Adenosine A\textsubscript{1} receptor activation reduces myocardial reperfusion effects on intrinsic cardiac nervous system

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\textsuperscript{1}Department of Anatomy and Neurobiology and \textsuperscript{2}Department of Surgery, Dalhousie University, Halifax, Nova Scotia B3H 4H7; and \textsuperscript{3}Department of Pharmacology, Faculty of Medicine, University of Montréal, Montréal, Québec, H4J 1C5 Canada

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Arora, R. C., and J. A. Armour. Adenosine A\textsubscript{1} receptor activation reduces myocardial reperfusion effects on intrinsic cardiac nervous system. Am J Physiol Regul Integr Comp Physiol 284: R1314–R1321, 2003; 10.1152/ajpregu.00333.2002.—The intrinsic cardiac nervous system is the final common integrator of regional cardiac function. The ischemic myocardium modifies this nervous system. We sought to determine the role that intrinsic cardiac neuronal P\textsubscript{1} purinergic receptors play in transducing myocardial ischemia and the subsequent reperfusion. The activity generated by ventricular neurons was recorded concomitant with cardiac hemodynamic variables in 44 anesthetized pigs. Regional ventricular ischemia was induced by briefly occluding (30 s) the ventral interventricular coronary artery distal to the arterial blood supply of identified ventricular neurons. Adenosine (100 μM) was administered to these neurons via their local arterial blood supply during or immediately after transient coronary artery occlusion. Occlusion was also performed following local administration of adenosine A\textsubscript{1} [8-cyclopropentyl-1,3-dipropylxanthine (DPCPX)] or A\textsubscript{2} [3,7-dimethyl-1-propargylxanthine (DMPX)] receptor blocking agents. The activity generated by ventricular neurons was modified by transient coronary artery occlusion and the subsequent reperfusion (∆| 112 ± 14 and 168 ± 34 impulses/min, respectively; \( P < 0.01 \) vs. preischemic states). Locally administered adenosine attenuated neuronal responses to reperfusion (−75%; \( P < 0.01 \) compared with normal reperfusion) but not ischemia. The neuronal stabilizing effects that adenosine elicited during reperfusion persisted in the presence of DMPX but not DPCPX. It is concluded that activation of neuronal adenosine A\textsubscript{1} receptors stabilizes the intrinsic cardiac nervous system during reperfusion.

myocardium; ventricular neurons

THE TERM REPERFUSION injury is used to describe a phenomenon that encompasses abnormal myocardial responses with reestablishment of oxygenated blood flow to the previous ischemic myocardium. The consequences of this response include myocardial cell injury, ranging from reversibly “stunned” myocardium to accelerated necrotic cell death (10). An additional clinical consequence is the generation of potentially lethal ventricular arrhythmias that can occur within seconds of the onset of reperfusion following coronary artery occlusion (10). The precise mechanisms involved in the generation of reperfusion-induced alterations in cardiac function remain elusive. One potential mechanism involves untoward activation of populations of intrinsic cardiac neurons. Excessive activation of specific populations of atrial neurons can induce negative cardiodynamic effects that include the genesis of cardiac arrhythmias (14). It is not known if ventricular myocardial ischemia/reperfusion can be transduced by the ventricular nervous system or whether the resultant neuronal responses (if any) can be modified pharmacologically. In the context of this investigation, the term transduction is employed to represent the capacity of the intrinsic cardiac nervous system to transduce cardiac sensory information, primarily by local circuit neurons, to efferent neurons regulating regional cardiac function.

Adenosine has received attention in the setting of reperfusion injury since it is known to exert cardioprotective and anti-arrhythmia effects (8). Adenosine is a byproduct of catabolism within the ischemic myocardium, increasing severalfold in the ischemic myocardium (17). In addition to cardiomyocyte modulation, adenosine has also been demonstrated to elicit neuromodulatory effects on the cardiac autonomic nervous system. Adenosine-sensitive cardiac afferent neurons are located in nodose and dorsal root ganglia (7, 12, 19). Adenosine can also modify the activity generated by atrial neurons in vitro (3) or in vivo (13). The neuromodulator effects that adenosine exerts on ventricular neurons have yet to be determined. Furthermore, if purinergic intrinsic cardiac neurons are involved in the transduction of myocardial ischemia/reperfusion from cardiac afferent neurons, it is important to determine whether purinoceptor specificity exists in that process. If such specificity does exist, the intrinsic cardiac nervous system may be capable of modification in the presence of such pathology to stabilize cardiac function.

Therefore, the present study was devised to determine 1) whether ventricular ischemia/reperfusion can be transduced by the ventricular nervous system and, if that does occur, 2) whether purinergic receptors are involved in such transduction. Furthermore, we sought...
to determine if the responsiveness of the intrinsic cardiac nervous system to myocardial ischemic/reperfusion injury could be modified by pharmacological means.

**MATERIALS AND METHODS**

**General methods.** All experiments were performed in accordance with the guidelines for animal experimentation described in "Guiding Principles for Research Involving Animals and Human Beings" by the American Physiological Society. Forty-four Hamshire-Duvoc pigs of either gender, weighing 20–30 kg, were studied. The animals were sedated with a combination of ketamine (80 mg/kg iv) and Pentothal Sodium (20 mg/kg iv). After the endotracheal intubation was performed, positive pressure ventilation was initiated with 0.95 FIO2 and 0.05 FICO2 using a Bird Mark 7A ventilator (Palm Springs, CA). Anesthesia was maintained with Pentothal Sodium (10–15 mg/kg iv every 5 min) during surgical preparation. Thereafter, during neuronal recording, anesthesia was maintained with α-chloralose (75 mg/kg iv bolus, with repeat doses of 12.5 mg/kg iv as required). The adequacy of anesthesia was assessed at regular intervals by applying noxious stimuli to a limb to determine reflex withdrawal responses of a limb as well as to monitor jaw tone. The animals, placed in a supine position, underwent a bilateral thoracotomy through the fifth intercostal space. Once completed, the animals were then placed in a right lateral decubitus position and a ventrolateral pericardiotomy was performed to expose the heart.

**Operative procedures.** A lead II ECG, left ventricular (LV) chamber pressure, and aortic pressure were monitored continuously throughout the experiments. LV chamber pressure was monitored via a Cordis (Miami, FL) #6 French pigtail catheter that was inserted into the outflow tract of the LV chamber via one femoral artery. Systemic arterial pressure was monitored via a Cordis #5 French catheter that was placed in the descending aorta via the other femoral artery. These catheters were attached to Bentley (Irvine, CA) Transec model 800 transducers. Intrinsic neuronal activity, ECG, and LV pressure were recorded concomitantly on an Astromed MT9500 8 channel rectilinear chart recorder.

**Recording neuronal activity.** A collection of porcine intrinsic cardiac neurons is located at the bifurcation of the left main as well as ventral interventricular (VIV) (analogous to the left anterior descending coronary artery in human) and circumflex coronary arteries (Fig. 1). Neurons in this ganglionic plexus, termed the VIV ganglionated plexus, are representative of those found throughout the porcine intrinsic cardiac nervous system. This ganglionated plexus was chosen for study because of the relative density of its neurons and the fact that its arterial blood supply is spared when the VIV coronary artery is occluded distal to its first diagonal branch as the multiple smaller arteries that supply blood to this collection of neurons originate cranial to that location.

The activity generated by ventral ventricular neurons was recorded by means of a tungsten microelectrode (Frederick Haer 25–10–3, Brunswick, ME) that had a 250-μm shank diameter and an exposed tip of 5 μm (impedance of 9–11 MΩ at 1,000 Hz). To minimize epicardial motion during each cardiac cycle, a circular ring of heavy gauge wire was gently placed around the epicardial fat on the cranioventral surface of the interventricular groove. Care was taken not to compromise the underlying coronary artery blood flow with this device. The tungsten microelectrode, mounted on a Marzhauser micromanipulator (model 25033–10, Fine Scientific Tools, North Vancouver, British Columbia), was used to explore the fat at varying depths ranging from the surface of the fat to regions adjacent to cardiac musculature. The reference electrode was attached to the adjacent pericardium. A grounding electrode was attached to the heavy gauge wire-stabilizing ring. Identified signals generated by ventricular neurons were differentially amplified by a Princeton Applied Research (Princeton, NJ) model 113 amplifier that had bandpass filters set at 300 Hz to 10 kHz and an amplification range of 100 to 500×. The output of this device, further amplified (50–200×) and filtered (bandwidth 100 Hz-2 kHz) by means of an optically isolated amplifier (Applied Microelectronics Institute, Halifax, N. S., Canada), was led to a Nicolet (Madison, WI) model 207 oscilloscope and to a Grass (Quincy, MA) AM8 Audio Monitor.

Loci in epicardial fat were identified in which action potentials with signal-to-noise ratios greater than 3:1 could be recorded. Individual units were identified by the amplitude and configuration of their action potentials. With the use of these techniques and criteria, the microelectrode does not record action potentials generated by axons of passage but rather records action potentials generated by somata and/or dendrites (4). Periodic motion at the recording site occurred due to cardiac and respiratory dynamics, thereby inducing minor fluctuations in the amplitude of individual action potentials generated by a given unit over time. Fluctuations in the amplitude of action potentials were found to vary by <10 μV over several minutes, retaining their same configurations over time. Thus, action potentials recorded in a given locus with the same configuration and amplitude (<10 μV) were considered to be generated by a single unit.

**Interventions.** Ventricular mechanical sensory inputs to identified neurons were tested by gently touching epicardial loci on the ventral epicardial surface of the right and left ventricles with a saline-soaked cotton applicator. Mech-
anosensoory inputs to identified neurons were further tested by transient occlusion (5 s) of the inferior vena cava. Chemo-
sensory inputs to these neurons were then tested by applying the Na$^+$ channel modifier veratridine (7.5 μM) and, subse-
sequently, adenosine (100 μM) to ventricular epicardial sen-
sory fields. These chemical agents were applied individually
to the epicardium for 60–120 s via 2-cm × 2-cm gauge squares soaked with one chemical (~0.5 ml). After each
chemical was removed, sensory fields were washed with normal saline (~2 ml/s) for 30 s, at least 5–10 min being
allowed to elapse before the next intervention. The applica-
tion of these chemicals to epicardial loci was repeated to ascertain whether spurious results could occur due to tachy-
phylaxis. Gauze squares soaked with room temperature nor-
al saline were also applied to identified epicardial sensory
fields to determine whether neuronal responses elicited by
epicardial chemical application were due to vehicle effects or
any mechanical effects elicited by the gauze squares.

Administration of chemicals to identified neurons via their
local arterial blood supply. To administer various chemicals to
the somata and dendrites of neurons identified in the ventral
venous arterial blood supplyplexus, a 24-French catheter was in-
serted in the VIV coronary artery at the level of its first diagonal
branch (Fig. 1, thick black arrow). The cannula was threaded
proximally (retrograde to flow) such that its tip was positioned
just cranial of the origin of the small arteries that supplied
to the ventralventricular ganglionated plexus. The posi-
tion of the catheter tip was confirmed by gentle palpation
through the artery wall. The cannula was fixed in place with
2–3 ml of adhesive applied to the arterial wall. PE-15 tubing
was inserted into this cannula, with a stopcock at its other end,
to permit the administration of chemicals into the local arterial
blood supply of identified neurons. Monitored hemodynamic
indexes were unaffected by the placement of this cannula.
Postmortem examination of appropriate catheter placement
was confirmed by injecting methylene blue dye via this catheter
into the regional coronary arteries.

The following pharmacological agents were infused via
this catheter into the local arterial blood supply of identified
neurons at rates of ~0.05 ml/s: adenosine (100 μM; 0.1 ml), the
selective adenosine A1 receptor antagonist 8-cyclopentyl-
1,3-dipropylxanthine (DPCPX; 1 mM, 1.0 ml), and the selec-
tive adenosine A2 receptor antagonist 3,7-dimethyl-1-propar-
glyxanthine (DMPX; 1 mM, 1.0 ml). Chemicals, when locally
administered, affected the somata of ventralventricular neu-
rons as well as tissues downstream such as ventricular sen-
sory neurites and regional cardiomyocytes. In four prelimi-
nary test animals, different doses of each pharmacological
agent were administered into the local coronary artery to
determine whether when applied in increasing doses they
entered the systemic circulation in sufficient quantities to
alter monitored cardiovascular indexes. In all experiments,
when a selective adenosine receptor antagonist was studied,
the effectiveness of adenosine receptor blockade was con-
ﬁrmed subsequently by administering adenosine (100 μM;
0.5 ml) into the local arterial blood supply 5 and 60 min after
administering DPCPX and DMPX.

Myocardial ischemia. A 3–0 silk ligation, passed around
the VIV coronary artery just distal to its ﬁrst diagonal branch (Fig.
1, black arrow), was led through a snare to occlude that artery
later in the experiments. Because investigated neurons derived
their arterial blood supply from arteries proximal to this liga-
ture, their arterial blood supply was not affected by these
transient coronary artery occlusions. A 30-s coronary artery
occlusion time was chosen to create each ischemic episode. This
relatively short period of occlusion was chosen for two reasons.
First, longer duration occlusions performed multiple times dur-
ing the course of each experiment might have induced precon-
ditioning. It has been demonstrated that myocardial precondition-
ing can be avoided if the periods of coronary artery occlusion
are less than 1 min in duration (20). Second, in four preliminary
experiments in which coronary artery occlusions of various
durations (15 and 30 s as well as 1 and 2 min) were tested, it
was found that multiple 30-s periods of occlusion elicited con-
sistent neuronal responses. Occlusions longer than 30 s did not
produce any further modulation of neuronal activity, whereas
occlusions less than 30-s duration produced inconsistent neuro-
nal responses. Thus, 30-s occlusion times were employed for the
remainder of the experiments. A minimum of 10 min was
allowed to elapse between each coronary artery occlusion to
allow neuronal and cardiovascular variables to return to
baseline values. To ensure reproducibility of the ischemic/
reperfusion responses, multiple occlusions were performed dur-
ing the course of each experiment. In a preliminary set of
experiments involving eight animals, adenosine and then
DPCPX plus DMPX were administered locally to investigated
neurons during the ischemic episodes (at concentrations de-
scribed above).

Myocardial reperfusion. In 15 separate pigs, following test-
ing the epicardial sensory inputs depicted above, adenosine
(100 μM, 0.1 ml) was administered into the arterial blood
supply of the ventral ventricular ganglionated plexus. The
local infusion of adenosine was begun at the time of onset of
myocardial reperfusion (immediately on release of the vascu-
lar occluder); its administration lasted for the ﬁrst 2–5 s after
coronary arterial ﬂow was reestablished.

Subsequently, the effects of preadministration of either
DPCPX or DMPX (1 mM; 1 ml administered in random order
into the local coronary artery blood) on neuronal activity re-
responses elicited during reperfusion were studied in the absence
or presence of adenosine. This was done to determine if local
administration of adenosine A1 or A2 receptor antagonists af-
fected the modulator effects that adenosine was capable of
exerting on ventricular neurons during reperfusion.

Data analysis. Heart rate and LV chamber systolic press-
ure were measured for 30-s periods of time before and
during the peak responses elicited by each intervention.
Similarly, the activity generated by identiﬁed ventricular
neurons was analyzed for 30-s periods of time before and
during each intervention. Data obtained before and during
each intervention are presented as means ± SE. Repeated-
tests with Bonferroni correc-
tion were used for statistical analysis of the effects elicited by
each intervention, where appropriate. Each chemical tested
elicited neuronal responses in each animal. As has been
found in the past (13), intrinsic cardiac neuronal activity
either increased or decreased when exposed to the interven-
tions described above, including exogenous adenosine admin-
istration, depending on the population of neurons investi-
gated. Thus, change in neuronal activity from baseline values
was also assessed during each intervention [expressed as
absolute value of the delta change (|Δ|) in neuronal activi-
ty]. Signiﬁcance values of P < 0.05, 0.02, or 0.01 were used
for these determinations.

RESULTS

Ventricular mechanosensory and chemo- sensory in-
puts. When a gentle touch was applied to the epicardial
surface of the right or left ventricles of nine pigs, the
activity generated by identiﬁed neurons changed in
each animal (Table 1). Neuronal activity increased or
decreased in response to gently touching a ventricular
epicardial locus, depending on the population of neu-
rons studied. Epicardial application of veratridine also altered neuronal activity, increasing or decreasing activity depending on the population studied. On the other hand, when adenosine was applied to epicardial loci at the dose studied, the activity generated by investigated neurons was not modified. Transient occlusion of the inferior vena cava induced minor reductions in LV chamber systolic pressure that were accompanied by concomitant changes in intrinsic cardiac neuronal activity. As has been found in the past (18), monitored indexes were unaffected when equal volumes of saline were administered into the blood supply of investigated neurons. Epicardial application of saline did not alter monitored indexes.

**Table 1. Responses of ventricular neurons to mechanical and chemical stimuli**

<table>
<thead>
<tr>
<th>Interventions</th>
<th>n</th>
<th>HR, beats/min</th>
<th>LVSP, mmHg</th>
<th>Neuronal Activity Change, impulses/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Touch LV</td>
<td>9</td>
<td>135 ± 11</td>
<td>131 ± 11</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Touch RV conus</td>
<td>9</td>
<td>134 ± 11</td>
<td>133 ± 12</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Touch RV sinus</td>
<td>9</td>
<td>134 ± 11</td>
<td>136 ± 11</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Veratridine (topical)</td>
<td>9</td>
<td>131 ± 10</td>
<td>140 ± 13</td>
<td>110 ± 2</td>
</tr>
<tr>
<td>Adenosine (topical)</td>
<td>6</td>
<td>141 ± 12</td>
<td>145 ± 10</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>Occlude IVC</td>
<td>6</td>
<td>123 ± 12</td>
<td>128 ± 15</td>
<td>115 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number of animals subjected to each intervention. HR, heart rate (beats/min); LVSP, left ventricular chamber systolic pressure; LV, left ventricular; RV, right ventricular; occlude IVC, inferior vena cave occlusion. Neuronal activity is presented as absolute change from baseline (control) values (impulses/min). *P < 0.01.

**Effects of ischemia on monitored indexes.** In 15 pigs, when the VIV coronary artery was occluded for 30 s, the activity generated by the more centrally located ventricular neurons increased in 10 animals (Fig. 2);

**Fig. 2. Effect of ischemia/reperfusion on intrinsic cardiac neuronal activity.** The activity generated by identified ventricular neurons (D) increased when the distal left ventral descending coronary artery was occluded (black bar below) for 30 s. Their excitability persisted during the reperfusion phase. The break in the diagram signifies a 3.5-min period removed from the recording. AP, aortic pressure; LVP, left ventricular pressure.
the activity generated by neurons identified in the five remaining animals decreased. Neuronal activity was changed from baseline values (|Δ| change) by the brief periods of coronary artery occlusion (Table 2). Activity changed even more during the early reperfusion period (Figs. 2 and 3). Heart rate remained unchanged throughout these brief periods of focal ventricular ischemia as well as during reperfusion (control 154 ± 11 beats/min; ischemia 156 ± 12 beats/min; reperfusion 155 ± 11 beats/min). During ischemia, LV chamber systolic pressure fell slightly (113 ± 4 to 101 ± 4 mmHg; P < 0.01). This index returned to baseline values immediately with reperfusion (114 ± 4 mmHg).

The effects of repeat coronary artery occlusions of 30-s duration on neuronal activity were tested in four separate animals to determine whether “preconditioning” effects could be elicited by multiple brief periods of regional ventricular ischemia. Each occlusion produced similar hemodynamic responses that were associated with cyanosis and dyskinesia that resolved with reperfusion. The absolute change in ventral ventricular neuronal activity from baseline values that occurred during each of the six episodes of regional ventricular ischemia was found to be similar, neuronal activity changes so induced ranging from 71 ± 8 to 105 ± 25 impulses/min. No significant differences were identified among the neuronal responses elicited when comparing each of the six transient coronary artery occlusions.

Adenosine and adenosine receptor antagonists. In the normally perfused hearts of 15 pigs when adenosine was administered into the local coronary artery blood supply of identified ventricular neurons, neuronal activity changed by a small amount (Table 2). No change

Table 2. Effects of ischemia alone as well as local administration of adenosine or DMPX and DPCPX on ventricular neuron activity

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Values are means ± SE. Note that neuronal activity changes during the periods of ischemia, changing even more during the reperfusion phase. Local arterial administration of adenosine altered neuronal activity modestly, as did the 2 blocking agents. *P < 0.05.

DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DMPX, 3,7-dimethyl-1-propargylxanthine; VCA, ventral descending coronary artery.

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Fig. 3. Effects of adenosine on reperfusion-induced neuronal responses. During the reperfusion period subsequent to that depicted in Fig. 2, when adenosine was administered locally to identified ventricular neurons (between arrows below), the activity they generated was suppressed.

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in cardiovascular variables was identified during this intervention (heart rate 138 ± 14 to 141 ± 16 beats/min; LVP systolic 116.9 ± 4.3 to 116.7 ± 3.7 mmHg). Local vehicle administration did not alter monitored variables.

Locally administered DPCPX and DMPX (n = 15) exerted minor effects on the activity generated by investigated neurons (Table 2). No hemodynamic alterations were detected following local arterial administration of DPCPX and DMPX (LV pressure 113.5 ± 3.1 to 113.9 ± 2.6 mmHg; heart rate 135 ± 11 to 136 ± 11 beats/min, \( P = \) not significant). The dose of DPCPX and DMPX employed produced effective local adenosine receptor blockade during the rest of experiments as local administration of adenosine failed to elicit neuronal responses (\( |\Delta| 8 ± 3 \) impulses/min compared with baseline values; \( P = \) not significant) in their presence.

Adenosine administered during ischemia. In eight separate animals, neuronal activity responses to ischemia were not affected by locally administered adenosine (92 ± 52 vs. 127 ± 41 |\( \Delta |\) impulses/min; \( P = \) not significant). Neither were ischemia-induced neuronal responses affected by the presence of DPCPX and DMPX (51 ± 18 and 63 ± 24 |\( \Delta |\) impulses/min, respectively; \( P = \) not significant). Therefore, for the rest of the experiments, we focused on neuronal responses elicited during the reperfusion (immediate postischemia) phase.

Adenosine administered during reperfusion. Local infusion of adenosine obtunded neuronal responses to reperfusion (n = 15 pigs; Fig. 3). Adenosine reduced their responsiveness to reperfusion by 75% (|\( \Delta |\) impulses/min of 167.7 ± 34.2 to 41.9 ± 10.7 impulses; \( P < 0.01 \)). Overall, adenosine returned intrinsic cardiac neuronal activity toward baseline (preischemic) values whether excitatory or depressive neuronal responses were induced initially during reperfusion (Fig. 4). Thus, in the 13 animals in which ventricular neuronal activity increased during reperfusion, local administration of adenosine suppressed neuronal activity during repeat reperfusion. In the two animals in which neuronal activity was diminished during the reperfusion phase, adenosine administered during reperfusion returned neuronal activity to baseline values (i.e., activity was greater than in the absence of adenosine).

Reperfusion and adenosine receptor blockade. Adenosine was tested during reperfusion in the presence of the A1 receptor antagonist DPCPX in seven other animals. After preadministered DPCPX, exogenously administered adenosine failed to modify the neuronal effects of reperfusion (230.3 ± 63.8 vs. 216.6 ± 94.7 |\( \Delta |\) impulses/min, comparing control responses elicited during reperfusion to those induced in the presence of DPCPX, \( P = \) not significant; Fig. 4). Conversely, preadministration of the A2 receptor antagonist DMPX to seven different animals did not diminish the capacity of adenosine to modify neuronal activity during reperfusion (230.9 ± 63 |\( \Delta |\) impulses/min before and 72.3 ± 41.3 |\( \Delta |\) impulses/min after DMPX; \( P < 0.02 \); Fig. 4).

**DISCUSSION**

As is found with canine atrial neurons (11), the porcine ventricular nervous system can transduce myocardial ischemia and the subsequent period of reperfusion injury (Fig. 2). This occurred despite the fact that the somata of investigated neurons were not directly involved in the ischemic process. The responsiveness (excitatory or inhibitory activity responses) of ventricular neurons to distal ventricular ischemia apparently was due to the summation of various inputs from an array of ventricular sensory neurites that were capable of transducing the mechanical and chemical milieu of the ventricle. Exogenously administered adenosine, acting principally via adenosine A1 receptors, modified ventricular neuronal responses to myocardial reperfusion but not to ischemia.

Adenosine, a by-product of catabolism within the ischemic cell, may arise from multiple cell types (15). Myocardial release of adenosine increases severalfold during myocardial ischemia (17). Adenosine has been shown to affect neurons in the central nervous system (2). It also affects neurons in autonomic ganglia associated with the gastrointestinal tract (6) and heart (3, 13) as well as sympathetic efferent postganglionic axons innervating coronary arteries (1). Adenosine affects cardiac sensory neurites associated with afferent neuronal somata in dorsal root (12) and nodose (19) ganglia. In contradistinction to these findings, adenosine did not modify ventricular sensory neurites that...
input to ventricular neurons identified in this study (Table 1). On the other hand, identified neurons did receive inputs from ventricular chemosensory neurites capable of transducing ion channel modifying agents such as veratridine (Table 1). These data imply that ventricular sensory inputs to investigated porcine ventricular neurites were capable of transducing chemicals other than adenosine, in addition to local deformation. The absence of neuronal responses elicited by epicardial application of adenosine, combined with an absence of hemodynamic perturbations when adenosine was administered directly to the somata and/or dendrites of ventral ventricular neurones (i.e., lack of efferent neuronal effects), suggests adenosine neuro-modulation was principally via the local circuit neurones (interneurons).

Data generated from these experiments demonstrated a differential response of the intrinsic cardiac nervous system to myocardial ischemia, as opposed to the subsequent reperfusion injury phase. The capacity of ventricular neurones to transduce regional ventricular ischemia was not affected by adenosine administered exogenously to investigated somata and/or dendrites immediately before or during the ischemic episode. Conversely, intrinsic cardiac neuronal activity was modified when adenosine administration began immediately with initiation of reperfusion (Fig. 3). Presumably, the lack of effect of adenosine induced during the ischemic period was due, in part, to the fact that mechanosensory inputs to the intrinsic cardiac nervous system secondary to ischemia-induced local myocardial dyskinesia (9) would not have been influenced by locally applied adenosine. Furthermore, oxygen free radicals liberated by the ischemic myocardium (21) are capable of activating ventricular sensory neurites (18). Thus, the enhancement of multiple sensory inputs to the intrinsic cardiac nervous system in such a state presumably overwhelmed any demonstrable effects that adenosine receptor modification elicited on identified ventricular neurones.

During reperfusion, locally administered adenosine affected intrinsic cardiac neuronal activity (Fig. 3). Under these circumstances, adenosine acted to restore neuronal activity toward baseline (preischemic) values (c.f., Fig. 2). The stabilizing effects that adenosine imparted to the intrinsic cardiac nervous system during reperfusion were abrogated by the presence of an A1 receptor but not an A2 adenosine receptor antagonist (Fig. 4). These data indicate that activation of intrinsic cardiac neuron adenosine A1 receptors obviates the responsiveness of ventricular neurones to reperfusion injury.

Adenosine has been shown to exert moderator effects on a number of cellular mechanisms associated with reperfusion injury. Specifically, adenosine A1 receptor activation produces alterations in local free radical generation (16) and K⁺-ATP channel activity (5). Both of these cellular mechanisms are known to affect intrinsic cardiac neurons (18). In the experiments described herein, adenosine apparently influenced intrinsic cardiac local circuit neurones that were involved in trans-lating myocardial reperfusion injury to cardiac effenter neurones, principally via their adenosine A1 receptors. As such, modulation of adenosine A1 receptors may be important in clinical situations such as during revascularization therapy (i.e., thrombolytic therapy or following coronary artery bypass grafting procedures) to suppress reperfusion-induced alterations in the intrinsic cardiac nervous system. Further studies are required to better elucidate the role of ventricular purinergic neurones involved in the reperfusion process to establish the possible clinical utility of adenosine receptor modulation in such circumstances.

**Perspectives**

Data derived from this study indicate that transient regional ventricular ischemia/reperfusion is transduced by the porcine ventricular nervous system. Stabilization of reperfusion-induced neuronal responses by adenosine A1 receptor activation indicates the importance of ventricular neuronal purinoreceptors during ventricular reperfusion injury. These data suggest that therapy devised to modify adenosine A1-sensitive ventricular cardiac neurones may be of benefit in reducing cardiac reperfusion injury sequelae.

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