Effect of ATP-sensitive potassium channel agonists on sympathetic hyperinnervation in postinfarcted rat hearts

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Kang C, Chen C, Lin C, Chang N, Lee T. Effect of ATP-sensitive potassium channel agonists on sympathetic hyperinnervation in postinfarcted rat hearts. Am J Physiol Heart Circ Physiol 296: H1949–H1959, 2009. First published April 3, 2009; doi:10.1152/ajpheart.00903.2008—Although the acute administration of ATP-sensitive potassium (KATP) channel agonists provides a neuroprotection, it is unclear whether similar benefits are found by modulating sympathetic innervation in chronic settings after myocardial infarction. We assessed whether KATP channel agonists can attenuate the sprouting of cardiac sympathetic nerves after infarction. Male Wistar rats after ligation coronary artery were randomized to either saline, nicorandil, pinacidil, glibenclamide, or a combination of 1) nicorandil and glibenclamide or 2) pinacidil and glibenclamide for 4 wk. To elucidate the role of mitochondrial KATP channels in modulating nerve growth factor, 5-hydroxydecanoate was assessed in an in vitro model. The measurement of myocardial norepinephrine levels revealed a significant elevation in saline-treated infarcted rats compared with sham-operated rats, consistent with excessive sympathetic innervation. Excessive sympathetic innervation was blunted after giving the rats either nicorandil or pinacidil, compared with saline, as assessed by the immunohistochemical analysis of tyrosine hydroxylase, growth associated protein-43, and neurofilament and Western blot analysis and real-time quantitative RT-PCR of nerve growth factor. The arrhythmic scores during programmed stimulation in the saline- or glibenclamide-treated infarcted rats were significantly higher than those of rats treated with KATP channel agonists. In contrast, the beneficial effects of nicorandil and pinacidil were abolished by administering either glibenclamide or 5-hydroxydecanoate. The sympathetic hyperinnervation after infarction is attenuated by the activation of mitochondrial KATP channels. The chronic use of mitochondrial KATP channel agonists after infarction may attenuate the arrhythmogenic response to programmed electrical stimulation.

Adenosine 5′-triphosphate-sensitive potassium channel; myocardial infarction; norepinephrine; sympathetic innervation

There is general agreement that the activation of ATP-sensitive potassium (KATP) channels in neurons has a protective effect during acute settings of cell-damaging conditions (53). In the cardiac tissues, the KATP channels are observed on cardiomyocytes (36) and pre- and postsynaptic membranes of sympathetic nerves, which can be inhibited by glibenclamide (18). Burgdorf et al. (5) have shown that the release of norepinephrine from sympathetic nerves supplying the heart can be modulated via KATP channels. In acute settings, the antiarrhythmic activity with KATP channel agonists is probably a secondary effect resulting from its anti-ischemic action and pharmacological preconditioning (46). Thus, although an acute administration of KATP channel agonists provides a neuroprotection, it is unclear whether similar benefits are observed by modulating sympathetic hyperinnervation in chronic settings after myocardial infarction (MI).

Nerve growth factor (NGF) is a trophic factor that is critical for the differentiation, survival, and synaptic activity of the peripheral sympathetic and sensory nervous systems (43). In ischemic hearts, an increase in cardiac NGF leads to a regeneration of sympathetic nerves (6, 21). The expression of cardiac NGF correlates with the density of sympathetic innervation in the heart (42), and the amount of NGF can affect the sympathetic nerve survival and synaptic transmission between neurons and cardiac myocytes (29). These results demonstrate the importance of NGF in the regulation of sympathetic innervation.

Increased sympathetic nerve density after myocardial injury has been shown to be responsible for the occurrence of lethal arrhythmias and sudden cardiac death in humans (7). During the chronic stage of MI, a regional increase of sympathetic nerves was commonly observed at the remote zone (56). Increased sympathetic nerve activity plays an important role in the generation of ventricular arrhythmia and sudden cardiac death (7). Cardiac myocytes contain two distinct KATP channels with one subtype located in the sarcolemma and the other in the inner membrane of the mitochondria (28). Mitochondrial KATP channels share some pharmacological properties with sarcolemmal KATP channels, while possessing a distinct pharmacological response. KATP channels are activated by numerous compounds with distinct chemical structures (39). These agents, collectively termed as KATP channel agonists, cause various biological effects through the modulation of the sarcolemmal and mitochondrial KATP channels. Pinacidil has been shown to activate both sarcolemmal and mitochondrial KATP channels, whereas nicorandil activates only mitochondrial KATP channels (39). The activation of KATP channels has been shown to attenuate the expression of endothelin-1 (ET-1) (55). ET-1 can upregulate NGF mRNA by augmenting the expression of the NGF promoter during the development of cardiac sympathetic innervation (16). The purpose of this study was 1) to investigate how two KATP channel agonists, nicorandil and pinacidil, affect sympathetic hyperinnervation; 2) to determine which subtype of KATP channels plays a role in sympathetic hyperinnervation by the use of 5-hydroxydecanoate (5-HD), a specific mitochondrial KATP channel blocker; and 3) to test whether ET-1 acts as a mediator of the effect of KATP channel agonists on NGF expression.
KATP CHANNELS AND NEURAL INNERVATION

METHODS

Animals: in vivo study. Male Wistar rats (300–350 g) were subjected to ligation of the anterior descending artery as previously described (26), resulting in the infarction of the left ventricular (LV) free wall. Rats were randomly assigned into six groups so as to have approximately the same number of survivors in each group: 1) saline group; 2) nicorandil (0.1 mg·kg\(^{-1}\)·day\(^{-1}\), Chugai Pharmaceutical), a specific mitochondrial K\(_{ATP}\) channel agonist; 3) pinacidil (0.1 mg·kg\(^{-1}\)·day\(^{-1}\), Sigma), a nonselective K\(_{ATP}\) channel agonist; 4) glibenclamide (1.4 mg·kg\(^{-1}\)·day\(^{-1}\)), a K\(_{ATP}\) channel blocker; 5) a combination of nicorandil and glibenclamide; and 6) a combination of pinacidil and glibenclamide. The doses of nicorandil (11), pinacidil (2), and glibenclamide (1) used in this study have been shown to specifically modulate K\(_{ATP}\) channels without the interference of hemodynamics. The drugs were started 24 h after infarction, during which the drugs can exert the maximum benefits at this timing window (50). The study duration was designed to be 4 wk because the majority of the myocardial remodeling process in the rat (70–80%) is complete within 3 wk (40). Sympathetic reinnervation has been shown to be present 6 days after injury (38). The drugs were given orally by gastric gavage once a day. To prevent hypoglycemic attacks during the administration of glibenclamide, 2.5% (wt/vol) sucrose in filtered tap water was supplied, and glucose examinations were performed once per week by the one-touch method. Sham operation served as controls to exclude the possibility of the drugs themselves to directly alter sympathetic hyperinnervation. In each treated group, the drugs were withdrawn at about 24 h before the end of the experiments to eliminate their pharmacological actions.

In vitro study. Although the results of the above study showed that K\(_{ATP}\) channel antagonist-induced sympathetic innervation was altered at 4 wk after infarction (see RESULTS), glibenclamide was used as an antagonist of K\(_{ATP}\) Channels, which can block both mitochondrial and sarcolemmal K\(_{ATP}\) channels and has multiple actions independent of K\(_{ATP}\) channels. Four weeks after the induction of MI by coronary ligation, the infarcted rat hearts were isolated and subjected to no treatment (vehicle), nicorandil (50 \(\mu\)M), pinacidil (50 \(\mu\)M), 5-HD (100 \(\mu\)M), nicorandil + 5-HD, or pinacidil + 5-HD. Each heart was perfused with a noncirculating modified Tyrode solution containing (in mM) 117.0 NaCl, 23.0 NaHCO\(_3\), 4.6 KCl, 0.8 NaHPO\(_4\), 1.0 MgCl\(_2\), 2.0 CaCl\(_2\), and 5.5 glucose, equilibrated at 37°C and oxygenated with a 95% O\(_2\)-5% CO\(_2\) gas mixture. The perfusion medium was maintained at a constant temperature of 37°C with a constant flow at 4 ml/min as previously described (24). The drugs were infused for 30 min. The doses of nicorandil (20), pinacidil (8), and 5-HD (34) used in this study have been shown to modulate K\(_{ATP}\) channels in an isolated heart. At the end of the study, all the hearts (\(n = 10\) in each group) were used for performing Western blot analysis from samples at the remote zone (>2 mm outside the infarct). The animal experiments were approved by the Chi-Mei Medical Center and conducted in accordance with its local guidelines for the care and use of laboratory animals. The investigation conformed with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

Echocardiogram. At 28 days after operation, the rats were lightly anesthetized with an intraperitoneal injection of ketamine-HCL (25 mg/kg). Echocardiographic measurements were done with a HP Sonos 5500 system with a 15-6L (6 –15 MHz, SONOS 5500; Agilent Technologies, Palo Alto, CA) probe. The M-mode tracing of the LV surface of the right ventricular outflow tract. Induced arrhythmias were effected using an electrical Bloom stimulator. To induce ventricular arrhythmias, eight paced beats at a cycle length of 120 ms (S\(_1\)) were applied, followed by one to three extrastimuli (S\(_2\), S\(_3\), and S\(_4\)) at shorter coupling intervals. The end point of ventricular pacing was the induction of ventricular tachycardia. Ventricular tachyarrhythmias including ventricular tachycardia and ventricular fibrillation were considered nonsustained when it lasted ≤15 beats and sustained when it lasted >15 beats. An arrhythmia scoring system was modified as previously described (35): 0, noninducible preparations; 1, nonsustained tachyarrhythmias induced with three extrastimuli; 2, sustained tachyarrhythmias induced with three extrastimuli; 3, nonsustained tachyarrhythmias induced with two extrastimuli; 5, nonsustained tachyarrhythmias induced with one extrastimulus; 6, sustained tachyarrhythmias induced with one extrastimulus; and 7, tachyarrhythmias induced during the eight paced beats. If the heart stopped before the pacing, the arrhythmia score assigned to that heart was 8. When multiple forms of arrhythmias occurred in one heart, the highest score was used. The experimental protocols were typically completed within 10 min.

Real-time RT-PCR of NGF. Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed from samples obtained from the remote zone with the TaqMan system (Prism 7700 Sequence Detection System, PE Biosystems) as previously described (26). For NGF, the primers were 5’-TCCCCACC-CCAGTCTTCCA-3’ (sense) and 5’-GCTTCTCTGCTGACACACA-3’ (antisense). For glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the primers were 5’-CTCCACACCAGTGGAAAGGC-3’ (sense) and 5’-GCGATGCTTGGTGTGCTG-3’ (antisense). Standard curves were plotted with the threshold cycles versus the log template quantities. For quantification, NGF expression was normalized to the expressed housekeeping gene GAPDH. Reaction conditions were programmed on a computer linked to the detector for 40 cycles of the amplification step.

Western blot analysis of NGF. Samples obtained from the remote zone were homogenized with a polytron blender. Homogenates were centrifuged at 10,000 \(g\) for 30 min to pellet the particulate fractions. The supernatant protein concentration was determined with the BCA protein assay reagent kit (Pierce). Protein (20 \(\mu\)g) was separated by 10% SDS-PAGE and electrotransferred onto a nitrocellulose mem-
brane. After incubation with rabbit polyclonal anti-NGF antibodies (Chemicon) at 1:500 dilution, the nitrocellulose membrane was then rinsed with a blocking solution and incubated for 2 h at room temperature. Antigen-antibody complexes were detected with 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium chloride (Sigma). Films were volume integrated within the linear range of the exposure using a scanning densitometer. Experiments were replicated three times, and the results were expressed as the mean value.

Immunohistochemical studies of tyrosine hydroxylase, growth associated protein-43, and neurofilament. To investigate the spatial distribution and quantification of sympathetic nerve fibers, an analysis of immunohistochemical staining for tyrosine hydroxylase, growth associated protein-43 (GAP-43, a marker peptide for neuronal regeneration and outgrowth), and neurofilament (a marker for sympathetic nerve; Ref. 31) was performed on LV muscle from the remote regions. Papillary muscles were excluded from the study because a variable sympathetic innervation has been reported (9). Paraffin-embedded sections were performed at a thickness of 5 μm. Tissues were incubated with anti-tyrosine hydroxylase antibodies (1:200; Chemicon), anti-GAP-43 (1:400; Chemicon), and anti-neurofilament (1:1,000; Chemicon) in 0.5% BSA in PBS overnight at 37°C. Immunostaining was performed using a standard immunoperoxidase technique (N-Histofine Simple Stain MAX PO kit, Nichirei, Tokyo, Japan). Isotype-identical directly conjugated antibodies served as a negative control. The experiments were replicated three times, and the results were expressed as the mean value.

The slides were coded so that the investigator was blinded to the rat identification. The nerve density was measured on the tracings by computerized planimetry (Image ProPlus, Media Cybernetics, Silver Spring, MD) as described previously (27). The density of nerve fibers was qualitatively estimated from 10 randomly selected fields at a magnification of ×400 and expressed as the ratio of labeled nerve fiber area to the total area.

Laboratory measurements. To determine the confounding roles of glucose and insulin in nerve remodeling, blood samples from the aorta were assayed at the end of the study. Plasma insulin concentration was measured by collecting 4 ml of blood in test tubes containing 2% ethylenediaminetetraacetic acid (80 μl/ml of blood). Blood samples were immediately centrifuged at 3,000 g for 10 min, and the plasmas were stored at −70°C until further analysis. Insulin was measured by ultrasensitive rat enzyme immunoassay (Mercodia, Uppsala, Sweden).

Because of a local release of norepinephrine after sympathetic innervation, the tissues from the border zone (0 to 2 mm outside the infarct) and remote zone were obtained for measurements of local norepinephrine levels at the end of the study. The myocardium was minced and suspended in a 0.4 N perchloric acid with 5 mmol/l reduced glutathione (pH 7.4), homogenized with a polytron homogenizer for 60 s in 10 vol. Samples were immediately centrifuged at 3,000 g for 10 min, and the supernatant was stored at −70°C until further analysis. The supernatant protein concentration was determined with the BCA protein assay reagent kit (Pierce). Total norepinephrine was measured using a commercial ELISA kit (Noradrenaline ELISA, IBL Immuno-Biological Laboratories, Hamburg, Germany).

To confirm the downstream pathways of the KATP channel, we determined the LV norepinephrine levels. Either nicorandil, pinacidil, or glibenclamide administration did not affect tissue norepinephrine concentrations in sham-operated rats. LV norepinephrine levels were significantly upregulated 1.7-fold at the border zone in the saline-treated infarcted rats than in the saline-operated rats (2.33 ± 0.20 μg/g protein, P < 0.0001). The expression was region dependent with a significant increase at the remote zone (2.95 ± 0.25 μg/g protein) compared with that at the border zone (2.33 ± 0.20 μg/g protein, P = 0.03) after infarction in the saline group. When compared with saline-treated infarcted rats, nicorandil- or pinacidil-treated infarcted rats had significantly lower LV norepinephrine levels at the remote regions. The beneficial effect of nicorandil and pinacidil on LV norepinephrine levels was reversed by administering glibenclamide. The changes of myocardial ET-1 at the remote regions were similar to those of LV norepinephrine (Table 2).

Echocardiographic data. When compared with sham-operated hearts, MI hearts showed structural changes such as increased LV diastolic and systolic diameters (Table 3), consistent with LV remodeling. Both LVEDD and LVESD in rats with MI were significantly reduced by nicorandil or pinacidil treatment (P < 0.0001). LV fractional shortening was significantly higher in the nicorandil- or pinacidil-treated infarcted group compared with the saline-treated infarcted group. Conversely, the rats to which glibenclamide was administered developed impaired LV systolic function and progressive LV dilatation than those in the nicorandil- and pinacidil-treated groups alone.

Immunohistochemical analyses. The tyrosine hydroxylase-immunostained nerve fibers appeared to be oriented in the longitudinal axis of adjacent myofibers (Fig. 1, top). Tyrosine hydroxylase-positive nerve density was significantly increased in the saline-treated infarcted rats compared with sham-operated rats (Fig. 1, bottom). Rats in the nicorandil- and pinacidil-treated groups showed less nerve density at the remote regions than that in saline-treated rats (0.06 ± 0.03%, 0.07 ± 0.05% vs.
### Table 1. Cardiac morphology and hemodynamics at the end of study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham Infarction with Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline Nic Pin Glib Nic + Glib</td>
</tr>
<tr>
<td>Number of rats</td>
<td>10 11</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>421 ± 24 410 ± 20 411 ± 15 408 ± 25 407 ± 16 412 ± 24</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>396 ± 25 409 ± 22 408 ± 15 385 ± 23 412 ± 18 412 ± 18</td>
</tr>
<tr>
<td>LVESP, mmHg</td>
<td>113 ± 7 102 ± 6 106 ± 10 108 ± 10 106 ± 11 110 ± 13</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>16 ± 2 5 ± 3 4 ± 3 5 ± 4 5 ± 3 5 ± 2</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>6.883 ± 482 6.373 ± 364 5.533 ± 612 5.524 ± 383 5.692 ± 549 5.524 ± 452</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>2.11 ± 0.21 2.21 ± 0.23 2.09 ± 0.20 2.17 ± 0.15 2.07 ± 0.24 2.25 ± 0.24</td>
</tr>
<tr>
<td>RVW/BW, mg/g</td>
<td>0.50 ± 0.04 0.53 ± 0.03 0.55 ± 0.12 0.47 ± 0.12 0.53 ± 0.12 0.52 ± 0.15</td>
</tr>
<tr>
<td>Lung W/BW, mg/g</td>
<td>4.12 ± 0.54 4.13 ± 0.47 4.53 ± 0.39 4.32 ± 0.45 4.18 ± 0.55 4.38 ± 0.28</td>
</tr>
</tbody>
</table>

Values are means ± SD. LVESP, left ventricular (LV) end-systolic pressure; LVEDP, LV end-diastolic pressure; +dP/dt, LV pressure rise; −dP/dt, LV pressure decline; BW, body weight; LVW, LV weight; RVW, right ventricular weight; Lung W, lung weight. *P < 0.05 compared with sham; †P < 0.05 compared with infarcted groups treated with saline and nicorandil (Nic) + glibenclamide (Glib); ‡P < 0.05 compared with infarcted groups treated with saline and pinacidil (Pin) + Glib.

### Table 2. Glucose, insulin, plasma, and tissue NE and tissue ET-1 concentration at the end of study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham Infarction with Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline Nic Pin Glib Nic + Glib</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>90 ± 7 85 ± 9 92 ± 8 92 ± 6 88 ± 9 90 ± 10</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>21 ± 8 19 ± 6 22 ± 10 89 ± 15 79 ± 12 85 ± 18</td>
</tr>
<tr>
<td>Plasma NE, ng/ml</td>
<td>2.7 ± 1.2 2.9 ± 2.1 3.2 ± 1.6 1.7 ± 1.5 2.5 ± 1.8 2.2 ± 2.2</td>
</tr>
<tr>
<td>Remote NE, µg/g protein</td>
<td>1.52 ± 0.42 1.36 ± 0.48 1.38 ± 0.74 1.33 ± 0.77 1.38 ± 0.49 1.67 ± 0.54</td>
</tr>
<tr>
<td>Border NE, µg/g protein</td>
<td>1.37 ± 0.35 1.15 ± 0.45 1.54 ± 0.30 1.57 ± 0.55 1.30 ± 0.36 1.44 ± 0.56</td>
</tr>
<tr>
<td>LV ET-1, pg/mg tissue</td>
<td>1.3 ± 0.2 1.1 ± 0.3 1.2 ± 0.3 1.5 ± 0.3 1.2 ± 0.4 1.2 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. NE, norepinephrine; ET-1, endothelin-1. *P < 0.05 compared with respective sham-operated rats; †P < 0.05 compared with respective infarcted groups without administering Glib; ‡P < 0.05 compared with the border zone within the same group.
compared with those in saline-treated infarcted group (both
ated in the nicorandil- and pinacidil-treated infarcted rats
neurofilament-positive nerve densities were significantly atten-
Similar to tyrosine hydroxylase results, GAP-43- (Fig. 2) and
infarcted rats showed a marked increase of NGF mRNA than
mRNA levels was similar between nicorandil- and pinacidil-
channel agonists-related NGF can be reversed to the levels
innervation in humans. The beneficial effect of KATP
channel agonists as antiarrhythmic agents were related to the
 remodeling of the sympathetic nervous system that may be
mediated by the KATP channel-ET-1-NGF pathway. Our results
were compatible with a recent finding of Kasama et al. (19),
showing that nicorandil administration attenuates sympathetic
innervation in humans.

The beneficial effect of KATP channel agonists on sympa-
thetic innervation was supported by three lines of evidence.
First, mitochondrial KATP channel activation plays a role in the
pathogenesis of sympathetic hyperinnervation after infarction.
The administration of KATP channel agonists attenuates sympa-
thetic hyperinnervation in chronically infarcted hearts. Either
pinacidil or nicorandil administration can attenuate sympa-
thetic hyperinnervation with a similar potency at the remote
zone, which may suggest that mitochondrial KATP channels
play a role in regulating the nerve sprouting. Although dissim-
lar structures, pinacidil and nicorandil appear to share a
common mediator, NGF, in which transcription levels of NGF
may play a role in the signal transduction pathway. The involve-
ment of NGF in mitochondrial KATP channel activation-
related sympathetic hyperinnervation after infarction was
further supported by administering a mitochondrial KATP an-
tagont (5-HD). The results are consistent with a recent
finding of Lou et al. (30), showing that mitochondrial KATP
channel activation is associated with neuroprotection. Second,
the beneficial effects of KATP agonists on attenuated sympa-
thetic hyperinnervation might be associated with reduced ET-1
levels. Rats in which KATP agonists were administered had
significant reductions of tissue ET-1 compared with saline-
treated infarcted rats, confirming the role of mitochondrial KATP
channel agonists-treated rats alone. These
morphic results mirrored those of norepinephrine con-
tents.

NGF protein and mRNA expression. Western blot analysis
showed that the NGF levels were significantly upregulated
8.3-fold at the remote zone in the saline-treated infarcted rats
compared with those in the sham-operated group (both
P < 0.0001 for GAP-43, and P < 0.0001 for neurofilament).
The rats to which glibenclamide was administered
developed stronger signals of an immunostained profile than
those in the KATP channel agonists-treated groups alone. These
morphometric results mirrored those of norepinephrine con-
tents.

Table 3. Echocardiographic findings

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham</th>
<th>Saline</th>
<th>Nic</th>
<th>Pin</th>
<th>Glib</th>
<th>Nic + Glib</th>
<th>Pin + Glib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality, n (%)</td>
<td>0 (0)</td>
<td>5 (31)</td>
<td>4 (25)</td>
<td>5 (33)</td>
<td>6 (40)</td>
<td>6 (38)</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Interventricular septum, mm</td>
<td>1.6±0.1</td>
<td>0.8±0.2*</td>
<td>0.8±0.2*</td>
<td>0.8±0.1*</td>
<td>0.7±0.3*</td>
<td>0.8±0.2*</td>
<td>0.8±0.2*</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>6.0±0.2</td>
<td>8.5±0.3*</td>
<td>7.3±0.2†</td>
<td>7.4±0.2‡</td>
<td>8.7±0.3*</td>
<td>9.0±0.2*</td>
<td>8.8±0.2*</td>
</tr>
<tr>
<td>LV posterior wall, mm</td>
<td>3.8±0.2</td>
<td>7.0±0.2*</td>
<td>5.5±0.3†</td>
<td>5.3±0.3‡</td>
<td>7.2±0.2*</td>
<td>7.3±0.2*</td>
<td>7.3±0.2*</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>16±4*</td>
<td>25±3‡</td>
<td>26±3‡</td>
<td>17±3‡</td>
<td>19±4*</td>
<td>17±3‡</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. LVESD, LV end-systolic dimension; LVESD, LV end-diastolic dimension. *P < 0.05 compared with the sham-operated group; †P < 0.05 compared with infarcted groups treated with saline and Nic + Glib; ‡P < 0.05 compared with infarcted groups treated with saline and Pin + Glib.

0.14 ± 0.07% in saline group, both P < 0.0001, respectively).
Similar to tyrosine hydroxylase results, GAP-43- (Fig. 2) and
neurofilament-positive nerve densities were significantly atten-
uated in the nicorandil- and pinacidil-treated infarcted rats
compared with those in saline-treated infarcted group (both
P < 0.0001 for GAP-43, and both P < 0.0001 for neurofilament).

Electrophysiological stimulation. To further elucidate the
physiological effect of attenuated sympathetic hyperinnerva-
tion, ventricular pacing was performed. The arrhythmia score
in the sham-operated rats was very low (0.1 ± 0.3) (Fig. 6). In
contrast, ventricular tachyarrhythmias consisting of ventricular
tachycardia and ventricular fibrillation were inducible by a
programmed stimulation in saline-treated infarcted rats. Nic-
orandil and pinacidil treatment significantly decreased the
inducibility of ventricular tachyarrhythmias compared with
that in the saline-treated infarcted group. Glibenclamide ad-
ministration significantly increased the arrhythmia scores in
KATP channel agonists-treated rats, compared with the KATP
channel agonists-treated rats alone.

DISCUSSION

Our present study shows for the first time that a chronic
treatment for 4 wk with KATP channel agonists leads to attenu-
synthetic hyperinnervation after MI. These results were concordant for beneficial effects of KATP channel ago-
sists, as documented structurally by a reduction in cardiac
nerve sprouting; molecularly, by myocardial NGF protein
and mRNA levels; biochemically, by tissue norepinephrine
and ET-1 levels; and functionally, by an improvement of fatal
ventricular tachyarrhythmias. The beneficial effects of KATP
channel agonists as antiarrhythmic agents were related to the
remodeling of the sympathetic nervous system that may be
mediated by the KATP channel-ET-1-NGF pathway. Our results
were compatible with a recent finding of Kasama et al. (19),
showing that nicorandil administration attenuates sympathetic
innervation in humans.
Fig. 1. **Top:** immunohistochemical staining for tyrosine hydroxylase from the remote regions (magnification, ×400). Tyrosine hydroxylase-positive nerve fibers (brown) are located between myofibrils and are oriented in a longitudinal direction as that of the myofibrils. Myocytes are not stained and appear pale in this view. Sham-operated group (A), saline group (B), nicorandil (Nic) group (C), pinacidil (Pin) group (D), glibenclamide (Glib) group (E), Nic + Glib (F), Pin + Glib group (G) are shown. Bar = 50 μm. **Bottom:** nerve density area fraction (in %) at the remote zone. Bars represent means ± SD. *P < 0.05 compared with saline-, Glib-, Nic + Glib-, and Pin + Glib-treated groups.
Third, the severity of pacing-induced fatal arrhythmias was associated with the degree of sympathetic innervation. The finding was consistent with the findings of Cao et al. (6, 7), showing that an increased postinjury sympathetic nerve density may be responsible for the occurrence of ventricular arrhythmia and sudden cardiac death in animals and patients.

**Mechanisms.** In this study, we demonstrated an attenuated sympathetic hyperinnervation in $K_{ATP}$ channel agonists-treated hearts. The mechanisms by which $K_{ATP}$ channel agonists affect sympathetic hyperinnervation remain undefined. However, several factors can be excluded. First, hemodynamics: Previous studies have shown a significantly reverse correlation between LV end-diastolic pressure and tyrosine hydroxylase-immuno- 
stained profiles (15). Neither nicorandil nor pinacidil exerted any hemodynamic effects at the dose used in this study. Pinacidil is an effective antihypertensive agent in treating patients with essential hypertension (44). The observation was not consistent with our stable hemodynamics throughout the study in pinacidil-treated rats. The discrepancy could be due to differences in doses, study population, and periods of treatment. Indeed, our results were consistent with the finding of Xu et al. (51), showing that nicorandil did not lower blood pressure even in a relatively high dose (6 mg·kg$^{-1}$·day$^{-1}$) in rats. Ito et al. (17) have shown that the neuroprotection effect of $K_{ATP}$ channel agonists was independent of changes in hemodynamics and appeared to be mediated by activating $K_{ATP}$ channels of cardiomyocytes. Second, differences in infarct sizes: The degree of sympathetic innervation was related to the infarct sizes (13). Successful fiber reinervation appears dependent on repopulating sheaths with Schwann cells, which would be injured according to the sizes of infarction. The possibility was excluded in this study due to similar infarct sizes among the groups.

![Fig. 2. A–G: immunohistochemical staining for GAP-43 from the remote regions (magnification, ×400). GAP-43-positive staining was markedly increased in groups treated with saline, Glib, Nic + Glib, and Pin + Glib (P < 0.001).](image-url)
Exactly how the activation of KATP channels leads to an attenuated sympathetic hyperinnervation cannot be determined from this study. Our results show that the activation of KATP channels in the reinnervated regions caused a relatively greater reduction in NGF expression. The finding was consistent with the results of Tyagi and Jose (48), showing that the activation of KATP channels attenuated the levels of NGF. KATP channel agonists attenuated tissue ET-1 levels in this study. Recently, we have shown that the inhibition of the ET system by administering ET receptor blockers attenuated sympathetic hyperinnervation after infarction (23). ET-1 significantly augmented the activity of the NGF promoter mediated by the ETA receptor, and the deletion of the activator protein-1 element of the NGF promoter markedly decreased this augmentation (16). Thus our results may suggest that the downregulation of NGF expression by KATP channel activation, probably through an ET-1 pathway, plays a role in attenuating sympathetic hyperinnervation after MI.

**Other mechanisms.** Although the present study suggests that the mechanisms of a KATP channel agonist-induced attenuation of sympathetic hyperinnervation may be related to attenuated NGF expression, other potential mechanisms need to be studied. Glibenclamide inhibits the activity of endogenous ecto-5'-nucleotidase and decreases the adenosine concentrations in the interstitial space of the ventricular muscles (38). In contrast, KATP channel agonists stimulate 5'-nucleotidase (47), which in turn resulted in increased adenosine concentrations. Increased interstitial adenosine levels have been shown to induce sympathetic apoptosis (49). Thus it is not surprising that the KATP channel agonists attenuate sympathetic hyperinnervation, whereas glibenclamide abolished the protection.
Besides, we demonstrated that the activation of K\textsubscript{ATP} channels leads to attenuated tissue norepinephrine levels, probably through an ET-1-dependent pathway. The K\textsubscript{ATP} channel is a high-fidelity metabolic sensor that adjusts the membrane potential-dependent cell functions to match the metabolic state (36). Previous studies have demonstrated an energetic insufficiency in the remote noninfarcted myocardium, such as reductions of ATP and the phosphocreatine-to-ATP ratio (54). Adenosine rapidly accumulates in the interstitial space in response to metabolic distress (33). In parallel with adenosine accumulation, the breakdown of intracellular ATP triggers the opening of K\textsubscript{ATP} channels in ischemic myocardium (12). Because K\textsubscript{ATP} conductance is present in noradrenergic neurons (10), the possibility arises that the K\textsubscript{ATP} channel opening in response to ischemia influences norepinephrine release from the heart. Burgdorf et al. (5) have shown that the activation of K\textsubscript{ATP} channels attenuated norepinephrine release directly at the presynaptic nerve ending during coronary low-flow ischemia. Thus we cannot exclude the possibility that K\textsubscript{ATP} agonists may have decreased the apparent norepinephrine content by reversing the metabolic compromise, independent of the ET-1 effect.

Clinical implications. So far, no studies have directly addressed the question of whether or not the long-term treatment with K\textsubscript{ATP} channel agonists may influence the susceptibility to ventricular arrhythmias after MI. Our results show that an association between K\textsubscript{ATP} channel agonist administration and ventricular arrhythmias is anatomically and functionally linked. After an acute MI, patients remain at high risk for recurrent cardiovascular events and mortality (4). The attenuation of sympathetic hyperinnervation prevents fatal ventricular arrhythmias. Thus the K\textsubscript{ATP} channel agonists may have important biological effects that prevent the occurrence of postinfarcted arrhythmias. Our results explained, at least in part, the clinical findings of the Impact of Nicorandil in Angina (IONA) study group (45), showing that a treatment with nicorandil improves the outcome in terms of reducing the incidence of fatal ventricular arrhythmias. Our finding examined in this study. It is possible that smaller MIs would yield a different profile of sympathetic innervation. Our finding cannot necessarily be extrapolated to animals with small to moderate infarction. Second, because the tissue norepinephrine levels were expressed with reference to gram protein, it may be doubtful whether a pathological modification of protein content per tissue volume in the remote regions might have contributed to the increase in norepinephrine tissue levels. We previously demonstrated a significant degree of LV hypertrophy after MI in the remote zone compared with that in sham-operated rats (25). Compensatory LV hypertrophy might decrease the apparent tissue norepinephrine content expressed per gram protein. Thus the increase in tissue norepinephrine content in the remote zone of infarcted rats should reflect the actual increase in regional norepinephrine levels. Finally, the administration of K\textsubscript{ATP} channel agonists not only attenuated sympathetic hyperinnervation but also prevented ventricular remodeling assessed by a decreased lung weight and right ventricular weight normalized to body weight, and reduced LVEDD and LVESD. K\textsubscript{ATP} channel agonists can attenuate ET-1 expression, which signaling pathway has been linked to not only attenuate sympathetic hyperinnervation but also improve ventricular remodeling, making it difficult to separate the two effects of ET-1-related action of K\textsubscript{ATP} channel agonists. The hypertrophic growth of the surviving myocytes may create a shift in the sympathovagal balance toward a sympathetic prevalence that leaves the myocardium in greater jeopardy for the development of life-threatening arrhythmias (22). One may imagine that the beneficial effects of K\textsubscript{ATP} channel agonists on arrhythmias are the result of their direct favorable ventricular remodeling. However, the improvement of an adverse ventricular remodeling after MI cannot necessarily be extrapolated to the antiarrhythmias. Previous studies have shown that the inducibility of ventricular arrhythmias can be reduced as a result of markedly different effects on ventricular remodeling, indicating that the relationship between ventricular remodeling and arrhythmias is more complex than previously thought (3). Further work is required to differentiate whether there is an effect of K\textsubscript{ATP} channel agonists on the nervous system or on the cardiac tissue directly with a secondary change in autonomic activity.

Conclusions. These data show that sympathetic hyperinnervation after infarction was attenuated by the activation of mitochondrial K\textsubscript{ATP} channels. These effects probably are functionally important because they are linked to the attenuated incidence of fatal arrhythmias. Further studies of the specific role of K\textsubscript{ATP} channels in the myocardium may contribute to the development of novel antiarrhythmic therapies.

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