Glibenclamide effects on reperfusion-induced malignant arrhythmias and left ventricular mechanical recovery from stunning in conscious sheep

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Received 18 August 2000; accepted 8 January 2001

Abstract

Introduction: Sulfonylureas have been associated with a high incidence of cardiovascular death in diabetic patients treated with these drugs. Although the evidence on the cardiovascular effects of sulfonylureas is contradictory and scarce, many experiments have shown that the second-generation compound glibenclamide has a protective effect on mechanical function and against generation of malignant arrhythmias. Objective: The purpose of this study was to assess whether glibenclamide elicits protection on postischemic myocardial functional recovery (stunning) and against reperfusion-induced arrhythmias in a conscious sheep model. Methods: Sheep were divided into three groups: control, glibenclamide (0.4 mg/kg) and vehicle. After a 12-min ischemic period, the heart was reperfused and recordings for index calculation were acquired during 2 h of reperfusion. Percent systolic wall thickening fraction (%WTH), radial diastolic compliance (CR), arrhythmia incidence and Bernauer’s arrhythmia severity index (ASI) were calculated for each group. Results: Glibenclamide infusion had a high proarrhythmic action (ASI: glibenclamide 143, control 54 and vehicle 23; ANOVA P < 0.001 drug vs. control and vehicle) and a detrimental effect on regional systolic (%WTH: glibenclamide 26.9 ± 6.7, control 65.7 ± 3.5 and vehicle 68.6 ± 5.6, ANOVA P < 0.01 drug vs. control and vehicle) and diastolic function (CR: glibenclamide 76.2 ± 7.8, control 104.7 ± 4.2 and vehicle 106 ± 4.9, ANOVA P < 0.05 drug vs. control and vehicle) during reperfusion. Conclusions: Glibenclamide infusion resulted in adverse cardiovascular effects. The combined deleterious effects on reperfusion-induced arrhythmias and on myocardial recovery from stunning could be the cause of the unexplained high mortality in diabetic patients treated with sulfonylurea derivatives. The mechanism involved seems to be the blockade of the cardiac ATP sensitive potassium (K-ATP) channel.

Keywords: Ischemia; K-ATP channel; Reperfusion; Stunning; Ventricular arrhythmias; Ventricular function

1. Introduction

Recently, modulators of ATP sensitive potassium (K-ATP) channels have received increasing interest because of their potential protective effect on the myocardium against myocardial postischemic dysfunction (stunning), infarction and prevention of malignant arrhythmias during ischemia and reperfusion [1–5].

Two types of K-ATP channel modulators have been used in patients. K-ATP channel openers (e.g. pinacidil and nicorandil) have been proposed in the treatment of hypertension and angina pectoris [6,7], while K-ATP channel blocking sulfonylurea derivatives (e.g. glibenclamide) have been administered for many years to patients suffering from type II diabetes mellitus [8].

Because sulfonylurea derivatives not only have specific actions on pancreatic K-ATP channels but also on cardiac K-ATP channels, they could have an important effect on the cardiovascular system [9] which might be either adverse [10] or beneficial [11,12]. For example, since K-ATP channels play a role in preserving cardiac function during ischemia [4] and in protecting against stunning [13], their blockade by sulfonylureas has been found to impair the contractile recovery of the heart after an ischemic episode [4,14]. On the contrary, K-ATP channel blockade by sulfonylureas and the subsequent prevention...
of action potential shortening [15], similar to a class III antiarrhythmic effect, have led to the hypothesis that blockade of K-ATP channels by these drugs might be antiarrhythmic [11,12]. However, controversial findings regarding the role of sulfonylureas in the protection against malignant arrhythmias [14,16–18] and on contractile recovery after an ischemic episode (myocardial stunning) [4,11,13,19] have been found in a variety of experimental models. Furthermore, these compounds have been shown to block myocardial protection afforded by preconditioning in humans [20] and have been associated with a high risk of malignant arrhythmias and death in cardiovascular patients treated with sulfonylureas for their diabetic disease [10,21]. The importance of sulfonylureas in the clinical setting and their potential deleterious cardiovascular effects would indicate that the widespread use of these drugs in cardiovascular patients should be reconsidered [21].

Although the effects of glibenclamide on arrhythmias and myocardial function have been assessed in a variety of animal models, there is no experience in a big conscious animal where the results are not masked or influenced by sedatives, anesthetics, changes in body temperature, etc., of particular importance in ischemia–reperfusion experiments. Thus, the purpose of this work was to study whether K-ATP channel blockade by the most representative sulfonylurea compound, glibenclamide, participates in ischemia and reperfusion-induced arrhythmias and in left ventricular recovery from stunning in a conscious sheep model.

2. Methods

2.1. Animal treatment

Fifty-seven male castrated Hampshire Down sheep aged 7–9 months, weighing 27–34 kg were used. On arrival to the animal house, they were deparasited with ivermectine, and vaccinated against tetanus, anthrax, gas gangrene and clostridial enterotoxemia. Adequate health condition was assessed by professional veterinary staff through clinical examination and laboratory tests. The animals were familiarized with the animal house personnel and the laboratory environment and treated according to the Guide for the Care and Use Laboratory Animals, published by the US National Institute of Health (NIH publication No. 85-23, revised 1996).

2.2. Surgical procedures

As previously described [22], after sedation with acepromazine maleate (0.3 mg/kg), anesthesia was induced with thiopental sodium (20 mg/kg). Following intubation and connection to mechanical ventilation (Neumovent 910, Córdoba, Argentina), anesthesia was maintained with 3% enflurane carried in oxygen and fentanyl citrate, 0.1 mg total dose. A sterile thoracotomy was performed at the fifth intercostal space. After pericardiotomy, a solid-state pressure microtransducer (Konigsberg P7, Pasadena, CA, USA) was inserted in the left ventricular cavity through a stab wound at the apical dimple to obtain left ventricular pressure (P). Tygon fluid-filled catheters were inserted in the internal mammary vein (for drug infusion) and in the left ventricle (for later calibration of the pressure microtransducer). The left anterior descending coronary artery (LAD) was dissected free from adjacent tissue just distal to the second or third diagonal branch, and a pneumatic cuff occluder was positioned around it. To obtain left ventricular wall thickness, a pair of piezoelectric crystals (5 mHz) was placed within the zone to be rendered ischemic. Finally, a pair of steel multifilament wires was tunneled subcutaneously to emerge between the scapulae. Cephalomicin 1 g/day i.m. was administered immediately and for 3 days after surgery. The venous and ventricular catheters were flushed daily with sodium heparin (5000 U) diluted in saline solution.

2.3. Drugs

Glibenclamide (Sigma, St. Louis, MO, USA) was dissolved in a vehicle containing 1 M NaOH (1 ml), ethanol (1 ml) and propylene glycol (1 ml). Then the solution was diluted with sodium chloride (290 mOsm/l) to a total volume of 20 ml. The dose for each animal was prepared on the day of the experiment and slowly infused for 10 min through a venous catheter. To avoid animal death, lidocaine (2 mg/kg, as a bolus) was infused at the start of reperfusion when malignant arrhythmias appeared.

2.4. Experimental protocol

Seven to ten days after surgery, the animals were studied in the conscious, unsedated state. The fluid-filled ventricular catheter was connected to an external pressure transducer (DT-XX, Viggo-Spectramed, Oxnard, CA, USA) previously calibrated using a transducer calibration system (Xcaliber, Viggo-Spectramed). The zero pressure point was set approximately at the level of the right atrium, and the signal generated by the Konigsberg transducer was adjusted to match that of the external transducer. The ultrasonic pair of crystals was connected to a sonomicrometer (Triton, San Diego, CA, USA) and calibrated in mm using the internal calibration. The electrocardiogram was recorded with an ECG Gould transducer. At each acquisition time all signals were digitized at a 4-ms interval for 15 s (3750 samples) using a personal computer equipped with an A/D converter (National Instruments Lab-PC, Austin, TX, USA) and software developed in our laboratory.

The animals were divided into three groups: control,
glibenclamide and vehicle treatment. An additional group of six animals underwent all three protocols to compare the drug and vehicle effects in the same animal. In the control group, after 20 min of basal recordings, the sheep underwent 12 min of complete ischemia followed by a reperfusion period of 120 min. In the glibenclamide group, glibenclamide at a dose of 0.4 mg/kg (maximum dose, 12 mg) was infused for 10 and 30 min before the 12-min ischemic period. In the vehicle group, drug vehicle [1 M NaOH (1 ml), ethanol (1 ml) and propyleneglycol (1 ml) diluted in sodium chloride solution (290 mOsm/l)] to a total volume of 20 ml] was infused at the same time before ischemia as glibenclamide. When the different experimental conditions were imposed on the same sheep, each protocol was separated by 6–7 days to ensure complete recovery of myocardial contractility (see Results) and performed at random to avoid any preconditioning-like effect and/or the effects of potential coronary collateral development.

Experiments with glibenclamide vehicle were made to rule out a hydroalcoholic solution effect on the hemodynamic parameters which might indirectly promote arrhythmia or a vehicle action on myocardial tissue which could directly trigger malignant arrhythmias.

The signals of 15–25 consecutive steady beats were recorded in each acquisition time. In each experimental condition, basal values were taken after stabilization of left ventricular pressure and dimensions. Then, measurements were acquired immediately before ischemia (preischemic values), at 11 min of the ischemic period (ischemic values), and during reperfusion, every 5 min during the first hour and every 10 min during the second hour.

To ensure fully reversible ischemia, a 12-min ischemic period was used, because this short-term regional ischemia induced considerable deterioration of myocardial function but did not result in myocyte death (as shown by anatomopathologic studies in preliminary experiments). Another reason for choosing this ischemic period was the relationship between reperfusion-induced malignant arrhythmias and the duration of the preceding ischemic time [23]. Even though 6 min was sufficient to trigger malignant arrhythmias in an anesthetized rat model [24], this period in sheep was insufficient either to trigger arrhythmias or to elicit mechanical deterioration. Thus, we decided to use 12 min of ischemia to study ventricular tachycardia (VT) and ventricular fibrillation (VF) since it was a period close to that previously shown to induce high incidence of VF [25], and not much longer than the ischemic time used in conscious sheep to study functional recovery after fully reversible regional ischemia [22].

2.5. Epicardial monophasic action potential

To evaluate glibenclamide action on K-ATP channels, the monophasic action potential was measured in a group of ten open chest sheep. A Ag–AgCl suction bipolar electrode was placed on the epicardium within the zone to be rendered ischemic. Control recordings were taken in five sheep during basal, preischemia, ischemia (at 11 min occlusion), and at 1 min of reperfusion. The remaining five sheep were treated with glibenclamide (0.4 mg/kg), 30 min before ischemia, and experimental recordings were acquired as in control. Monophasic action potential duration (APD) was determined at a repolarization of 50% (APD_{50}) and 90% (APD_{90}) of maximal plateau amplitude.

2.6. Data analysis

End diastole was defined to occur at the onset of the rapid upstroke of the digitally obtained time derivative of left ventricular pressure (dP/dt). End systole was defined as the time point where dP/dt reached 10% of its minimal value. This point was similar to that obtained in previous experiments where the end-systolic point was defined as the maximal value of the pressure/diameter ratio. End ejection was established to occur at peak negative dP/dt.

Percent wall thickening fraction (%WTH) was calculated as

\[ \% \text{WTH} = 100 \left( \frac{\text{WTH}_e - \text{WTH}_d}{\text{WTH}_d} \right) \]

where WTH_e is maximum wall thickness between end systole and end ejection, and WTH_d is end-diastolic wall thickness.

End-diastolic radial compliance (CR) was defined as [26]

\[ \text{CR} = \frac{d \epsilon}{d \sigma} = -\frac{dP/d}{d(\ln \text{WTH})} \]

where \( \epsilon \) is radial strain and \( \sigma \) is radial stress. CR was calculated as the slope of the linear fit of the pressure vs. ln WTH relationship using the last 15–30 samples of each beat depending on heart rate (HR).

At each acquisition time, end-systolic pressure (P_{es}), end-diastolic pressure (P_{ed}), HR, %WTH and CR were calculated from each recorded beat and the average of processed beats was the value of the corresponding acquisition time. The value assigned to reperfusion for these variables was the mean of the total reperfusion period, an analysis previously employed to study the behavior of functional recovery [27]. This temporal mean was calculated as the time integral (trapezoidal rule) of the variable divided by total reperfusion duration.

To study the evolution of left ventricular regional function throughout the experiment and its recovery during reperfusion, %WTH and CR were referred to their basal values considered as 100%.

Arrhythmias were detected from the electrocardiogram and the left ventricular pressure signal during ischemia and reperfusion, and diagnosed in accordance to the Lambeth convention as VT, VF or other type of abnormal rhythm as single extrasystoles, salvos, etc. [28]. The severity of arrhythmias was evaluated by Bernauer’s arrhythmia se-
verity index (ASI) [16], an index which allows the statistical comparison and calculation of significance in experiments with tachyarrhythmias.

2.7. Exclusion criteria

The animals were excluded if they had poor-quality electrocardiographic, wall thickening or pressure signals. Sheep with VT at the onset of reperfusion received an intravenous bolus of lidocaine (2 mg/kg) to revert the malignant arrhythmia. When VT evolved to VF, cardioversion was done with one electric shock of 200 J (Rhomicron defibrillator, mod. 790, Argentina). If VT was reverted or defibrillation was successful, the animal continued the experiment and recordings were acquired over the reperfusion period for hemodynamic and mechanical measurements. The animal was not included for any study if more than one electric shock was needed or if it died. If after three consecutive electric shocks VF was not reverted, the animal was sacrificed.

2.8. Statistical analysis

Multiple comparisons between groups for hemodynamic parameters, mechanical function, arrhythmia severity (Bernauer’s index) and APD were performed by the appropriate ANOVA test (see tables and figures). When the F ratio was found to exceed the corresponding critical value for P<0.05, Scheffé’s test was used to compare pairs of mean values. To study arrhythmia incidence, a χ² test with Yate’s correction for continuity was used. Values were expressed as mean±S.E.M.

3. Results

Of fifty-seven operated sheep, five were discarded due to loss of signals or malfunctioning occluder. Of the remaining fifty-two animals (control, n = 16; glibenclamide, n = 20 and vehicle, n = 10; additional group = 6) fifteen died during the reperfusion period. The results of thirty-seven animals (control, n = 12; glibenclamide, n = 10; vehicle, n = 9; additional group = 6) are thus reported for hemodynamic and mechanical studies.

3.1. Hemodynamic data

Hemodynamic data are summarized in Tables 1 and 2. Although a significant rise in Pd was observed during ischemia, it returned immediately to its preischemic values during reperfusion. HR and Pes remained unchanged among treatments (control, glibenclamide and vehicle) and throughout the four experimental times (basal, preischemia, ischemia and reperfusion). These results might be explained on the basis of the small ischemic area (less than 20% of the total left ventricular mass).

3.2. Arrhythmias

Table 3 shows that glibenclamide had a high proarrhythmic effect when arrhythmia incidence and Bernauer’s index were calculated. When the same group of sheep was studied in the three experimental conditions, Bernauer’s index was significantly higher in glibenclamide vs. control and vs. vehicle animals, but arrhythmia incidence did not show differences between experiments due to the small number of animals (Table 4). Glibenclamide not only intensified the appearance of reperfusion-induced

| Table 1 |
| Temporal evolution of hemodynamic parameters in the three experimental protocols using different groups of animalsa |

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Preischemia</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>104.63±3.82</td>
<td>101.5±3.51</td>
<td>102.4±4.56</td>
<td>100.25±3.47</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>100.17±2.99</td>
<td>102.19±3.34</td>
<td>108.47±4.38</td>
<td>98.6±2.63</td>
</tr>
<tr>
<td>Vehicle</td>
<td>98.63±1.81</td>
<td>99.23±3.1</td>
<td>98.95±4.33</td>
<td>97.02±2.68</td>
</tr>
<tr>
<td>Pes (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.46±0.87</td>
<td>9.61±0.63</td>
<td>14.67±1.73†</td>
<td>9.3±1.09</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>9.12±0.53</td>
<td>9.88±0.47</td>
<td>14.69±0.82†</td>
<td>10.04±1.12</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9.45±0.58</td>
<td>9.16±0.64</td>
<td>15.02±0.92†</td>
<td>8.86±0.73</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>77.67±3.3</td>
<td>78.04±3.26</td>
<td>80.7±4.42</td>
<td>79.01±4.5</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>80.34±3.4</td>
<td>81.53±4.06</td>
<td>87.64±3.88</td>
<td>80.91±3.75</td>
</tr>
<tr>
<td>Vehicle</td>
<td>80.45±4.19</td>
<td>78.18±6.37</td>
<td>82.03±6.35</td>
<td>76.95±5.31</td>
</tr>
</tbody>
</table>

a Basal: hemodynamic values at the start of the experiment; Preischemia: values immediately before ischemia; Ischemia: values at 11 min of the ischemic period; Reperfusion: values expressed as the average of the total reperfusion period. There were no differences between groups for any parameter in the four experimental steps. Pes increased during ischemia. Data are mean±S.E.M.

†, P<0.01 (one-way ANOVA followed by Scheffe), when compared to basal and preischemic values.
Table 2
Temporal evolution of hemodynamic parameters when the same animal was subjected to the three experimental protocols (additional group, n=6)\(^a\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>Preischemia</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P_e) (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>107.7±6.8</td>
<td>103.2±6.2</td>
<td>107.4±7.82</td>
<td>104.1±6.42</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>97.02±3.23</td>
<td>98.11±2.98</td>
<td>104.06±2.97</td>
<td>97.06±3.9</td>
</tr>
<tr>
<td>Vehicle</td>
<td>95.2±1.9</td>
<td>92.5±2.5</td>
<td>96.71±4.97</td>
<td>92.8±2.22</td>
</tr>
<tr>
<td>(P_s) (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.52±1.54</td>
<td>9.42±1.54</td>
<td>16.6±2.54†</td>
<td>11.13±1.48</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>9.63±0.8</td>
<td>10.13±0.75</td>
<td>14.9±1.2†</td>
<td>10.67±2.26</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9.31±0.71</td>
<td>9.14±0.69</td>
<td>14.8±1.1†</td>
<td>8.71±0.67</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80.52±5.63</td>
<td>75.88±5.41</td>
<td>80.87±6.88</td>
<td>77.71±7.49</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>84.84±4.78</td>
<td>82.2±5.56</td>
<td>90.22±7.54</td>
<td>84.85±6.29</td>
</tr>
<tr>
<td>Vehicle</td>
<td>72.44±5.29</td>
<td>69.45±4.99</td>
<td>73.6±7.07</td>
<td>74.59±6.57</td>
</tr>
</tbody>
</table>

\(^a\) Data are mean±S.E.M. Basal, hemodynamic values at the start of the experiment; Preischemia, values immediately before ischemia; Ischemia, values at 11 min of the ischemic period; Reperfusion, values expressed as the average of the total reperfusion period. There were no differences between experiments for any parameter in the four experimental steps. \(P_s\) increased during ischemia. \(^\dagger\), \(P<0.01\) (two-way ANOVA for repeated measures followed by Scheffe) when compared to basal and preischemic values.

Table 3
Arrhythmia incidence and arrhythmia severity (Bernauer’s index) in the three groups of animals during ischemia and reperfusion\(^a\)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>n</th>
<th>Ischemia [x/n]</th>
<th>Reperfusion [x/n]</th>
<th>Bernauer’s index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VT VF Other</td>
<td>VT VF Other</td>
<td>Isch. Rep.</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>– – –</td>
<td>7/16 6/16 5/16</td>
<td>0 54</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>20</td>
<td>– – 4/20</td>
<td>17/20(^*) 16/20(^\dagger) 16/20(^\dagger)</td>
<td>4 143(^\ddagger)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>– – –</td>
<td>3/10 2/10 2/10</td>
<td>0 23</td>
</tr>
</tbody>
</table>

\(^a\) VT, ventricular tachycardia; VF, ventricular fibrillation; Other, ventricular extrasystoles or salvos; n, animal number for each group; x/n, number of animals over total number of animals. \(^*\), \(P<0.025\) glibenclamide vs. control, \(^\dagger\), \(P<0.01\) glibenclamide vs. vehicle (\(\chi^2\) test), \(^\ddagger\), \(P<0.001\) glibenclamide vs. control and vs. vehicle (one-way ANOVA followed by Scheffe).

Arrhythmias, but also had some pro-arrhythmic action during ischemia (a few premature ectopic beats were seen in five animals). When tachyarrhythmias occurred, they appeared during the first 20 s of reperfusion and usually began with premature ectopic beats or salvos and sudden appearance of VT which turned into VF. In most cases, VT started without previous ectopic beats or salvos and turned suddenly into VF. Although mortality due to VF in the glibenclamide group (50%) was greater compared to control (25%) and vehicle (10%), it did not reach statistical significance (\(\chi^2\) test).

3.3. Glibenclamide effects on action potential duration

Fig. 1 and Table 5 show that in control, ischemia shortened APD (percent change with respect to preischemia, APD\(_{50}\) –37.65±3%; APD\(_{90}\) –30.05±1.03%) and that this response was abolished by glibenclamide.

Table 4
Arrhythmia incidence and arrhythmia severity (Bernauer’s index) during ischemia and reperfusion in the animals submitted to the three different experimental conditions (n=6)\(^a\)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>n</th>
<th>Ischemia [x/n]</th>
<th>Reperfusion [x/n]</th>
<th>Bernauer’s index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VT VF Other</td>
<td>VT VF Other</td>
<td>Isch. Rep.</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>– – –</td>
<td>3/6 1/6 2/6</td>
<td>0 17</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>6</td>
<td>– – 1/6</td>
<td>6/6 4/6 4/6</td>
<td>1 44(^\dagger)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>– – –</td>
<td>2/6 0/6 2/6</td>
<td>0 8</td>
</tr>
</tbody>
</table>

\(^a\) VT, ventricular tachycardia; VF, ventricular fibrillation; Other, ventricular extrasystoles or salvos; n, animal number for each group; x/n, number of animals over the total number of animals. \(^\dagger\), \(P<0.01\) glibenclamide vs. control and \(^\ddagger\), \(P<0.001\) glibenclamide vs. vehicle (one-way ANOVA for repeated measures followed by Scheffe).
Fig. 1. Lines 1 and 2: monophasic action potential recordings from control and glibenclamide (0.4 mg/kg) treated sheep at preischemia, ischemia (11 min) and reperfusion (1 min). Note that the action potential duration did not change with glibenclamide (see APD_{50} and APD_{90} in Table 5) during ischemia and at 1 min of reperfusion due to K-ATP channel blockade. Line 3: APD during ischemia and APD changes during reperfusion due to VT in a glibenclamide-treated sheep. Note the progressive increase in heart rate during VT (at 30 s, 240 beats/min, compared with 15 s, 180 beats/min). At 40 s VT is reverted by lidocaine infusion. Line 4: action potential recordings during VF in a glibenclamide-treated animal different from the one shown in line 3.

Table 5
Action potential duration (ms) during control and 30 min after glibenclamide injection

<table>
<thead>
<tr>
<th>Group</th>
<th>APD_{50} Basal</th>
<th>Preischemia</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>178±6</td>
<td>175±5</td>
<td>118±6†§</td>
<td>176±6</td>
</tr>
<tr>
<td></td>
<td>234±2</td>
<td>231±2</td>
<td>172±4†§</td>
<td>229±4</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>197±1</td>
<td>194±3</td>
<td>198±2</td>
<td>195±4</td>
</tr>
<tr>
<td></td>
<td>265±5</td>
<td>262±7</td>
<td>264±5</td>
<td>260±8</td>
</tr>
</tbody>
</table>

* Action potential duration (ms) in control (n=5) and after glibenclamide (n=5) infusion at repolarization of 50% (APD_{50}) and 90% (APD_{90}) of maximal plateau amplitude was measured in open chest sheep using a Ag–AgCl suction electrode placed in the ischemic region (see text). †, P<0.01 control vs. drug treatment; §, P<0.01 ischemia vs. preischemia (two-way ANOVA for repeated measures followed by Scheffe). Data are mean±S.E.M.

(percent change with respect to preischemia, APD_{50} 0.69±1%; APD_{90} 1.3±0.96%). Note that the recovery of APD at 1 min of reperfusion was completely achieved in control and in glibenclamide-treated sheep, showing no changes compared with preischemic data.

3.4. Contractile behavior during ischemia and reperfusion

Table 6 shows regional mechanical parameters for

Table 6
Regional mechanical parameters measured at the start of the protocol (basal condition) in the same animal undergoing the three different experimental protocols (n=6)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glibenclamide</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>% WTH</td>
<td>34.4±5.3</td>
<td>32.6±6.2</td>
<td>35.8±4.4</td>
</tr>
<tr>
<td>CR (mmHg⁻¹)</td>
<td>0.0096±0.0011</td>
<td>0.0104±0.0014</td>
<td>0.0097±0.0015</td>
</tr>
</tbody>
</table>

* %WTH, percent wall thickening fraction; CR, radial diastolic compliance. There were no differences among groups (two-way ANOVA for repeated measures). Data are mean±S.E.M.
systolic (%WTH) and diastolic (CR) function at the start of the protocols (basal conditions) in the group of animals undergoing the three different experimental protocols. The data show that complete recovery of regional mechanical function among protocols was achieved with a 12-min ischemic period. Thus, since stunning must be studied only in conditions allowing full reversibility after an ischemic episode [29], this result enabled use of the same animal for the different experimental protocols.

Fig. 2 shows that glibenclamide strongly affected the recovery of left ventricular regional systolic and diastolic mechanical function (measured as percent recovery of %WTH and CR, respectively) after a fully reversible ischemic episode. Although there was regional mechanical deterioration during ischemia and reperfusion, global systolic function remained unchanged (Tables 1 and 2). Results in the three different groups of sheep showed a large decrease in the percentage recovery of %WTH (P < 0.01) and CR (P < 0.05) in the glibenclamide (26.9 ± 6.7 and 76.2 ± 7.8%) vs. control (65.7 ± 3.5% and 104.7 ± 4.2%) and vs. vehicle (68.6 ± 5.6 and 106 ± 4.9%) groups, (Fig. 2A and B). Similar results were found when the same sheep were submitted to the three different experimental protocols — glibenclamide strongly affecting percent recovery of cardiac parameters of both systolic (%WTH: 27.2 ± 6.7% vs. control 66.3 ± 5.2% and vs. vehicle 64.4 ± 8%, P < 0.01) and diastolic function (CR: 72.3 ± 5.8 vs. control 103.6 ± 6.5, P < 0.05 and vs. vehicle 105.2 ± 8, P < 0.05) (Fig. 2C and D).

Although in our model, the combined VT and VF duration before successful cardioversion did not last more than 40 s, arrhythmia appearance could have had an effect on the subsequent myocardial functional recovery, per se or due to lidocaine infusion and/or lidocaine plus electric cardioversion. Thus, to make sure that these possibilities did not have any effect on measured functional recovery, percent recovery of %WTH and CR were compared in animals without arrhythmias against those with malignant arrhythmias (VT) plus lidocaine (Fig. 3) and in animals without arrhythmias at the start of reperfusion against those with lidocaine plus electric cardioversion due to VT and VF (Fig. 4). Even though a statistical analysis could not be applied (groups with very low number of animals), the figures indicate the apparent absence of differences in recovery from stunning between animals, indicating that neither lidocaine nor combined lidocaine plus cardioversion had an additive deleterious effect on the depressant action of glibenclamide on %WTH and CR.

![Fig. 2. Left ventricular regional function in preischemia, ischemia and its recovery during reperfusion measured as percent recovery of the percentage wall thickening fraction (%WTH) and percent recovery of radial diastolic compliance (CR) (both parameters referred to basal values considered as 100%). (A) Percent recovery of %WTH and (B) percent recovery of CR when three different groups of animals (control n = 12, glibenclamide n = 10 and vehicle n = 9) were considered (one-way ANOVA for repeated measures followed by Scheffé). (C) Percent recovery of %WTH and (D) percent recovery of CR when the same animals underwent the three different protocols (n = 6) (two-way ANOVA for repeated measures followed by Scheffé). Data are mean ± S.E.M.](image-url)
Fig. 3. Temporal evolution of global ($P_{max}$) and regional (%WTH and CR) parameters measured in animals with lidocaine (2 mg/kg in bolus) due to VT and in animals without lidocaine in control [(D) $P_{max}$], (E) %WTH and (F) CR] and glibenclamide-treated (D) $P_{max}$, E:%WTH and F: CR) experiments. Only the first 30 min are shown. The first point indicates the end of ischemia; $n=3$ in each of the four different groups. Statistical analysis was not possible. However, the figure shows the apparent lack of differences between animals. Data are mean±S.E.M.

4. Discussion

The present study shows that the administration of glibenclamide results in a proarrhythmic effect at the start of reperfusion in chronically instrumented conscious sheep subjected to a sustained and reversible ischemic episode. Our results agree with those of Bernauer [16] in isolated rat hearts and Cole et al. [4] and Shigematsu et al. [14] in guinea pig right ventricular walls. Conversely, many authors have demonstrated that glibenclamide protects against malignant arrhythmias [3,5,30] or at least diminishes the duration of arrhythmic episodes and increases the chance of spontaneous reversion of malignant arrhythmias [19,24,25]. Another relevant finding is that the drug improves left ventricular mechanical recovery during reperfusion. Since the persistence of myocardial contractile dysfunction (stunning) after an ischemic episode strongly affects morbidity and mortality [29], the detrimental action of the drug on mechanical function is of major clinical interest. Although recovery from stunning has been studied in conscious animals [29], there is no experience about glibenclamide and posts ischemic functional improvement. Moreover, recovery from stunning has been studied in most cases as recovery of systolic function (measured as ventricular developed pressure, %WTH or percent segment shortening) and only a few studies have analyzed the recovery of diastolic function [22,31]. The present paper is the first, to our knowledge, to report on the action of glibenclamide on left ventricular regional function in a conscious animal model. Glibenclamide deteriorated both systolic and diastolic function, a result similar to that previously observed by Cole [4] and Shigematsu et al. [14] in guinea pig right ventricular wall and Mitani et al. [2] in rat hearts. In contrast, Bril et al. [19] found a protective effect of this drug on ventricular function in rat hearts, albeit only during ischemia, and Auchampach et al. [13]
did not observe any glibenclamide action per se on postischemic systolic functional recovery in anesthetized dogs using a dose similar to that employed here. The conflicting results are probably due to species differences, the experimental model (global versus regional ischemia, anesthetized versus conscious animal) and the employed dose of glibenclamide.

### 4.1. Animal model

Sheep were chosen for several reasons: (a) they have been widely used to study the cardiovascular system in a variety of experimental models; (b) experimental studies have shown a similar pattern in heart development and coronary circulation of sheep and humans [32,33]; (c) the conscious sheep is a well-established animal model to study regional mechanical function [22,34]; (d) sheep are very docile animals and remain conscious and calm without sedation throughout the whole experiment; (e) percent thickening fraction [%WTH] (31.5±10.64, mean pooled data of preischemic values) was similar to that recorded for pigs [35] and dogs [36], suggesting that the sheep is an adequate species to study regional mechanics; (f) studies in sheep Purkinje fibers resemble the behavior of action potential changes seen in cardiomyocytes [37] during ischemia, and have shown a similar mechanism in afterdepolarization-induced cardiac arrhythmias [38] observed in other animal models. Thus, we think that within rationale limits, our conscious animal model is adequate to study ischemia and reperfusion-induced arrhythmias and mechanical dysfunction.

### 4.2. Glibenclamide dosage

Although there is no reported effect of glibenclamide in sheep, the employed dose (0.4 mg/kg) resulted in a 2.5 μM plasma level, which was within the pharmacologic range [39] and effectively blocked the sarcolemmal [40] (Fig. 1 and Table 5) and mitochondrial K-ATP channels [41]. Furthermore, it was also similar to doses that had deleterious cardiovascular actions in humans [20] (10 mg total dose) and in dogs [13] and rabbits [40] (0.3 mg/kg). However, the used dose was lower than that reported in other studies in dogs (10 mg/kg) [30], anesthetized rabbits (3, 6, 12 and 24 mg/kg) [42] and closed chest rats [24] (5 mg/kg) which are well beyond the range likely to be encountered in the clinical setting [39]. Regarding the hypoglycemic effect of the drug, it is noteworthy that although a significant decrease was mentioned in dogs [43] [probably due to the used dose (1 mg/kg) or a species-
dependent effect] 0.4 mg/kg did not greatly affect plasma glucose levels in sheep (a slight decrease, less than 15% from basal values, was observed; data not shown) allowing for a complete conscious and calm state.

4.3. Mechanisms of glibenclamide-induced reperfusion arrhythmias and myocardial stunning

Although the genesis of reperfusion arrhythmias and the pathophysiology of stunning are not fully understood, many studies have suggested transient Ca\(^{2+}\) overload as one of the causes [29,44]. Measurements of Ca\(^{2+}\) in isolated myocytes, papillary muscles and perfused ferret and rat hearts have shown an association between an increase in intracellular Ca\(^{2+}\) and the induction of ventricular arrhythmias. Effectively, in hearts submitted to 10 min ischemia, VT and VF at the beginning of reperfusion were preceded by prominent elevations in Ca\(^{2+}\) levels. Conversely, the reduction in intracellular Ca\(^{2+}\) resulted in a significant decrease of VT and VF upon reperfusion [44]. Afterdepolarization-induced triggered activity due to altered Ca\(^{2+}\) inward current could be involved in sheep [38]. Afterdepolarization is a complex mechanism which implies two components [38]: (a) action potential lengthening (first or initiating phase) and (b) altered ionic inward current (second or depolarization phase). It is noteworthy that glibenclamide by K-ATP channel blockade leads to action potential lengthening (first phase) as observed in the present study (Fig. 1) which might result in Ca\(^{2+}\) overload [4] (second phase). Thus, as demonstrated in sheep Purkinje fibers [45], Ca\(^{2+}\)-induced triggered arrhythmias during reperfusion appear to be the arrhythmogenic mechanism in our animal model. Regarding this Ca\(^{2+}\)-related pathophysiology, it has been postulated that the K-ATP channel protective effect on myocardial contractile function [1,4,13] might be due to action potential shortening as a consequence of K-ATP channel opening, leading to a reduction in the time of Ca\(^{2+}\) influx via voltage-gated channels and to an increase in the time during which the Na\(^{+}\)-Ca\(^{2+}\) exchanger might operate to extrude Ca\(^{2+}\) from the cell. This would help to maintain intracellular Ca\(^{2+}\) at physiological levels and might also indirectly reduce the consumption of high energy phosphates stores which could be later used in contractile recovery [4]. Thus, glibenclamide due to its K-ATP channel blocking effect leads to action potential lengthening (Fig. 1) which would finally result in Ca\(^{2+}\) overload and a subsequent injury of the contractile apparatus. However, we cannot assure that Ca\(^{2+}\) overload is the main and sole mechanism of glibenclamide-induced stunning, since Ca\(^{2+}\) overload could secondarily lead to oxyradical genesis [29] which has also been mentioned as a cause of stunning and reperfusion-induced arrhythmias. However, both Ca\(^{2+}\) and/or oxyradical mechanisms are not mutually exclusive and may represent different features of the same pathophysiological sequence. Although the preceding explanation emphasizes that the deleterious effects of glibenclamide on reperfusion-induced arrhythmias and on mechanical function are a cause of sarcolemmal K-ATP channel blockade, the drug also inhibits mitochondrial K-ATP channels [41], which have recently attracted attention as being the most important pathway in the cardioprotection afforded by K-ATP channel openers [41]. However, the hypothesis of Ca\(^{2+}\) overload appears as the most probable mechanism, since Ca\(^{2+}\) channel antagonists protect against arrhythmias during reperfusion (including glibenclamide-triggered arrhythmias [17]) and against stunning [29]. Moreover, K-ATP channel openers having a similar action have been called ‘indirect Ca\(^{2+}\) channel blockers’ [41].

It is also important to consider other drug effects on the cardiovascular system that might affect cardiac contractile recovery or trigger malignant arrhythmias; for example (1) glibenclamide stimulates glycolytic ATP synthesis in rat hearts without changes in the inotropic state or in oxygen consumption [46]. However, impaired posts ischemic recovery should not be attributed to this metabolic effect because an increase in ATP is more likely to improve, and not to impair, cardiac function; (2) a decrease in coronary blood flow [19,30,43] might be also considered as a cause of arrhythmogenesis and/or stunning. In rat hearts, a high decrease in flow was described [16,19] and correlated with the occurrence of VF. However, counteracting the glibenclamide-induced vasoconstriction with sodium nitroprusside, a real antiarrhythmic effect was not achieved [16]. Moreover, many authors have found a protective effect against arrhythmias even though there was a high decrease in coronary flow [3,30,43]. As for stunning and the probable flow restriction by glibenclamide, we did not find any differences in wall thickening and compliance measurements before and after drug infusion (see preischemic values compared to basal values in Figs. 2 and 4). In our conscious model we cannot entirely rule out a vasoconstrictive drug effect, but, if it existed, it was small enough not to produce any detrimental effect on myocardial perfusion as corroborated by the maintenance of ventricular dynamics (Tables 1 and 2). Work by Auchampach et al. using similar doses in anesthetized dogs seems to confirm our assumption [13]. Moreover, these authors showed a detrimental action of the drug on myocardial recovery when a high dose (1 mg/kg) was used, although there were no changes in myocardial perfusion. Conversely, in the same animal and with the same glibenclamide dose, Billman et al. [43] reported a reduction of preischemic flow and hyperemic response. However, these authors showed that, although glibenclamide affects hyperemia, the coronary blood flow quickly returned to its preischemic values, indicating that glibenclamide had no additional effects on vascular resistance during reperfusion. Thus, these conflicting results about the effect of glibenclamide on myocardial blood flow suggest that this is not the main mechanism involved in ischemia and reperfusion-induced arrhythmias and stunning. Finally, the
deleterious effect of glibenclamide on mechanical recovery cannot be explained either by the appearance of arrhythmias or their electric cardioversion (Fig. 4) nor by a combined deleterious interaction between drugs when lidocaine was infused (Fig. 3).

5. Conclusions

Our paper reinforces the cardioprotective role of K-ATP channels in the setting of ischemia and reperfusion, and shows for the first time that, although glibenclamide is a widely used and apparently safe drug, it has combined proarrhythmic and cardiodepressant effects in conscious sheep. These effects can be largely explained by the glibenclamide blocking effect on cardiomyocyte sarcoplasmal K-ATP channels (resulting in Ca$^{2+}$ overload secondary to action potential lengthening) and/or mitochondrial K-ATP channels.

Although it is probable that the reported undesired actions only take place in the setting of ischemia and early reperfusion when cardiac K-ATP channels are activated, it appears advisable to be cautious in sulfonylurea administration for the treatment of type II diabetic patients.

Acknowledgements

We thank Julio Martínez and Fabián Gauna for surgical and technical help. Animal care provided by veterinarians María I. Besansón, Pedro Iguain and Marta Tealdo and veterinary assistants Juan Mansilla, Juan Ocampo and Osvaldo Sosa is gratefully acknowledged.

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