Propranolol and Lidocaine Inhibit Neural Norepinephrine Release in Hearts With Increased Extracellular Potassium and Ischemia

Xiao-Jun Du, PhD; Rudolph A. Riemsma, PhD; Keith A.A. Fox, MD; Anthony M. Dart, MRCP, PhD

Background. Propranolol and lidocaine are effective antiarrhythmic drugs in myocardial ischemia and infarction. As sympathetic nerve activation and norepinephrine release in ischemic hearts are arrhythmogenic, we tested the possibility that both agents inhibit neural norepinephrine release following sympathetic activation in the ischemic environment.

Methods and Results. The model used was an in situ perfused innervated rat heart. Norepinephrine release was induced by electrical stimulation of the left cervicothoracic stellate ganglion and analyzed using radioenzymatic assay or high-performance liquid chromatography. In normoxically perfused hearts, evoked norepinephrine release was not affected by either of the two agents at doses of 1 to 10 μmol/L but dose-dependently reduced at 10 μmol/L.

K+ (D,L-propranolol: −53±4% at 1 μmol/L and −64±6% at 10 μmol/L; lidocaine: −37±11% at 0.1 μmol/L, −67±5% at 1 μmol/L, and −75±6% at 10 μmol/L). At 10 μmol/L K+, norepinephrine release was not affected by timolol or atenolol (both 10 μmol/L but was equally inhibited by D- or L-propranolol at 10 μmol/L (−56±5% and −53±9%, respectively), indicating a β-blocking-independent mechanism. In hearts with metabolic acidosis (pH 6.85) at K+ of 4 mmol/L, neural norepinephrine release was also reduced by propranolol at 10 μmol/L (−37%). Finally, in hearts perfused with 4 mmol/L K+ and subjected to 6-minute periods of ischemia, neural norepinephrine release was similarly suppressed by D,L-propranolol (−38±6% at 0.1 μmol/L, −44±5% at 1 μmol/L, and −78±3% at 10 μmol/L) or lidocaine (−39±7% at 0.1 μmol/L, −58±9% at 1 μmol/L, and −91±3% at 10 μmol/L).

Conclusions. These data indicate that propranolol and lidocaine inhibit neural norepinephrine release via a Na+ channel-blocking mechanism that is synergistic with changes induced by ischemia, primarily raised extracellular K+. This mechanism may contribute to the anti-ischemic and antiarrhythmic properties of both agents in acute myocardial ischemia, which induces increased extracellular K+ and sympathetic activation. (Circulation. 1995;88[part 1]:1885-1892.)

Key Words • potassium • ischemia • nervous system • sodium • β-adrenergic receptors • membranes

The antiarrhythmic and anti-ischemic effects of propranolol in acute myocardial ischemia and infarction have been well established.1-4 In the setting of ischemia, sympathetic activation with norepinephrine (NE) release in the ischemic myocardium is considered of importance in mediating ventricular arrhythmias.5 The protective effects of β-adrenergic antagonists are generally attributed to blockade of β-adrenoceptors on myocytes. Whether the antiarrhythmic effect may be partly due to inhibition of neural norepinephrine release in the ischemic myocardium is unknown. Although it is well known that activation of presynaptic β-adrenoceptors facilitates norepinephrine release, studies examining the effect of β-antagonists on norepinephrine release yield conflicting results.5 In addition, some β-antagonists, like propranolol, possess a nonspecific membrane stabilizing activity, including the blockade of voltage-gated Na+ channels.6 This activity, however, is generally considered of minor importance, as it requires concentrations far above those achieved clinically.

Acute myocardial ischemia induces regional hyperkalemia and acidosis within minutes of interruption of coronary flow.8-9 Elevated extracellular K+ concentration ([K+]o) is known to interfere with the conduction of the action potential in Purkinje fibers or myocytes10-13 and perhaps also in adrenergic nerves.14 Interestingly, studies in vitro have revealed a potentiation, by a raised [K+]o, or by acidosis, of inhibitory effects of lidocaine on Na+ channels of myocardium.10-12,15,16 As depolarization of the neural plasmalemma by Na+ influx via voltage-gated Na+ channels is necessary for action potential propagation and subsequent norepinephrine release,17 an inhibition of Na+ channels of the neural membrane in ischemia may suppress norepinephrine release and hence the intensity of the subsequent adrenergic stimulation to the ischemic myocardium. This possibility, however, has never been tested.
Using a perfused, innervated rat heart model, we have therefore studied the effects of propranolol and lidocaine on neural norepinephrine release during simulated ischemia (ie, increased [K⁺], and acidosis) and stop-flow ischemia. We chose lidocaine as a reference agent for propranolol because it is a well-defined Na⁺ channel blocker with effects known to be enhanced by increased [K⁺], or by acidosis and also because it is an effective agent for the acute treatment of ischemic arrhythmias.

Methods

Preparation

Male Sprague-Dawley rats (280 to 350 g) were used for this study. Experiments were carried out using a perfused, innervated rat heart model previously described in detail. Rats were anesthetized with pentobarbital (60 mg/kg IP) and heparinized (200 U per rat IV). The chest was opened and a metal cannula inserted into the ascending aorta to start coronary perfusion in situ. The perfusate was a modified Krebs-Henseleit solution containing (in mmol/L) Na⁺ 145, K⁺ 4.0, Ca²⁺ 1.85, Mg²⁺ 1.05, HCO₃⁻ 25, PO₄³⁻ 0.5, glucose 11, and EDTA 0.027. The buffer was continuously gassed with 95% O₂-5% CO₂ (pH 7.4) and warmed to 37°C. Perfusion flow rates were controlled by a peristaltic pump and set at 5 mL·g⁻¹·min⁻¹ by the estimated heart weight.

The left cervicothoracic stellate ganglion, with the cardiac nerves attached, was separated and mounted on a pair of bipolar electrodes for subsequent electrical stimulation using a model S88 or a model SD9 stimulator (Grass Instrument Co, Quincy, Mass). The nerves were continuously superfused with warm and oxygenated buffer except when stimulated. Stimuli had a pulse width of 2 milliseconds, a current of 0.8 mA, and a frequency of 5 Hz.

After ligation of bilateral pulmonary vessels and the superior vena cava, a catheter was inserted via the inferior vena cava into the right atrium for the collection of coronary venous effluent. The recovery of coronary effluent was between 85% and 100%.

The left ventricle was cannulated via the apex, and the ventricular pressure was derived from a pressure transducer (Elcomatic, Glasgow, UK) or a microtip catheter pressure transducer (model SPR-249, Millar Instruments Inc, Houston, Tex) and was recorded on a TA2000 recorder (Gould Inc, Cleveland, Ohio) or a model 7 polygraph (Grass Instrument). Heart rate was measured from ventricular pressure traces.

Alteration of K⁺ levels in the perfusate was achieved by infusion, via a model 22 or a model 901A pump (Harvard Apparatus, South Natick, Mass), of KCl solution, and final K⁺ concentrations achieved were ascertained by measuring K⁺ levels in the perfusate and in the venous effluent collected in the absence of nerve stimulation using a model 501 Na/K analyzer (Instrumentation Laboratory, Milan, Italy). Global heart ischemia was induced by stopping of perfusion, and the myocardial temperature was kept at 37°C by covering hearts with a thermostatic chamber. Coronary effluent was collected during the first 2 minutes of the restoration of coronary flow to the preischemic level.

Norepinephrine Assay

Two norepinephrine analysis methods—radioenzymatic assay and high-performance liquid chromatography (HPLC) with electrochemical detection—were used in this study. Samples collected from one experiment were always assayed with the same method and, whenever possible, in a single assay run. In experiments carried out in Edinburgh, effluent samples were immediately cooled on ice and mixed with an equal volume of perchloric acid (final concentration, 0.3N) and stored at −40°C until assayed. Concentrations of norepinephrine were analyzed radioenzymatically in duplicate, and the average of the two measurements was used. The intra-assay coefficient of variation at 2 pmol/mL was 7%. For those experiments carried out in Melbourne, effluent samples were immediately frozen on dry ice and stored at −70°C until analyzed using an HPLC method. Norepinephrine was extracted using alumina adsorption, separated by HPLC, and quantified by electrochemical detection. The intra-assay coefficient of variation was 3%.

Drugs Used

Desipramine, atenolol, timolol, d,l-propranolol, d-propranolol, l-propranolol (all from Sigma Chemical Co, St Louis, Mo), butoxamine (provided by Dr A. Ungar, Department of Pharmacology, University of Edinburgh), and lidocaine chloride (Delta West Ltd, Bentley, Australia) were used.

Protocols

A 20-minute period of perfusion with normal perfusate was allowed to stabilize preparations before the experiment. All experiments were carried out in the presence of desipramine (final concentration of 0.1 μmol/L) to inhibit neural norepinephrine reuptake. Sympathetic ganglion stimulation was performed at 5 Hz for periods of 30 or 60 seconds with 15-minute intervals between stimuli. The first stimulus (S₁) served as a reference for each experiment. Drugs or KCl were infused into the heart at least 10 minutes before the subsequent stimulation. Coronary effluent was collected for a period of 2 minutes immediately before or during and after nerve stimulation. In two groups of hearts (n=8 each) perfused with either 4 or 10 mmol/L K⁺, the reproducibility of norepinephrine release in response to five episodes of nerve stimuli (30 seconds) was examined.

Propranolol and norepinephrine release. The effect of propranolol and other β-antagonists on norepinephrine release was examined in seven separate groups of hearts perfused with various perfusate K⁺ concentrations (Table 1). Hearts in group 1 were initially perfused with 4 mmol/L K⁺. After a control nerve stimulus (S₁, 30 seconds), d,l-propranolol was infused into the heart at 1 μmol/L throughout the subsequent experiment, and another four stimuli (30 seconds each) were performed at K⁺ concentrations of 4 (S₂), 7 (S₃), 10 (S₄), and 13 mmol/L (S₅), respectively. In group 2, hearts were perfused with 10 mmol/L K⁺, and four nerve stimuli (30 seconds each) were given in the absence (S₁) and presence of d,l-propranolol at 0.1 (S₂), 1 (S₃), and 10 μmol/L (S₅), respectively.
The effect of D- and L-isomers of propranolol on norepinephrine release was tested in groups 3 and 4 with perfusate K+ of 10 mmol/L. The first control stimulus (S1, 30 seconds) was followed by another two stimuli (30 seconds each) in the presence of D- or L-propranolol (10 μmol/L, respectively, S2) or a combination of D- and L-propranolol (5 μmol/L each, S3).

In groups 5 and 6, hearts were perfused with 10 mmol/L K+, and three stimuli (30 seconds each) were performed in the absence (S1) and presence (S3 and S4) of β-antagonists. The agents presented at S3 and S4 were atenolol (10 μmol/L, S3) and timolol (10 μmol/L, S4) for group 5 and butoxamine (10 μmol/L, S3) and D,L-propranolol (10 μmol/L, S3) for group 6. In comparison, hearts in group 7 were perfused with 4 mmol/L K+, and sympathetic ganglion was stimulated twice (30-second duration each) without (S1) and with (S3) D,L-propranol at 10 μmol/L.

The protocols for this series of experiments are also summarized in Table 1 for clarity.

**Table 1. Protocols for Experiments Examining the Effect of Propranolol and Other β-Antagonists on Neural Norepinephrine Release**

<table>
<thead>
<tr>
<th>Group</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=7)</td>
<td>4/−</td>
<td>4/1 (D,L-propranolol)</td>
<td>7/1 (D,L-propranolol)</td>
<td>10/1 (D,L-propranolol)</td>
<td>13/1 (D,L-propranolol)</td>
</tr>
<tr>
<td>2 (n=8)</td>
<td>10/−</td>
<td>10/0.1 (D,L-propranolol)</td>
<td>10/1 (D,L-propranolol)</td>
<td>10/10 (D,L-propranolol)</td>
<td>...</td>
</tr>
<tr>
<td>3 (n=8)</td>
<td>10/−</td>
<td>10/10 (L-propranolol)</td>
<td>10/5+5 (L-propranolol)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>4 (n=8)</td>
<td>10/−</td>
<td>10/10 (D-propranolol)</td>
<td>10/5+5 (D-propranolol)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>5 (n=7)</td>
<td>10/−</td>
<td>10/10 (atenolol)</td>
<td>10/10 (timolol)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6 (n=8)</td>
<td>10/−</td>
<td>10/10 (butoxamine)</td>
<td>10/10 (D,L-propranolol)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>7 (n=7)</td>
<td>4/−</td>
<td>4/10 (D,L-propranolol)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Values in the table denote concentrations of perfusate K+ in mmol/L and agents tested in μmol/L (mmol/L/μmol/L).

Propranolol, lidocaine, and norepinephrine release in ischemia. Effects of D,L-propranolol and lidocaine on norepinephrine release in ischemic hearts were tested. Five groups of hearts (n=7 to 10) were perfused with 4 mmol/L K+ and subjected to three periods of 6-minute total ischemia separated by 15-minute intervals of perfusion at 5 mL·g⁻¹·min⁻¹. Nerve stimulation (5 Hz, 1 minute) were performed in the final minute of ischemic periods. Group 1 served as control and received no drug treatment. In the remaining four groups, propranolol (two groups) or lidocaine (two groups) were infused 10 minutes before the second and third periods of ischemia (0.01 and 0.1 μmol/L for one group and 1 and 10 μmol/L for one group, respectively). Previous studies have shown that there is no ischemia-induced norepinephrine release by periods of total ischemia less than 10 minutes.²³

**Statistical Analysis**

Results are expressed as mean±SEM. Whenever possible, each animal served as its own control to eliminate between-animal variation in quantitative norepinephrine release and to improve statistical power. Therefore, norepinephrine data are presented both in absolutes and in percentage of individual values measured before and after an intervention. Differences were tested for statistical significance by one- or two-way ANOVA, followed by paired- or unpaired Student’s t test. Bonferroni’s correction was performed for comparison of repetitive measurements between groups. P<.05 was considered significant.

**Results**

Basal norepinephrine release was always low (0 to 1.3 pmol·g⁻¹·min⁻¹) and not influenced by perfusion with increased K+ concentrations (4 mmol/L: 0.7±0.2 pmol·g⁻¹·min⁻¹, 10 mmol/L: 1.0±0.1 pmol·g⁻¹·min⁻¹, P=NS). In control hearts, quantities of norepinephrine release evoked by S5/S6 were not significantly different within or between groups (P=NS by ANOVA or paired t test: 4 mmol/L K+, 34.0±3.9, 36.2±4.1, 33.8±4.6, 34.4±5.8, and 33.5±5.5 pmol/g, respectively; 10 mmol/L K+, 37.1±5.0, 39.9±6.2, 34.6±5.8, 33.1±4.8, and 31.9±4.3 pmol/g, respectively). The percentages of S3, S5, and S6 (individual control) were 108±4%, 99±7%, 97±10%, and 95±8%, respectively, for the group with 4 mmol/L K+ and 107±7%, 91±6%, 89±5%, and 86±6%, respectively, for...
the group with 10 μmol/L K* (P=NS for within- and between-group comparisons).

**Propranolol and Norepinephrine Release at Increased K***

D,L-Propranolol at 1 μmol/L failed to modify norepinephrine release induced by nerve stimulation during perfusion with 4 mmol/L K* but showed a progressively enhanced inhibition of such release with increasing perfusate K* from 7 to 13 mmol/L (F=6.91, P<.01 by ANOVA, 7 mmol/L: −44±11%, 10 mmol/L: −50±7%, and 13 mmol/L: −59±9%, all P<.01 by paired t test versus S1, Fig 1). At 10 mmol/L perfusate K*, inhibition of norepinephrine release by propranolol was dose dependent (F=25.8, P<.001; 0.1 μmol/L: −7.7±10.3%, P=NS; 1 μmol/L: −53.5±4.0%, P<.01; and 10 μmol/L: −63.7±6.0%, P<.001, Fig 1).

At 4 mmol/L K*, D,L-propranolol of 10 μmol/L was ineffective in modifying norepinephrine release with an S2/S1 ratio of 110±8%. With 10 mmol/L K*, norepinephrine release was not influenced by atenolol or timolol at 10 μmol/L but was inhibited moderately by 10 μmol/L butoxamine (−32±9%, P<.05) and markedly by 10 μmol/L D,L-propranolol (−69±5%, P<.001, Fig 2). Administration of D- or L-isomers of propranolol at 10 μmol/L reduced basal heart rate to a similar extent to that observed by the combination of the two isomers (−55±5% versus −59±8% and −53±9% versus −59±8%, both P=NS, Fig 2). There was a good correlation between the reduction of norepinephrine release produced by either of the two isomers and that by simultaneous infusion of both isomers (r=.80, P<.01, n=16).

**Lidocaine and Norepinephrine Release at 4 and 10 mmol/L K***

In hearts perfused with 4 or 10 mmol/L K*, basal norepinephrine overflow remained low and not affected by lidocaine even at 100 μmol/L (0.68±0.22 versus 1.01±0.11 pmol·g⁻¹·min⁻¹, P=NS). There was no significant difference in norepinephrine release by S1 in groups with 4 or 10 mmol/L K* (49.3±6.7 versus 41.2±9.5 pmol/g). With 4 mmol/L K*, norepinephrine release in response to nerve stimulation was not significantly affected by lidocaine until the concentration was 10 μmol/L or above (P<.02, Fig 3). In contrast, in hearts with 10 mmol/L K*, there was a dose-dependent suppression of norepinephrine release by lidocaine that was statistically significant starting at 0.1 μmol/L (F=8.54, P<.001 by two-way ANOVA for overall difference at a dose of 0.1 to 100 μmol/L, Fig 3). The average dose required to suppress norepinephrine release by 50% of control (IC₅₀) was about 70 μmol/L with 4 mmol/L K* and 0.5 μmol/L with 10 mmol/L K*.

**Effects of Propranolol and Lidocaine on Basal Heart Rate at 4 and 10 mmol/L K***

Basal heart rate remained stable in hearts perfused with 4 or 10 mmol/L K* (244±6 and 232±9 beats per minute, P=NS, combined data from 20 and 47 hearts, respectively). Both D,L-propranolol and lidocaine reduced basal heart rate, in a concentration-dependent manner, with 4 or 10 mmol/L K*. Interestingly, for both agents, the dose-response curves of heart rate reduction were similarly shifted leftward by an increased perfusate K* concentration (Fig 4). At 10 mmol/L K*, D- and L-isomers of propranolol at 10 μmol/L reduced basal heart rate to a similar extent to that produced by 10 μmol/L D,L-propranolol (−162±6 and −151±8 versus −165±6 beats per minute, P=NS).

**Propranolol, Lidocaine, and Norepinephrine Release: Effect of Metabolic Acidosis**

Norepinephrine release evoked by the first stimulus (S₁) at normal pH was similar in all three groups. In the
control group, norepinephrine release was not affected by 10-minute perfusion (104±7% of S1, P=NS) but was reduced by 25-minute perfusion with the acidic buffer (79±8% of S1, P<.05, Fig 5). In groups receiving drug treatment, D,L-propranolol or lidocaine at 1 μmol/L did not reduce norepinephrine release after 10-minute acidic perfusion (101±6% or 93±5% of S1, respectively, all P=NS). A 25-minute acidic perfusion together with propranolol of 10 μmol/L suppressed norepinephrine release to 43±7% of S1 (P<.01), a value significantly lower than the control group without drug treatment (P<.01). In the presence of lidocaine at 10 μmol/L, norepinephrine release by S1 tended to be lower than the control group (56±10%, P=.09), but this was statistically insignificant. Acidic perfusion profoundly suppressed the systolic ventricular pressure during basal and nerve stimulation (data not shown).

Propranolol, Lidocaine, and Norepinephrine Release in Myocardial Ischemia

In hearts preperfused with 4 mmol/L K+ and subsequently undergoing three periods of 6-minute total ischemia, norepinephrine release evoked by nerve stimulation was reproducible in the control group (Table 2). Treatment with D,L-propranolol or lidocaine before ischemia resulted in a significant and dose-dependent inhibition of norepinephrine release starting at a concentration of 0.1 μmol/L versus S1 without drug (Table 2 and Fig 6). In the control group, each nerve stimulation (S1, S2, S3) during ischemia induced a significant increase in heart rate (+32±9, +32±9, and +36±10 beats per minute, respectively). This chronotropic response to nerve stimulation in the ischemic heart was partly or totally inhibited by lidocaine and propranolol (data not shown).

Discussion

The present study demonstrates a dose-dependent inhibition of neural norepinephrine release by propranolol only at an increased [K+]t, and, to a lesser extent, at a reduced extracellular pH. Lidocaine, a specific Na+ channel blocker, shows a very similar inhibitory effect on norepinephrine release with a marked potentiation.
TABLE 2. Effects of D,L-Propranolol (0.01 to 10 μmol/L) and Lidocaine (0.01 to 10 μmol/L) on the Neural Norepinephrine Release (pmol/g) in Hearts Perfused With 4 mmol/L K+ and Subjected to 6-Minute Periods of Total Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=7)</td>
<td>None</td>
<td>33.0±5.2</td>
<td>27.7±4.3</td>
<td>30.2±4.5</td>
</tr>
<tr>
<td>2 (n=9)</td>
<td>Propranolol</td>
<td>43.8±3.1</td>
<td>38.3±4.0</td>
<td>27.3±3.6*</td>
</tr>
<tr>
<td>3 (n=10)</td>
<td>Propranolol</td>
<td>31.0±4.3</td>
<td>12.7±2.9*</td>
<td>5.8±0.6*</td>
</tr>
<tr>
<td>4 (n=9)</td>
<td>Lidocaine</td>
<td>45.3±5.7</td>
<td>33.7±5.7</td>
<td>28.1±5.2*</td>
</tr>
<tr>
<td>5 (n=8)</td>
<td>Lidocaine</td>
<td>46.4±6.8</td>
<td>17.4±4.6*</td>
<td>3.1±0.4*</td>
</tr>
</tbody>
</table>

Note: (S1,S2) were applied in the last minute of ischemic periods. S1 served as a reference without drug treatment.

*P<.001 vs S1, in the same group by paired t test.

by raised [K+]o. Finally, norepinephrine release in ischemic hearts is dose dependently suppressed by both agents.

**Mechanism of the Inhibition of Norepinephrine Release by Propranolol or Lidocaine at High [K+]o.**

Several mechanisms could account for the inhibition of neural norepinephrine release by propranolol. As this inhibition is observed only at raised [K+]o of 7 to 13 mmol/L, an inhibition by high K+ of norepinephrine release is possible. Although in some models hyperkalemia alone can inhibit evoked norepinephrine overflow and postsynaptic responses,14,24,25 previous studies and the present study with this model show no effect on norepinephrine release by increasing perfusate K+ up to 13 mmol/L.19 Blockade of facilitatory presynaptic β-adrenoceptors could lead to a reduced norepinephrine release.6 In this model with a normal K+, however, propranolol fails to suppress norepinephrine release according to our previous and the present studies.18 Further evidence against such a possibility comes from the findings that the inhibitory effect of propranolol is not shared by timolol, a potent nonselective β-blocker, and that the two isomers of propranolol, with and without β-blocking activity, are equally effective in the suppression of norepinephrine release at an increased [K+]o. Finally, the inhibition of norepinephrine release by lidocaine is also potentiated by a raised [K+]o, and this again does not indicate a presynaptic β-adrenergic mechanism. A mild inhibition of norepinephrine release was observed by butoxamine, a relatively selective β2-antagonist, at 10 mmol/L K+. This is probably due to effects other than blockade of presynaptic β2-adrenoceptors, and the properties of butoxamine are still only partly understood.

Propranolol also possesses membrane stabilizing activity, including the inhibition of Ca2+ and Na+ channels.7 Theoretically, blockade of either channel (N-type Ca2+ channels for neuronal tissues) could lead to an inhibited neurotransmission, as action potential propagation along the axons is mediated by Na+ influx and norepinephrine exocytosis is the result of Ca2+ influx at the nerve varicosities. The dependency of the effect of propranolol on [K+]o suggests that Ca2+ channels are not involved as they function at near-zero membrane potential and therefore should not be sensitive to increased [K+]o to the levels studied. In contrast, the functional state of Na+ channels depends on the resting membrane potential, which is in turn determined by the K+ gradient across the membrane.9 The similar effect of lidocaine on both norepinephrine release and heart rate at increased [K+]o provides further support for a Na+ channel–dependent mechanism. In our study, propranolol is also synergistic with an increased [K+]o, in the suppression of the basal heart rate. Sinus automaticity is determined by the rate of spontaneous depolarization of the resting membrane potential,26 and the main ionic currents involved in this pacemaking are a background inward Na+ current (I_{Na}) and a diminishing outward K+ current (I_{K}).26 Thus, an inhibition of the Na+ inward current may suppress the automaticity of the sinoatrial node.

**Fig 6. Bar graph of inhibition of neural norepinephrine (NE) release by concentrations of D,L-propranolol and lidocaine in the ischemic heart.** Data have been presented as percentages of the NE overflow by a control nerve stimulation in the absence of drug treatment (S1=100%) calculated from the absolutes presented in Table 2. In the control group (n=7), there was no significant difference in the amount of NE released by S2 and S3 compared with that by S1 (92.8±7.0% and 102.3±13.7% of S1, respectively). Note the dose-dependent inhibition of NE release by both agents in the ischemic conditions. *P<.001 versus individual control values released by S1 in the same group (paired t test).
The mechanism for the inhibition of norepinephrine release with raised [K+]o, may relate to changes in the functional state of Na+ channels with a resultant change in the affinity of the channel for the two drugs. Na+ channels exist in three functional states: resting (R), open (O), and inactivated (I).27-30 Blockade of Na+ channels by drugs is more likely when the channel is in I form, perhaps due to an increased affinity and accessibility of agents to channel-binding sites.27-30 According to the K+ equilibration potential EK, an increase in [K+]o from 4 to 10 mmol/L may lead to a 20-mV depolarization of the resting membrane potential,9 and this could result in an inactivation of about 90% of Na+ channels. There is evidence that lidocaine binds mainly to Na+ channels in I form, thereby blocking channels and inhibiting the transition of functional states from I to R. In addition, after binding with lidocaine, channel reactivation requires a much more negative membrane potential.27-30 This is largely prevented by a high [K+]o-induced partial depolarization, leading to a progressively increased channel blockade. Studies have indeed shown that an increased [K+]o enhances the depressant effects of lidocaine and other class I antiarrhythmic agents on the electrophysiology of myocytes.10-12,28 Thus, we speculate that a synergistic inhibition of Na+ channels by propranolol or lidocaine and an increased [K+]o is the ionic mechanism for the inhibition of norepinephrine release observed. Further studies are required to confirm this mechanism in neuronal preparations.

**Contribution of Metabolic Acidosis**

Reduction of external pH to 6.85 for 25 minutes had only a minor effect on neural norepinephrine release, in keeping with our previous observation.31 At the reduced pH, propranolol at 10 μmol/L reduced norepinephrine release by 37%, although no such synergism was observed at 1 μmol/L. The same tendency was also found for lidocaine. This could be due to both higher drug concentration and development of intracellular acidosis, via an enhanced H+/K+ and inhibited H+/Na+ exchange,9,32 following longer acid diffusion. Potentiation by acidosis of the electrophysiological effects of lidocaine has been previously demonstrated.15,16,28 Reduction in pH reduces Vma and the resting membrane potential, which is due to an enhanced K+ efflux via H+/K+ exchange and a suppressed Na+/K+, ATPase.9,11,15,16,28 Following reduction in the resting membrane potential, the fraction of inactivated Na+ channels may increase and hence the blockade of the Na+ channel by drugs.11,28 In addition, acidosis lengthens the action potential duration, which may increase the time constant of recovery for the blocked and inactivated channels.11,28 The present study provides data showing that the synergism of acidosis and Na+ channel blocker, observed previously in Purkinje fibers or myocytes, also pertains to the presynaptic adrenergic nerves.

**Inhibition of Neural Norepinephrine Release in Ischemic Heart**

Acute ischemia rapidly leads to an increase in [K+]o and acidosis,8,32 and propranolol has no effect on the rise in [K+]o.8,33 Our results indicate that propranolol and lidocaine, while having little effect on norepinephrine release under physiological conditions, show a potent and dose-dependent inhibition of neural norepinephrine release in the ischemic heart with a threshold effective dose of 0.1 μmol/L. The extent of the inhibition of norepinephrine release by either propranolol or lidocaine at 10 μmol/L is more pronounced in ischemia (−88.5% and −90%, respectively) than during normoxic perfusion with 10 mmol/L K+ (−59−−69% for propranolol and −75% for lidocaine), suggesting that additional factors in ischemia, such as acidosis and hypoxia,11,15,16,28,34 may also contribute to the observed drug effect. Our experiments with acidosis support this view. However, a raised [K+]o, is by far the most important component.

Interestingly, our results are in keeping with the findings from a study in in vivo rats with coronary artery occlusion.35 In that study, the myocardial norepinephrine content was unchanged, but a marked reduction in the density of fluorescing adrenergic fibers was found in the ischemic area after 60 minutes of local ischemia, indicating a local release and accumulation of norepinephrine in the ischemic zone. These changes could be partly or completely prevented by pretreatment with different doses of lidocaine.35 Our study provides direct evidence for the mechanism of this inhibited norepinephrine release in vivo.

**Clinical Implications**

Propranolol and lidocaine are effective in the suppression of malignant arrhythmias and cellular damage in the acute phase of myocardial infarction.1-5,20 and adrenergic involvement in these processes has been supported by a large number of studies.5,9,19 However, few of the previous studies have linked these therapeutic effects with norepinephrine release in the ischemic myocardium. The inhibition of neural norepinephrine release demonstrated in this study is likely to contribute to the therapeutic effects of propranolol and lidocaine in myocardial ischemia. This may also be the case for other class I and III antiarrhythmic agents. Our data may also help to explain the clinical findings that the class I antiarrhythmic agents, although particularly effective in suppressing ischemic arrhythmias, are much less effective in the attenuation of ventricular arrhythmias induced when exogenous catecholamines are given.36-38 This difference in efficacy of antiarrhythmic activity may indicate that suppression of endogenous norepinephrine release is an additional mechanism for the potent antiarrhythmic property of class I agents in ischemia. In addition, although clinical studies have shown that β-blockers without membrane stabilizing activity reduce sudden cardiac death in patients after acute myocardial infarction,3 it is not known if they are as effective as those with such activity in the inhibition of ventricular arrhythmias during acute myocardial ischemia.

The generally accepted concept that the membrane stabilizing activity of β-antagonists is of little therapeu- tic importance is based primarily on data collected under physiological conditions. By simulating the metabolic changes seen in acute myocardial ischemia, we have shown a suppression of norepinephrine release by propranolol at concentrations found clinically.39-41 The importance of the experimental environment in the assessment of drug effects in cardiovascular tissues has been stressed by previous studies.9-16,28 The present study, concerning neural norepinephrine release during myocardial ischemia, provides another example of this
issue whereby ischemic changes amplify drug effects leading to unexpected pharmacological actions.

Acknowledgments
The Cardiovascular Research Unit is supported by the British Heart Foundation. The part of this project conducted in Baker Medical Research Institute was supported by grants from the Alfred Group of Hospitals, Melbourne, and BP Australia Ltd. The excellent technical assistance of Miss Margaret Millar and Mrs Jean Samuel is greatly acknowledged. We thank Dr A. Unger for his generous supply of butoxamine. We are grateful to Professor James Angus and Professor Murray Esler for their support with laboratory facilities. We also wish to thank Miss Helen Cox and Miss Andrea Turner for their help in the catechol assay.

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Circulation. 1993;88:1885-1892
doi: 10.1161/01.CIR.88.4.1885

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