Ventricular fibrillation in preconditioned pig hearts: role of $\text{K}_{\text{ATP}}^+$ channels

Gilles Rioufol, Michel Ovize, Joseph Loufoua, Calin Pop, Xavier André-Fouët, and Yves Minaire.

Ventricular fibrillation in preconditioned pig hearts: role of $\text{K}_{\text{ATP}}^+$ channels. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2804-H2810, 1997.—ATP-dependent potassium ($\text{K}_{\text{ATP}}^+$) channels play a role in the infarct size-limiting effect of preconditioning in pigs. We previously demonstrated that preconditioning shortens monophasic action potential duration (MAPD) and accelerates the time to ventricular fibrillation (VF) during a prolonged ischemia in pigs. We sought to determine whether the mechanism of the reduced time to VF in preconditioned pigs is a consequence of $\text{K}_{\text{ATP}}^+$ channel activation. Pigs underwent 40 min of coronary occlusion and 2 h of reperfusion. Before this, animals received either no intervention (control), 10 min of ischemia and 10 min of reperfusion (preconditioned), or an intravenous infusion of nicorandil, a $\text{K}_{\text{ATP}}^+$ channel opener. Additional control, preconditioned, and nicorandil-treated pigs were pretreated by glibenclamide, an antagonist of $\text{K}_{\text{ATP}}^+$ channels. Because 1) the $\text{K}_{\text{ATP}}^+$ channel activator nicorandil did not produce shorter time to VF, 2) the $\text{K}_{\text{ATP}}^+$ channel inhibitor glibenclamide did not block the acceleration of VF by preconditioning, and 3) there was no relationship between time to VF and infarct size or MAPD, the major conclusion is that reduced time to VF in preconditioned animals is not a consequence of $\text{K}_{\text{ATP}}^+$ channel activation.

EXPOSING THE HEART to sublethal periods of ischemia renders it more resistant to subsequent sustained ischemic insult (11). This protection, termed preconditioning, can delay ischemic irreversible cell death in dogs, rats, rabbits, and pigs (10, 11, 15, 18). Numerous studies clearly demonstrated that preconditioning also reduces ischemia and reperfusion-induced ventricular arrhythmias in the rat model (9, 19). However, whether preconditioning protects against ventricular arrhythmias in larger animal species remains controversial. Vegh et al. (21) demonstrated that preconditioning reduces ventricular tachycardia and ventricular fibrillation (VF) during a 25-min coronary artery occlusion in dogs. In contrast, Murry et al. (11) and Przyklenk and Kloner (16) failed to demonstrate similar protection during a sustained ischemic insult and the following reperfusion in the canine heart. We recently showed that preconditioning reduces infarct size but fails to prevent VF in the pig model (14). Rather, preconditioning accelerated the time to VF during the prolonged coronary artery occlusion (14). This detrimental effect was accompanied by a rapid and significant shortening of the monophasic action potential duration (MAPD) and a decrease in the VF threshold in the first minutes of the sustained ischemic insult (14).

A large body of evidence indicates that activation of ATP-dependent potassium ($\text{K}_{\text{ATP}}^+$) channels plays a crucial role in the infarct size-limiting effect of preconditioning (18). However, activation of $\text{K}_{\text{ATP}}^+$ channels shortens MAPD, which may trigger lethal arrhythmias (2).

We therefore proposed that activation of $\text{K}_{\text{ATP}}^+$ channels might be favorable, on the one hand, by limiting infarct size and detrimental, on the other hand, by triggering ventricular arrhythmias during an acute ischemic insult. This is a major clinical issue because many patients suffering an acute myocardial infarction may have been preconditioned by preceding episodes of angina or may be under treatment with the $\text{K}_{\text{ATP}}^+$ channel opener nicorandil, which is widely used in Europe and J anpan (7, 12, 13). Thus the objective of the present study was to determine whether glibenclamide, a specific blocker of $\text{K}_{\text{ATP}}^+$ channels, might alter the incidence and the time to onset of VF in pig hearts that had been previously preconditioned or pretreated by the $\text{K}_{\text{ATP}}^+$ channel opener nicorandil. We addressed this important issue using a previously described pig model of acute myocardial infarction, which allows simultaneous assessment of incidence of VF, MAPD, and infarct size (14).

MATERIALS AND METHODS

All experiments performed in this study conform to the “Guiding Principles for Research Involving Animals and Human Beings” approved by the American Physiological Society.

Surgical Preparation

Fifty-seven farm pigs, weighing 28.5 ± 0.4 kg, were premedicated with droperidol (1 mg/kg sc) and anesthetized with α-chloralose (80 mg/kg iv). Additional intravenous administration of α-chloralose (20 mg/kg) was performed when needed. Pigs were ventilated with room air through a tracheotomy tube, and tidal volume and rate were adjusted to provide physiological pH and blood gases. Body temperature was monitored via a rectal thermometer and kept constant (range 38.0–39.0°C) by means of a heating pad because it has been demonstrated that temperature may alter infarct size (3). Cannulas were inserted into the right jugular vein (for administration of drugs and fluids) and the carotid artery (for measurement of blood pressure). Electrocardiographic limb leads and arterial pressure were monitored continuously throughout the experiment on a Gould recorder (Gould, Cleveland, OH).

A thoracotomy was performed in the fourth left intercostal space, and a segment of the left anterior descending coronary artery (LAD) was isolated, approximately midway between the left main ostium and the apex. One unipolar catronic ORX electrode (Plastimed, Saint Leu La Forêt, France), used to
measure monophasic action potentials (MAPs), was positioned via a small scalp incision into the subepicardium in the center of the soon-to-be ischemic LAD bed. One bipolar pacing electrode was inserted in the left ventricular subepicardium in the vicinity of the circumflex coronary artery and used to pace the heart during MAP recordings. The animals were allowed 15 min to stabilize after these surgical procedures.

Experimental Design

After the surgical procedures, all pigs were randomly assigned to one of six groups (Fig. 1). The randomization procedure took into account the fact that some animals developed intractable VF and could not be analyzed for infarct size. Thus, in case of intractable VF, the randomization paper was put back into the randomization box.

All animals underwent a 30-min treatment period consisting of one of the following protocols. 1) The control group (C; n = 11) received intravenous infusion of vehicle for 10 min followed by no intervention for 20 min. 2) The control/glibenclamide group (C/Gli; n = 9) received intravenous infusion of 0.5 mg/kg glibenclamide for 10 min followed by 20 min without any intervention. 3) The preconditioning group (PC; n = 8) received intravenous infusion of vehicle for 10 min followed by 10 min of LAD occlusion and 10 min of reperfusion. 4) The preconditioned/glibenclamide group (PC/Gli; n = 12) received intravenous infusion of 0.5 mg/kg glibenclamide for 10 min followed by 10 min of LAD occlusion and 10 min of reperfusion. 5) The nicorandil group (Nic; n = 9) received intravenous infusion of vehicle for 10 min followed by intravenous infusion of 0.5 mg/kg nicorandil for 10 min and then 10 min without any intervention. 6) The nicorandil/glibenclamide group (Nic/Gli; n = 8) received intravenous infusion of 0.5 mg/kg glibenclamide for 10 min followed by intravenous infusion of 0.5 mg/kg nicorandil for 10 min and then 10 min without any intervention.

At the end of the 30-min treatment period, all pigs underwent 40 min of coronary artery occlusion followed by 2 h of reperfusion.

Infarct Size Determination

At the end of the 2-h reperfusion, the LAD was reoccluded and 0.5 mg/kg Uniporser blue pigment (Ciba-Geigy, Hawthorne, NY) was injected intravenously to delineate the in vivo area at risk, as previously described (15). With this technique, the previously nonischemic myocardium appears blue, whereas the previously ischemic myocardium (area at risk) remains unstained. Under deep anesthesia, the hearts were stopped by intravenous injection of potassium chloride (20 meq), excised, and cut into five to seven transverse slices, parallel to the atrioventricular groove. After right ventricular tissue had been removed, the heart slices were weighed. The basal surface of each slice was photographed for later measurement of the area at risk. Then each slice was incubated for 10 min in a 1% solution of triphenyltetrazolium chloride at 37°C. This method reliably distinguishes necrotic myocardium (which appears pale) from viable myocardium, which stains brick red (23). The slices were then rephotographed. Enlarged projections of these slides were traced for determination of the boundaries of the area at risk and area of necrosis. Extent of the area at risk and area of necrosis was quantified by computerized planimetry and corrected for the weight of the

Nicorandil (Rhoˆne-Poulenc Rorer, Vitry-sur-Seine, France) was dissolved in saline (0.9%). Glibenclamide (Sigma, L’Isle D’Abeau, France) was dissolved in a vehicle prepared on the day of the experiment; 10 ml vehicle contained 0.5 ml NaOH (1.0 N), 0.5 ml propylene glycol, 0.5 ml ethanol, and 8.5 ml saline (0.9%).

Hemodynamics

Heart rate and arterial blood pressure were measured and averaged over five continuous cardiac cycles in sinus rhythm for each sample period. Measurements of heart rate and arterial blood pressure were made at baseline (i.e., before treatment), after drug infusion, and immediately before the sustained occlusion in all groups. In the preconditioned groups, hemodynamics were measured during the brief 10-min preconditioning occlusion. In all groups, hemodynamics were then monitored throughout the sustained occlusion and at frequent intervals after reperfusion.

VF and MAPD

The incidence of spontaneous VF during the preconditioning period, sustained occlusion, and reperfusion was quantified for all groups. The time to VF, defined as the time elapsed from the beginning of the sustained LAD occlusion to the first spontaneous VF, was recorded. Each time VF occurred, defibrillation was achieved by means of internal paddles in <20 s with direct current shock (energy: 10 W·s) from a Scard F defibrillator (Siemens, Erlangen, Germany). When defibrillation could not be achieved after three shocks, VF was considered intractable.

MAP recordings were performed on a Gould recorder. Impulses were delivered by a programmed stimulator (Hugo Sachs, Freiburg im Brisen, Germany) with a rate of 180 beats/min, a duration of 5 ms, and an intensity of 1 mA, as previously described (14). MAP duration (MAPD) was measured under pacing at a constant rate (180 beats/min) at 50% repolarization (MAPD50). MAPD50 was measured in the ischemic zone at frequent intervals throughout the experiment. MAPD50 was measured and averaged over three separate cardiac cycles under steady-state conditions (i.e., 30–45 s after the beginning of pacing) for each sample period.
tissue slices. Total weight of the area at risk and the area of necrosis was then calculated and expressed as a percentage of total left ventricular weight.

Blood Glucose

Because glibenclamide is known to influence blood glucose levels, arterial blood samples were obtained in four controls, four preconditioned, five glibenclamide-treated controls, five glibenclamide-treated preconditioned, and six glibenclamide-treated nicorandil animals. Samples were taken at baseline, at the end of the treatment period, and at the end of the final reperfusion. The measurement of blood glucose levels was performed by using a glucometer (Glucometer 4, Bayer Diagnostics, Puteaux, France).

Exclusion Criteria

Pigs that developed intractable VF, i.e., VF that could not be reversed after three direct current shocks, were excluded from the infarct size study. However, MAPD and VF data for these animals have been included in the final analysis.

Statistics

All measurements are expressed as means ± SE. Comparison of hemodynamics and blood glucose was performed by two-factor analysis of variance and post hoc Tukey’s test. Comparison of infarct size, MAPD, and the time to VF was performed by nonparametric Kruskall-Wallis and post hoc Wilcoxon tests (24). Comparison of the incidence of VF was performed by a $\chi^2$ test. A P value < 0.05 was considered statistically significant.

RESULTS

Mortality and Exclusions

Among the 57 pigs that entered this study, 14 developed intractable VF during the prolonged coronary occlusion ($n = 11$) or the final reperfusion ($n = 3$): 3 controls, 3 preconditioned, 1 glibenclamide-treated control, 4 glibenclamide-treated preconditioned, 1 nicorandil-treated, and 2 glibenclamide-treated nicorandil pigs (Table 1).

Hemodynamics

Heart rate and systolic arterial pressure did not differ among groups at baseline (Fig. 2, A and B, respectively). Nicorandil-treated animals exhibited a transient decrease in blood pressure during drug infusion (Fig. 2B). At 10 min of nicorandil perfusion, systolic blood pressure averaged 71 ± 4 vs. 96 ± 4 mmHg at baseline ($P < 0.05$). However, this reduction in pressure was short lived; during the prolonged occlusion, arterial pressure no longer differed from...
baseline or control values. Glibenclamide blunted (although not significantly) this blood pressure decrease in the Nic/Gli group. Hemodynamics were similar among the six groups during the following 40-min occlusion and 2-h reperfusion.

Blood Glucose

Blood glucose levels were similar among groups at baseline. Glibenclamide did not significantly alter these values in control, preconditioned, or nicorandil pigs.

VF

Incidence of VF. No pig in the control or C/Gli groups developed VF during the treatment period. In the PC group, three (38%) pigs fibrillated during the brief LAD occlusion and three pigs (38%) during the following brief reperfusion. In the PC/Gli group, 1 of 12 pigs (8%) developed VF during the brief episode of ischemia and 4 of 12 (33%) fibrillated during the following brief reperfusion [P = not significant (NS) between PC and PC/Gli groups]. One of nine (11%) nicorandil-treated animals developed VF just before the sustained coronary artery occlusion. No pig in the Nic/Gli group developed VF during the treatment period.

As expected in this model, control hearts exhibited a high incidence of VF (73%) during the 40-min LAD occlusion (Fig. 3). Unlike infarct size, incidence of VF in the preconditioned and nicorandil-treated groups was not significantly different from that of the control group, averaging 88 and 78%, respectively. Glibenclamide per se did not significantly alter the incidence of VF (78% in the C/Gli group during the prolonged coronary artery occlusion; P = NS vs. control). Although it significantly attenuated infarct size limitation, glibenclamide did not reduce the incidence of VF in the preconditioned and nicorandil groups, which averaged 92 and 88%, respectively, during the sustained ischemic insult (P = NS vs. control).

During the final reperfusion period, the incidence of VF was not different among groups, ranging from 9 to 37% (P = NS among groups; Fig. 3).

Fig. 3. Incidence of ventricular fibrillation (VF). Data of all pigs that entered the study are included in this analysis. For incidence of VF during the 2-h reperfusion, data are expressed as % of no. of animals that survived the 40-min occlusion period (see Table 1). Incidence of VF did not significantly differ among groups during the 40-min ischemic insult or the following 2-h reperfusion.

MAPD

MAPD$_{90}$ was comparable among groups at baseline, ranging from 182 ± 1 to 190 ± 4 ms (P = NS). During the treatment period, MAPD in the control group did not vary significantly (183 ± 2 ms), whereas preconditioned and nicorandil-treated hearts displayed a significant shortening of MAPD that averaged 162 ± 6 and 151 ± 11 ms, respectively (P < 0.05 vs. baseline and control group). In both groups, glibenclamide blunted this effect (Fig. 5). In each group, MAPD$_{90}$ returned to near baseline values just before the sustained coronary artery occlusion (P = NS vs. baseline and among groups).

During the prolonged coronary artery occlusion, MAPD$_{90}$ significantly decreased in the control, preconditioned, and nicorandil-treated hearts (P < 0.05 vs. baseline). Glibenclamide prevented (in controls) or blunted (in preconditioned and nicorandil groups) this effect of the sustained ischemia (Fig. 5). Interestingly, preconditioned hearts exhibited a larger shortening of MAPD$_{90}$ at 5 min of the prolonged ischemia than during the preceding preconditioning episode; this effect was prevented by glibenclamide (Fig. 5). During the final reperfusion, MAPD$_{90}$ did not vary significantly in any group compared with the end of the prolonged coronary artery occlusion (data not shown).

Infarct Size

In the present study, infarct size was considered as a secondary end point, used to ensure that precondition-
The control group (41.7 ± 2.9%) was significantly smaller than that observed in ischaemia or nicorandil infusion: mean infarct size averaging that had been triggered either by the 10-min ischemia. Preconditioning and Nic infusion significantly attenuated infarct size limitation of preconditioning in control, PC, and Nic-treated hearts. *P < 0.05 vs. control.

Additional reduction of MAPD50 occurred in the PC group at 5 min of the prolonged ischemia. Gli blunted or prevented MAPD50 shortening during the 40-min coronary artery occlusion in control, PC, and Nic-treated hearts. *P < 0.05 vs. control.

Area at risk and infarct size data are presented for those animals that did not develop intractable VF: eight controls, eight glibenclamide-treated controls, five preconditioned, eight glibenclamide-treated preconditioned, eight nicorandil, and six glibenclamide treated nicorandil pigs (Table 1). Area at risk (expressed as percentage of left ventricular (LV) weight) did not differ among the six groups, ranging from 15 ± 1 to 16 ± 1% of LV weight (Table 2). As expected, infarct size (expressed as percentage of the risk region) in both the PC group (7.5 ± 3.4%) and the Nic group (11.0 ± 2.9%) was significantly smaller than that observed in the control group (41.7 ± 8.8%; P < 0.05). Infarct size in the C/Gli group (41.9 ± 5.6% of the risk region) was not significantly different from that in the control group, indicating that glibenclamide per se did not alter infarct size (Table 2). As expected, glibenclamide significantly attenuated infarct size limitation of preconditioning that had been triggered either by the 10-min ischemia or nicorandil infarction: mean infarct size averaged 31.8 ± 3.9% and 35.1 ± 4.5% of the risk region in the PC/Gli and Nic/Gli groups, respectively (P = 0.05 vs. PC and P = NS vs. control for both groups; Table 2).

**DISCUSSION**

Recent studies suggest that repeated episodes of angina, which often occur within the days preceding an acute myocardial infarction, may precondition the human heart, possibly through activation of K_ATP channels (7, 12, 13). In addition, many patients with coronary artery disease are currently receiving the K_ATP channel opener nicorandil, a drug now available in Europe or Japan for the treatment of stable angina pectoris. VF is probably the first cause of sudden death after an acute coronary artery occlusion. With respect to this, whether activation of K_ATP channels might trigger lethal ventricular arrhythmias is a major unresolved issue.

In the present study, we showed that K_ATP channel activation reduces infarct size but this activity is not related to the shortening of MAPD or the time to VF during the prolonged ischemia.

Is the Proarrhythmic Activity Observed in Preconditioned Pig Hearts Related to Activation of K_ATP Channels?

We previously demonstrated that preconditioning does not prevent VF in the pig heart but rather accelerates the time to VF during the prolonged ischemic insult, and this is associated with a simultaneous significant shortening of MAPD and decrease in VF threshold (14). These observations led us to question whether activation of K_ATP channels, known to shorten MAPD, might be responsible for this proarrhythmic effect of preconditioning in the pig heart.

There is no doubt that hearts that underwent the initial 10 min of ischemia or nicorandil infusion were preconditioned because they developed significantly smaller infarcts than controls. This protection was likely mediated by K_ATP channels because it was prevented by administration of glibenclamide, as previously demonstrated by other investigators (4, 5, 17, 18).

Indirect evidence suggests that activation of K_ATP channels during preconditioning is not arrhythmogenic. Rohmann et al. (17) and Van Winkle et al. (20) failed to find any difference in the incidence of VF during a sustained period of ischemia and the following reperfusion among control or preconditioned pigs (17, 20). In both studies, pretreatment of preconditioned hearts with glibenclamide or 5-hydroxydecanoate, both potent antagonists of K_ATP channels, did not affect rhythm disturbances. Koning et al. (8) demonstrated that rapid ventricular pacing preconditions the pig heart through activation of K_ATP channels without affecting the incidence of VF (8). Vegh et al. (22) reported that glibenclamide partially prevented the antiarrhythmic effect of preconditioning on ventricular tachycardia but failed to alter VF in preconditioned dogs subjected to a 25-min prolonged LAD occlusion (22). However, none of these studies specifically investigated both the arrhythmogenic role of K_ATP channel activation and its anti-

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**Table 2. Area at risk and infarct size**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Area at Risk, % of LV wt</th>
<th>Infarct Size, % of area at risk</th>
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<tr>
<td></td>
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<tr>
<td>C</td>
<td>16 ± 2</td>
<td>41.7 ± 8.8</td>
</tr>
<tr>
<td>C/Gli</td>
<td>15 ± 1</td>
<td>41.9 ± 5.6</td>
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<tr>
<td>PC</td>
<td>16 ± 1</td>
<td>7.5 ± 3.4*</td>
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<tr>
<td>PC/Gli</td>
<td>16 ± 1</td>
<td>31.8 ± 3.9</td>
</tr>
<tr>
<td>Nic</td>
<td>15 ± 1</td>
<td>11.0 ± 2.9*</td>
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<tr>
<td>Nic/Gli</td>
<td>15 ± 1</td>
<td>35.1 ± 4.5</td>
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Values are means ± SE. LV, left ventricular. *P < 0.05 vs. control.
crotic activity in preconditioned hearts. In the present study, we demonstrated that glibenclamide prevents ischemic preconditioning but does not affect the incidence of VF during a prolonged ischemic insult in preconditioned pig hearts. This strongly suggests that endogenous activation of K\textsubscript{ATP} channels is not responsible for the proarrhythmic effect of preconditioning in the pig heart.

Some investigators found that K\textsubscript{ATP} Channel openers might be arrhythmogenic in some circumstances. Wollenbe et al. (25) demonstrated that pinacidil and BRL-34915 hasten (but glibenclamide delays) the time to VF during low-flow ischemia in isolated rat hearts (25). Chi et al. (1) demonstrated in the canine heart that pinacidil increases the incidence of VF in response to ischemia in a region remote from a previous myocardial infarction. Yao and Gross (27) showed that incremental doses of intracoronary bimakalim similarly reduce infarct size after 60 min of ischemia and 4 h of reperfusion, yet only the highest dose triggers ischemia-induced VF (27). The present study demonstrated that nicorandil is able to reduce infarct size without increasing the incidence of lethal arrhythmias. In addition, blockade of K\textsubscript{ATP} channels by glibenclamide attenuated the infarct size limitation but failed to alter the incidence of VF in nicorandil-treated hearts.

The present study indicates that activation of K\textsubscript{ATP} channels is cardioprotective but not proarrhythmic in this model.

Does the Increased Shortening of MAPD during the Sustained Occlusion Predict VF in the Preconditioned Pig Heart?

Activation of K\textsubscript{ATP} channels classically results in a shortening of MAPD that is prevented by sulfonylureas like glibenclamide (2). Several studies, including the present one, reported that preconditioning significantly shortens MAPD during the first minutes of a sustained coronary artery occlusion (14, 18, 26).

Shortening of MAPD is usually believed to facilitate reentry and trigger type 1a arrhythmias, including VF (6). We recently demonstrated that the maximal incidence of VF occurred near the 5th minute of the sustained ischemic episode in preconditioned hearts, a time of maximum action potential shortening (14). From this observation, it was hypothesized that shortening of action potential secondary to the activation of K\textsubscript{ATP} channels might be responsible for the increased susceptibility to VF in the early minutes of the sustained ischemic insult in preconditioned animals. The present study does not confirm this hypothesis. Glibenclamide prevented the shortening of action potential in preconditioned hearts, yet it did not modify the time distribution of VF during the 40 min of ischemia. The present results suggest that shortening of MAPD is not the trigger of VF in this model. This apparent discrepancy may be due to the well-known fact that VF is always very sudden in the ischemic pig heart; it happens usually after one or two premature beats, rather than being preceded by ventricular tachycardia (a rare event in this model; 14).

Conclusion

The present study demonstrates that the K\textsubscript{ATP} channel modulators nicorandil and glibenclamide fail to alter time to VF in preconditioned animals, although they significantly modify infarct size or MAPD. This suggests that the reduced time to VF in preconditioned pigs is not a consequence of K\textsubscript{ATP} channel activation.

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Address for reprint requests: M. Ovize, Hôpital Cardiovasculaire et Pneumologique Louis Pradel, Unité 81, 59 Bd Pinel, 69394 Cedex 03 Lyon, France.

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