are considered to be standard inhibitors of Na^+/H^+ exchange. Another investigator (Maron, 1989) has found cardioprotective effects in ischaemic hearts by using the Na^+/H^+ exchange inhibitor methylisobutyl amiloride (MIBA). Altogether these studies seem to indicate that cardioprotection in ischaemia and reperfusion could be a common effect of Na^+/H^+ exchange inhibitors which would suggest that the exchanger plays a crucial role under these conditions.

Several investigators have already proposed that Na^+/H^+ exchange might be important in the pathophysiology of cardic ischaemia (Frelin *et al.*, 1984; 1985; Lazdunski *et al.*, 1985). This assumption was based on the observation of intracellular Na^+ overload that occurs during ischaemia as the consequence of an over-activated Na^+/H^+ exchange. Due to the decreased energy stores in this condition, the Na^+ ions cannot be extruded sufficiently by the ATP-dependent Na^+/K^+ pump. The resulting high concentration of intracellular Na^+ impairs in turn the outward Ca^{2+} transport by Na^+/Ca^{2+} exchange which might even switch to operate in the reversed mode and could cause an additional Ca^{2+} influx (Frelin *et al.*, 1985; Lazdunski *et al.*, 1985; Rabkia, 1989; Lederer *et al.*, 1990; Pierce *et al.*, 1990; Tani & Neely, 1990). Thus, a vicious cycle of intracellular Na^+ and Ca^{2+} overload and a decrease of high energy phosphates could continue during the onset of ischaemia to a point where Na^+/H^+ exchange would be inhibited by extracellular acidification. This inhibition of Na^+/H^+ exchange is to be expected when the decrease of extracellular pH has reached about 6.0 (Frelin *et al.*, 1984; Lazdunski *et al.*, 1985; Wallert & Fröhlich, 1989; Weissenberg *et al.*, 1989), which is supposed to take several minutes (Cobbe & Poole-Wilson, 1980). In the case of reperfusion the extracellular acid would be washed out, which would reactivate Na^+/H^+ exchange and the vicious cycle could start again leading to arrhythmias and cardiac necrosis. From this consideration, an intervention at the step of the Na^+/H^+ exchanger with an inhibitor would retard necrosis of the tissue during ischaemia and reperfusion.

In the present experiments the benzoyl guanidine, Hoe 694, has been characterized as a potent and specific inhibitor of Na^+/H^+ exchange in different tissues. The cardioprotective and antiarrhythmic effects of the substance are probably due to the interference with the exchange system during cardiac ischaemia and reperfusion. It is suggested that the inhibition of Na^+/H^+ exchange might become a new approach in the therapy of cardiac ischaemia.

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