Protection against ventricular arrhythmias and cardiac death using adenosine and lidocaine during regional ischemia in the in vivo rat

Sarah J. Canyon and Geoffrey P. Dobson
Department of Physiology and Pharmacology, School of Biomedical Sciences, James Cook University, Townsville, Queensland, Australia 4811

Submitted 18 March 2004; accepted in final form 15 April 2004

Canyon, Sarah J., and Geoffrey P. Dobson. Protection against ventricular arrhythmias and cardiac death using adenosine and lidocaine during regional ischemia in the in vivo rat. Am J Physiol Heart Circ Physiol 287: H1286–H1295, 2004; 10.1152/ajpheart.00273.2004.—Despite decades of research, there are few effective ways to treat ventricular fibrillation (VF), ventricular tachycardia (VT), or cardiac ischemia that show a significant survival benefit. Our aim was to investigate the combined therapeutic effect of two common antiarrhythmic compounds, adenosine and lidocaine (AL), on mortality, arrhythmia frequency and duration, and infarct size in the rat model of regional ischemia. Sprague-Dawley rats (n = 49) were anesthetized with pentobarbital sodium (60 mg·ml⁻¹·kg⁻¹ ip) and instrumented for regional coronary occlusion (30 min) and reperfusion (120 min). Heart rate, blood pressure, and a lead II electrocardiogram were recorded. Intravenous pretreatment began 5 min before ischemia and extended throughout ischemia, terminating at the start of reperfusion. After 120 min, hearts were removed for infarct size measurement. Mortality occurred in 58% of saline controls (n = 12), 50% of adenosine only (305 µg·kg⁻¹·min⁻¹, n = 8), 0% in lidocaine only (608 µg·kg⁻¹·min⁻¹, n = 8), and 0% in AL at any dose (152, 305, or 407 µg·kg⁻¹·min⁻¹) 1 min after ischemia plus 608 µg·kg⁻¹·min⁻¹ lidocaine, n = 7, 8, and 6). VT occurred in 100% of saline controls (18 ± 9 episodes), 50% of adenosine-only (11 ± 7 episodes), 83% of lidocaine-only (23 ± 11 episodes), 60% of low-dose AL (2 ± 1 episodes, P < 0.05), 57% of mid-dose AL (2 ± 1 episodes, P < 0.05), and 67% of high-dose AL rats (6 ± 3 episodes, P < 0.05). VF occurred in 75% of saline controls (4 ± 3 episodes), 100% of adenosine-only-treated rats (3 ± 2 episodes), and 33% lidocaine-only-treated rats (2 ± 1 episodes) of the rats tested. There was no deaths and no VF in the low- and mid-dose AL-treated rats during ischemia, and only one high-dose AL-treated rat experienced VF (25.5 sec). Infarct size was lower in all AL-treated rats but only reached significance with the mid-dose treatment (saline controls 61 ± 5% vs. 38 ± 6%, P < 0.05). We conclude that a constant infusion of a solution containing AL virtually abolished severe arrhythmias and prevented cardiac death in an in vivo rat model of acute myocardial ischemia and reperfusion. AL combinational therapy may provide a primary prevention therapeutic window in ischemic and nonischemic regions of the heart.

Address for reprint requests and other correspondence: G. P. Dobson, Dept. of Physiology and Pharmacology, School of Biomedical Sciences, James Cook Univ., Townsville, Queensland, Australia 4811 (E-mail: geoffrey.dobson@jcu.edu.au).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
ular region and Lido primarily targets the ventricles. Our study design therefore is very different from Homeister et al. (21), who administered an intravenous bolus of Lido in open-chested dogs 1 min before a 90-min occlusion of the left circumflex coronary artery and again 1 min before reperfusion, immediately followed by adenosine infusion at reperfusion and continued for 1 h. In the present study, AL was administered simultaneously in a single preparation 5 min before and during regional ischemia, not separately and sequentially. Moreover, we (12) have recently reported that, at higher concentrations, the single combination of AL acts as a highly effective non-depolarizing surgical cardioplegia that arrests the heart in asystole by “clamping” the membrane potential in its resting state at around −83 mV. We report that an intravenous infusion of an AL solution administered 5 min before and 30 min during ischemia rat protects from death, ventricular arrhythmias, and cell necrosis in the rat model of regional ischemia.

METHODS

Animals and reagents. Male Sprague-Dawley rats (330–400 g) from the James Cook University Breeding Colony were fed ad libitum and housed at 12:12-h light-dark cycle. On the day of the experiment, the animals were artificially ventilated at 75–80 strokes/min on a humidified room air using a Harvard Small Animal Ventilator (Harvard Apparatus) to maintain blood PO2, PCO2, and pH in the normal physiological range (Ciba-Corning 865 blood gas analyzer). Body temperature was maintained at 37°C (Homeothermic Blanket Control Unit, Harvard Apparatus). The left femoral vein was cannulated using polyethylene-50 tubing for drug infusions while the left femoral artery was cannulated for blood collection and blood pressure monitoring (UFI 1050 BP coupled to a MacLab).

A left thoracotomy was performed through the fourth and fifth intercostal spaces. The pericardium was opened, and the heart was gently exteriorized. A 6-0 suture was threaded under the left coronary artery (LCA) located between the base of the pulmonary artery and left atrium. LCA ties were attached to a custom-designed snare occluder fastened to the cradle via a 20-in. teflon tube attached to a detachable 10-g weight. By adding or removing the weight, a constant ligation pressure could be applied and easily released. Leads were implanted subcutaneously in a lead II ECG configuration. Rats were stabilized for 15–20 min before occlusion. Any animal that had dysrhythmias and/or a sustained fall in mean arterial blood pressure below 80 mmHg was discarded from the study. Ischemia was confirmed by regional cyanosis downstream of the occlusion, and reperfusion was confirmed by lack of cyanosis in that region.

Experimental design. Animals (n = 49) were randomly assigned into six treatment groups: 1) saline controls (0.9% saline, n = 12); 2) Adeno (305 µg·kg⁻¹·min⁻¹, n = 8); 3) Lido only (608 µg·kg⁻¹·min⁻¹, n = 8); 4) low-dose AL (Adeno: 152 µg·kg⁻¹·min⁻¹ and Lido: 608 µg·kg⁻¹·min⁻¹, n = 7); 5) mid-dose AL (Adeno: 305 µg·kg⁻¹·min⁻¹ and Lido: 608 µg·kg⁻¹·min⁻¹, n = 8); and 6) high-dose AL (Adeno: 457 µg·kg⁻¹·min⁻¹ and Lido: 608 µg·kg⁻¹·min⁻¹, n = 6). The AL solutions were prepared on the day of the experiment in physiological saline (0.9%). Drugs were infused intravenously at 1 ml/h (210 infusion pump, Stoelting). All rats received continuous infusion for 5 min before and throughout 30 min of regional ischemia. The treatment was ceased when the coronary ligature was released at the onset of reperfusion after 30 min of ischemia and animals reperfused for 120 min for infarct sizing. In the present study, we chose a range of Adeno concentrations that have led to improved function or reduced necrosis in animal models (55, 56, 68) as well as provided a therapeutic benefit to humans (33, 61, 67). Lido was kept constant at the antiarrhythmic dose of 608 µg·kg⁻¹·min⁻¹. Only one dose of Lido was used to avoid a large number of groups from a statistical standpoint.

The primary end points used to assess the cardioprotective effects of AL solution were mortality, episodes and durations of ventricular arrhythmias, and infarct size. On the basis of the binomial distribution for an episode of VF cited in the Lambeth Conventions, our study protocol required at least four animals in each group to survive for sufficient statistical power to test the primary end points (64). The secondary end points included heart rate (HR), mean arterial pressure (MAP = systolic pressure − diastolic pressure/3 + diastolic pressure), and the rate-pressure product (RPP = HR × systolic pressure).

Arrhythmia analysis. With the use of a lead II ECG tracing, the occurrence of premature ventricular beats (PVB), VT, VF, VT and VF were recorded and the duration of each episode of bigeminy, VT, and VF were measured. Arrhythmias were defined according to the Lambeth Conventions (Fig. 1) (64). PVBs were defined as discrete and identifiable premature QRS complexes, and an episode of bigeminy was recognized as a variant of PVBs and characterized by the minimum sequence: P, QRS, PVB, P, QRS, PVB (64). Salvos were defined as two or three consecutive PVBs, whereas VT was defined as a run of four or more consecutive ventricular premature beats. An episode of VF was defined as a signal where individual QRS deflections could not easily be distinguished from each other and where rate could no longer be measured (64). The duration of each episode was measured in seconds, and the sums of these during 30-min ischemia and 30-min reperfusion were analyzed. Occasionally, when VF occurs in a background of VT, it is difficult to determine whether the signal is from VT or VF. For this reason, the number of episodes and durations of VT and VF were summed and analyzed separately under the variable VT + VF (48).

Analysis of the ischemic area at risk and infarct size. After 120 min of reperfusion, the coronary artery was reocluded, the heart was excised, and blue dye [3 ml copper (II) phthalocyanine-tetrasulfonic acid tetrasodium salt] was flushed retrograde through the aorta at a flow rate of −18 ml/min to demarcate the ischemic risk zone. The heart was sliced transversely into six or seven slices of uniform thickness (2 mm) using a custom-made, equal-spaced, multiscalpel blade slicer. The slices were weighed and digitally photographed, and area measurements were performed using the Image J (NIH) image analysis program. The “area at risk” (AAR) per left ventricle (LV) was defined as the area left unstained by the blue dye, whereas the blue-stained region was defined as the perfused area not at risk of suffering ischemic damage. The slices were then incubated at 37°C for 15 min in a 1% solution of TTC (16) and briefly immersed in formalin for color enhancement. The slices were rephotographed. The region of the slice unstained by TTC (white) was defined as the area of necrosis (AN), whereas the infarcted region was defined as the area of the slice stained by TTC (brick red). The ratio of the AN to the AAR defined infarct size (AN/AAR) and was expressed as a percentage.

Statistical analysis. Data are expressed as means ± SE. Arrhythmias have a non-Gaussian distribution; therefore, arrhythmia episodes and duration were compared using a Mann-Whitney U-test (48). Infarct size and hemodynamic data (Figs. 1A, 2A, and 3A) were analyzed using a one-way ANOVA with a least-significance difference post hoc test. Differences were considered significant if P ≤ 0.05.
RESULTS

Four rats were excluded from our study: one animal’s MAP was <70 mmHg before treatment (Lido only), a second animal’s ventilation tubing became clogged (mid-dose AL), a third rat from the Lido-only group died before the end of the experiment from severe hypotension (no ventricular arrhythmias were involved), and a fourth rat was excluded from the experiment from severe hypotension (no ventricular arrhythmias were involved). Data from seven of the twelve saline control rats (n = 5, surviving) and four of eight Adeno-only-treated rats (n = 4, surviving) died during the ischemic period from an episode of VF. No deaths occurred in the Lido-only-treated rats (n = 6) or in any group where the AL solution was infused (n = 19). Only data from surviving rats were further analyzed (n = 34).

Arrhythmias during ischemia. Arrhythmias occurred with great variability among groups (Figs. 2, A–D). Treatment with Adeno-only, Lido-only, or high-dose AL did not significantly change episodes or durations of arrhythmias of any type from saline controls during ischemia or reperfusion. Episodes of PVBs occurred in all groups: saline controls (60 ± 22 episodes), Adeno only (19 ± 4 episodes), Lido only (104 ± 21 episodes), low-dose AL (48 ± 10 episodes), mid-dose AL (35 ± 12 episodes), and high-dose AL (57 ± 20 episodes); yet no group resulted in significant differences from saline controls (Fig. 2A). Compared with Lido-only treatment, low-dose AL and mid-dose AL significantly reduced the number of PVBs (104 ± 21 vs. 48 ± 10 and 35 ± 12 episodes, P < 0.05).

Although salvos occurred during the 30 min of ischemia in all treatment groups: saline controls (15 ± 7 episodes), Adeno only (10 ± 4 episodes), Lido only (49 ± 21 episodes), low-dose AL (20 ± 13 episodes), mid-dose AL (11 ± 5 episodes), and high-dose AL (32 ± 20 episodes), no significant differences were found. Episodes of bigeminy also occurred in all groups: saline controls (12 ± 4 episodes), Adeno only (9 ± 3 episodes), Lido only (23 ± 10 episodes), low-dose AL (8 ± 3 episodes), mid-dose AL (12 ± 5 episodes), and high-dose AL (14 ± 5 episodes) (Fig. 2B). Over the 30 min of ischemia, the cumulative durations of bigeminy were as follows: saline controls (23 ± 12 s), Adeno only (20 ± 7 s), Lido only (59 ± 26 s), low-dose AL (13 ± 7 s), mid-dose AL (20 ± 8 s), and high-dose AL (63 ± 53 s). No significant differences in bigeminy episodes and cumulative durations were found (Fig. 2B).

The mean number of VT episodes in saline controls was 18 ± 9, affecting 100% of animals, whereas 75% experienced VF (4 ± 3 episodes) (Fig. 2C). Treatment with Adeno only resulted in VT in 50% of the rats tested (11 ± 7 episodes), and 100% of rats had VF (3 ± 2 episodes). In Lido-only treatment, VT occurred in 83% (23 ± 11 episodes) and VF occurred in 33% (2 ± 1 episodes) of rats tested. Of the subjects treated with low- and mid-dose AL solution, 60% and 57% of the treated rats, respectively, had at least 1 episode of VT (2 ± 1 episodes), whereas 67% of high-dose AL-treated rats experienced 6 ± 3 episodes of VT. However, low- and mid-dose AL completely protected against VF during ischemia, whereas only one rat treated with high-dose AL experienced VF (25.5 s) (Fig. 2C).
Rats infused with mid-dose AL solution experienced not only a significant reduction in VTs but also a significant reduction in the durations of VT (2 ± 1 s) and VT + VF (2 ± 1 s) compared with saline controls (P < 0.05) (Fig. 2D). The durations of VT and VT + VF for saline controls were 106 ± 45 and 156 ± 72 s and for Lido-only treatment were 31 ± 18 and 37 ± 22 s, respectively (Fig. 2D). Low-dose AL resulted in a mean VT duration of 9 ± 8 s, and high-dose AL resulted

Fig. 2. A: episodes of PVBs and salvos in saline controls and the five treatment groups during 30-min ischemia. Significance was shown between PVBs in lidocaine (Lido)-treated rats compared with Adenosine (Adeno)-alone and low- and mid-dose Adeno + Lido (AL)-treated rats (P < 0.05). Surviving rats are as follows: saline, n = 5; Adeno only, n = 4; Lido only, n = 6; low-dose AL, n = 5; mid-dose AL, n = 7; and high-dose AL, n = 6. B: episodes and durations of bigeminy in saline controls and the five treatment groups during 30-min ischemia. Surviving rats are as follows: saline, n = 5; Adeno only, n = 4; Lido only, n = 6; low-dose AL, n = 5; mid-dose AL, n = 7; and high-dose AL, n = 6. C: sum of episodes of VT and VF in saline controls and the five treatment groups during 30-min ischemia. There was no VF for low- or mid-dose AL-treated rats and virtually none in high-dose AL-treated rats. Surviving rats are as follows: saline, n = 5; Adeno only, n = 4; Lido only, n = 6; low-dose AL, n = 5; mid-dose AL, n = 7; and high-dose AL, n = 6. D: sum of durations of VT and VF in saline controls and the five treatment groups during 30-min ischemia. Surviving rats are as follows: saline, n = 5; Adeno only, n = 4; Lido only, n = 6; low-dose AL, n = 5; mid-dose AL, n = 7; and high-dose AL, n = 6. *Statistically significant difference (P < 0.05).
in a mean VT duration of 4 ± 2 s. In addition, infusion of mid-dose AL solution significantly reduced the durations of the VT episodes compared with Adeno-only-treated rats (27 ± 18 s, P < 0.05) (Fig. 2D). It was noted that, with the exception of the low- and mid-dose AL solution treatments, a high variability in arrhythmia frequency and duration across the other treatment groups was apparent (Fig. 2A). Only the infusion of AL solution provided consistent reductions of VT or VF frequency or duration without large variability between samples.

Early reperfusion arrhythmias. Within the first minute of reperfusion, 80% of saline controls, 75% of the adeno-only-treated animals, and 16% of Lido-only-treated animals experienced at least one episode of VT of 0.6- to 35-s duration, yet no animals treated with AL solution at any dose of Adeno endured reperfusion arrhythmias. Neither treatment with Adeno only or Lido only differed significantly from each other or from saline controls (P < 0.05). An episode of VF occurred in one of the five saline controls within the first minute and lasted 16 s. There were no episodes of VF in the Adeno-only or Lido-only treatment groups during 30-min reperfusion. Rats treated with low- or mid-dose AL solution experienced no ventricular arrhythmias (PVB, salvos, bigeminy, VT, or VF) at or during reperfusion. High-dose AL treatment prevented VT, VF, salvos, and bigeminy, yet three rats experienced 26 ± 23 episodes of PVB within the first 30 s. The number of episodes of VT from saline controls and the Adeno-only-treated animals was found to be significantly higher than mid-dose AL solution-treated animals (P < 0.05). Additionally, the durations of VT and VT + VF durations in the Adeno-only group (11 ± 8 s for both) were significantly longer than treatment with mid-dose AL treatment (P < 0.05).

Infarct size. Mean AAR (AAR/LV), AN (AN/LV), and infarct size (AN/AAR) expressed as a percentage of the LV are shown in Fig. 3, A and B. The AARs for saline controls, Adeno-only-, Lido-only-, low-dose AL-, mid-dose AL-, and high-dose AL-treated animals were 63 ± 7%, 58 ± 8%, 56 ± 8%, 43 ± 6%, 48 ± 8%, and 66 ± 8%, respectively, and not significantly different (P < 0.05) (Fig. 3A). Overall, the mean risk area was 55 ± 0.03% (n = 33). The AN in mid-dose AL-treated animals was 18 ± 4% and significantly lower than saline controls (38 ± 5%) and Adeno-only (33 ± 7%) and Lido-only (33 ± 3%) treatments (P < 0.05) but was not different from low-dose AL (21 ± 6%) or high-dose AL (30 ± 5%) (Fig. 3A). The mean infarct size of mid-dose AL (38 ± 6%) was also significantly reduced from saline controls, (61 ± 5%) and Adeno-only (56 ± 4%) and Lido-only (66 ± 8%, P < 0.05) animals but not from low-dose AL (45 ± 9%) and
high-dose AL animals (45 ± 6%) (Fig. 3B). There was no significant change in mean infarct size between saline controls, Adeno-only, or Lido-only treatments (Fig. 3B).

Systemic hemodynamics. HR, MAP, and RPP are shown in Fig. 4. There were no significant differences among groups at baseline before pretreatment. After pretreatment at preocclusion, the HRs of Lido-only (357 ± 16 beats/min), low-dose AL (328 ± 8 beats/min), mid-dose AL (327 ± 13 beats/min), and high-dose AL (322 ± 9 beats/min) animals were all significantly reduced compared with saline control (429 ± 13 beats/min) and Adeno-only (405 ± 18 beats/min) animals. The preocclusion MAPs and RPPs of saline control (98 ± 9 mmHg and 36,400 ± 3,900 beats·min⁻¹·mmHg⁻¹), Adeno-only (73 ± 18 mmHg and 24,600 ± 8,300 beats·min⁻¹·mmHg⁻¹), and Lido-only (81 ± 12 mmHg and 25,900 ± 5,500 beats·min⁻¹·mmHg⁻¹) animals were significantly greater than low-dose AL (49 ± 4 mmHg and 12,000 ± 1,200 beats·min⁻¹·mmHg⁻¹), mid-dose AL (45 ± 4 mmHg and 10,000 ± 1,000 beats·min⁻¹·mmHg⁻¹), and high-dose AL (40 ± 3 mmHg and 8,500 ± 900 beats·min⁻¹·mmHg⁻¹) animals. By 25 min of ischemia, the HRs of Lido-only (303 ± 22 beats/min), low-dose AL (305 ± 15 beats/min), mid-dose AL (314 ± 16 beats/min), and high-dose AL (303 ± 18 beats/min) animals were significantly lower than Adeno-only animals (405 ± 18 beats/min) and saline controls.
ADENOSINE AND LIDOCAINE PROTECT AGAINST VENTRICULAR ARРHYTHMIAS DURING ISCHEMIA

(427 ± 16 beats/min, P < 0.05). At 25 min of ischemia, the MAPs and RPPs of saline control (98 ± 15 mmHg and 37,100 ± 6,700 beats·min⁻¹·mmHg⁻¹) and Adeno-only (65 ± 9 mmHg and 21,900 ± 2,300 beats·min⁻¹·mmHg⁻¹) animals were maintained significantly higher than those of Lido-only (56 ± 4 mmHg and 13,400 ± 1,600 beats·min⁻¹·mmHg⁻¹), low-dose AL (54 ± 7 mmHg and 12,500 ± 1,700 beats·min⁻¹·mmHg⁻¹), mid-dose AL (49 ± 5 mmHg and 10,500 ± 1,800 beats·min⁻¹·mmHg⁻¹), and high-dose AL (46 ± 6 mmHg and 9,400 ± 1,600 beats·min⁻¹·mmHg⁻¹) animals (P < 0.05). Throughout reperfusion, the hemodynamics in all groups rose toward pretreatment values. However, within the 120-min reperfusion period, no treatment reached starting baseline values in any group (Fig. 4).

DISCUSSION

Our study shows in the in vivo rat model that a single solution of two antiarrhythmic drugs, AL, administered before and during 30 min of regional ischemia, leads to protection from cardiac death, severe arrhythmias, and tissue necrosis. In our study, 58% of saline controls and 50% of Adeno-only-treated animals died during ischemia secondary to VF. In Lido-treated animals, although there were no deaths, the drug was unable to decrease the incidence of arrhythmias or infarct size compared with saline controls (Figs. 2, C and D, and 3). In contrast, AL administration (at all combinations studied) prevented cardiac death, virtually abolished VT and VF, and reduced infarct size from 61% to 38% (mid-dose AL) (Fig. 2, A and B). Our work may have clinical relevance because 50% of sudden death in humans is due to VT or VF and the remainder to bradyarrhythmias or electromechanical dissociation (25).

AL solution’s antiarrhythmic actions and survival benefit. The ability of AL solution, especially the mid-dose AL, to virtually abolish arrhythmias and reduce cell death may be linked to lower cardiac excitability and better stabilization of electrophysiological and metabolic functions in the ischemic heart, although the latter were not measured in the present study. Experimental support for lower demand was reflected in the reduced HR and other hemodynamic parameters in AL-treated groups (Fig. 4), which also may have played a role in infarct size reduction (Fig. 3). However, at least for Adeno, no consistent link has been demonstrated between MAP and infarct size reduction in a number of small animal models, including the rat (8, 10). For example, Casati et al. (8) carefully showed that many cardioprotective actions of the A 1 agonist 2-chloro-N6-cyclopentyladenosine were independent of changes to MAP and other hemodynamic parameters (see Limitations of the study for further discussion).

The mechanisms for AL solution’s protection are not known. They may involve Adeno’s slowing of the sinoatrial node (negative chronotropy), slowing atrial contractility (negative inotropy) and delaying of A-V nodal impulse conduction (negative dromotropy) (15), and Lido’s ability to downregulate voltage-dependent Na⁺ fast channels in cardiac myocytes and intercalated discs (52) and its negative inotropic effect via action potential shortening (39, 66). Adenosine may also stabilize myocardial excitability by activating the A 1 receptor subtype (and perhaps A 3), blunt the stimulatory effects of catecholamines (inhibiting sarcoclemmal Na⁺/H⁺ and Na⁺/Ca²⁺ exchangers), and inhibit norepinephrine release from nerve terminals (15, 18). These electrophysiological and receptor-linked effects may have “downstream” metabolic effects leading to reduced Na⁺ and Ca²⁺ entry via channels and exchangers (39, 58, 61) and improved handling of these cations by the sarcoplasmic reticulum (41) and mitochondria (17).

AL solution’s protection may also relate to shortening of the action potential duration: Adeno by opening of the ATP-sensitive K⁺ (KATP) channels (and possibly other K⁺ channels) through enhanced phase 3 repolarization (17, 29, 37, 45), and Lido’s effect through its interaction with cardiac sarcolemmal Ca²⁺ channels (39, 66). Because increased K⁺ channel activation and rapid action potential duration shortening are known to cause arrhythmias (9), the AL combination may “clamp” ischemic cells at more polarized potentials than either Adeno or Lido alone (12). If left “unchecked,” depolarization of the resting membrane potential could lead to chaotic firing of some cells in the ischemic region and act as a potential substrate for arrhythmogenesis. Thus AL’s overall effect to dampen a rapid decline in depolarization (or excitability) and excessive action potential duration shortening may assist to regulate Na⁺ and Ca²⁺ entry and thereby improve Ca²⁺ handling in ischemic regions of the heart. Of particular relevance, Hu et al. (22) have recently proposed that Adeno may act like a brake on the rapid decline of membrane depolarization via PKC-mediated, dynamin-dependent KATP channel internalization, and Olchewski et al. (47) have reported that Lido in some cases may partially inhibit KATP channels. It should be added, however, that the involvement of sarcolemmal KATP channels in early myocardial ischemia arose from studies using the nonspecific blocker glibenclamide (17, 28) and the highly selective sarcolemmal KATP blocker HMR-1,883 (6), but much controversy remains (17). Other potential sites of Adeno’s actions, and perhaps Lido’s actions, include the mitochondria (17, 46) and sarcoplasmic reticulum (41).

In summary, we speculate that the antiarrhythmic effect and survival benefit of AL therapy may relate to a better matching of membrane excitability and myocardial metabolism in both ischemic and nonischemic regions. Reentry arrhythmias could be reduced by AL’s ability to reduce abnormal regional heterogeneity of action potential waveforms and refractory periods in the different chambers of the heart, including transmural, transepicardial, interventricular, and supraventricular regions (25, 27, 43). In the normal heart, heterogeneities in action potential durations and refractory periods are important to the synchronization of the heart as a pump (7, 44). Therefore, by reducing myocardial excitability and energy demand, and by synchronizing action potential shortening and dispersion through better handling of intracellular Na⁺ and Ca²⁺, the combination of AL may limit the formation and dispersion of refractoriness in the ischemic and border zones. Because AL solution was infused before and during regional ischemia, further experiments are required to test whether the antiarrhythmic therapy could break newly formed ventricular reentry circuits when administered either as a bolus or intravenous infusion without prior treatment.

Adeno and Lido alone during occlusion were unable to decrease the incidence of ventricular arrhythmias. Our study showed that Adeno alone or Lido alone were unable to decrease the incidence of ventricular arrhythmias during the occlusion compared with saline controls in our rat model of regional ischemia. It was surprising that the infusion of Adeno
alone (305 μg·kg⁻¹·min⁻¹) failed to protect from VT and VF because 100% of the surviving animals were affected, and those animals that died, died from VF. These results may relate to the dosages employed in our model because Adeno is used clinically for acute management of supraventricular tachycardia with good outcomes (19). However, there have been a number of reported cases where Adeno has been associated with life-threatening ventricular arrhythmias at doses lower than those used in the present study (≤150 μg·kg⁻¹·min⁻¹) (49, 65). To our knowledge, death during regional ischemia with Adeno infusion has not been reported before in the rat, dog, pig, or human.

Similarly, when Lido was infused at 608 μg·kg⁻¹·min⁻¹, the incidence of VT and VF was not decreased, yet the durations of these arrhythmias were reduced during ischemia compared with saline controls (Figs. 2, C and D). Although the arrhythmias did not lead to cardiac death, there was a surprisingly high number of episodes of PVBs, runs of bigeminy, and VT and VF during ischemia (Figs. 2, A–D). Interestingly, Lido treatment led to the highest incidence of PVBs (104 ± 21 episodes). The routine use of Lido to treat acute ventricular arrhythmias has come under scrutiny in recent years for two main reasons: a number of studies have shown that Lido increases the electrical defibrillation threshold in a concentration-dependent manner by slowing conduction and generating reentry circuits (3), and others have implicated the drug in fatal bradyarrhythmias in patients with acute myocardial infarction (20, 40). Under some circumstances, Lido causes arrhythmias that compete with its antiarrhythmic effect mostly in the ischemic zone (3). Despite its potential drawbacks, Lido is still used in the surgical setting to treat ventricular tachyarrhythmias in patients with acute myocardial ischemia when they interfere with hemodynamic status. Our data also suggest that a combination of Adeno and Lido may ameliorate some of the untoward effects of Lido alone in the clinical setting.

Reperfusion arrhythmias. Compared with ischemia-induced arrhythmias, reperfusion arrhythmias were minimal in our study, although significant differences were found between treatment groups. Rats receiving Adeno only or Lido only experienced VT or VF similar to saline controls upon reperfusion, whereas no animal receiving any dose of AL experienced reperfusion-induced arrhythmias. It is possible that protection afforded by AL solution against reperfusion-induced arrhythmias may have occurred via attenuation of reactive oxygen species at the onset of reperfusion (15, 50, 60).

Infarct size reduction. Consistent with virtually no severe arrhythmias in AL-treated rats, the infarct size was also significantly lower (38 ± 6%) in mid-dose AL animals than saline control (61 ± 5%), Adeno-only (56 ± 5%), and Lido-only animals (66 ± 8%) (Fig. 3). Interestingly, varying doses of Adeno with Lido did not change infarct size significantly, although infarct size tended to be higher in high-dose AL-treated rats (45 ± 6%). This may suggest a dose-dependent AL infarct size reduction but more studies are required. Again, it was surprising that Adeno infusion alone did not reduce infarct size or HR compared with control values (Figs. 3 and 4), and this may have related to the dosages employed in our study. Lido is also known to reduce infarct size (36, 57, 58), but infarct size in our study was not significantly different from controls or from Adeno alone. Our data show that for either Adeno or Lido to have a profound cardioprotective effect, they had to be coadministered and constantly infused before and during ischemia. Infusion of both drugs together before the induction of ischemia may also have had a preconditioning-like effect on the myocardium and possibly the coronary vasculature, which may further result in a reduction in the inflammatory response and other reperfusion-related injuries. Further work using both in vitro and in vivo models of regional ischemia is required to test these hypotheses.

Although the early work of Homeister et al. (21) showed that the sequential and separate administration of Lido before and during ischemia and Adeno at reperfusion in dogs reduced infarct size (48 ± 7% in controls to 21 ± 6% in the AL-treated group), very little data were presented on the incidence of arrhythmias during ischemia or reperfusion. Therefore, the different study design and drug delivery regimes of Homeister and colleagues precludes meaningful comparisons with our data. It should also be noted that Vander Heide and Reimer (59) could not repeat Homeister et al.’s (21) findings in the dog model, and much controversy remains. In the present study, Lido was not administered as a bolus but infused continuously in the presence of Adeno before and during ischemia, and this combination appears to be key to the cardioprotective and survival benefit of AL.

In conclusion, clinical management of arrhythmias appears limited today by a lack of selective and safe antiarrhythmic drugs (23, 24). In the present study, we report that intravenously administering a solution of AL before and during ischemia in the in vivo rat model led to exceptional protection from cardiac death, suppression of severe arrhythmias, and reduced tissue necrosis. The antiarrhythmic and other cardioactive properties of AL solution during ischemia and reperfusion may involve opening A1 receptor-linked KATP channels and modulating the voltage-dependent Na⁺ fast channels and improved intracellular Na⁺ and Ca²⁺ handling. The new AL combinational therapy may have clinical relevance by reducing abnormal regional electrical heterogeneity and, therefore, life-threatening arrhythmias in the ischemic heart.

Limitations of the study. Although we show remarkable survival benefit of coadministering AL in the rat model, we are aware that attempts to develop better antiarrhythmic drug therapies are hampered by understanding of the underlying electrical and molecular mechanisms of arrhythmogenesis and translation from experimentally induced ischemia in animal models to human patients with multiple underlying pathologies (26). One possible limitation of AL therapy may be the effect on hemodynamics. This would be less of a problem in the surgical setting or during percutaneous transluminal coronary interventions (balloon and stent) where hemodynamic changes could be avoided by using intracoronary infusions (67). In humans, intracoronary infusions of Adeno up to 240 μg/min causes a minimal decrease in systemic arterial pressure, HR or ECG variables (67). Other considerations using animal models and their applicability to humans include differences in the scaling of mass-specific metabolic rates (11), differences in timing of ischemia-induced arrhythmias and electrophysiological properties (48), and differences in functional morphology such as in the collateral circulation (26, 55). Despite species differences, knowledge obtained from antiarrhythmic drug studies in mouse, rat, guinea pig, or rabbit models, and their underlying electrophysiological and metabolic mechanisms of action, has been instrumental in devising new diagnostic and
ADENOSINE AND LIDOCAINE PROTECT AGAINST VENTRICULAR ARHYTHMIAS DURING ISCHEMIA

therapeutic strategies in the treatment of arrhythmias and sudden death in humans.

ACKNOWLEDGMENTS

The authors thank Prof. Andre Kleber, Department of Physiology, University of Bern, Bern, Switzerland, for valuable comments on the interpretation of the data.

GRANTS

This study was supported in part by Australian Heart Foundation Grant G 00B0547 (to G. P. Dobson).

REFERENCES