Enhancement by norepinephrine of automaticity in sheep cardiac Purkinje fibers exposed to hypoxic glucose-free Tyrode’s solution: a role for \(\alpha\)-adrenoceptors?

ALESSANDRO MUGELLI, M.D., SANDRA AMERINI, M.SC., GABRIELLA PIAZZESI, M.SC.,* ELISABETTA CEBBAI, M.SC., AND ALBERTO GIOTTI, M.D.

ABSTRACT  A period of drive in the presence of norepinephrine (NE) may be followed by the induction or acceleration of spontaneous activity. Experiments were carried out in sheep cardiac Purkinje fibers to determine whether the effects of NE on automaticity were modified during superfusion with hypoxic glucose-free Tyrode’s solution and to assess the possible contribution of \(\alpha\)-adrenergic influences on automaticity under these conditions. The following results were obtained: (1) Low concentrations of NE (10\(^{-7}\) and 3 \(\times\) 10\(^{-7}\)M) were able to induce automaticity after a period of drive in normal oxygenated (97% \(\text{O}_2\), 3% \(\text{CO}_2\)) Tyrode’s solution. (2) Superfusion with hypoxic (97% \(\text{N}_2\), 3% \(\text{CO}_2\)) glucose-free Tyrode’s solution enhanced NE-induced automaticity. (3) Practolol, in concentrations able to block the effects of NE in normal oxygenated solution, did not counteract the effects of NE in hypoxic glucose-free solution. (4) Yohimbine, but not prazosin, antagonized the effects of NE in hypoxic glucose-free solution. At the same concentration, yohimbine did not affect transmembrane potentials or automaticity induced by isoproterenol. It is concluded that \(\alpha\)-adrenergic responsiveness appears to be enhanced during superfusion in vitro with hypoxic glucose-free solution, and that \(\alpha\)-adrenoceptors belonging to the \(\alpha_2\)-subtype in sheep cardiac Purkinje fibers might influence abnormal automaticity, possibly through an effect on oscillatory potentials.


ADRENERGIC CONTROL of automaticity is generally considered to be under the influence of \(\beta\)-adrenoceptors. Cardiac \(\alpha\)-adrenoceptor stimulation does not appear, in fact, to directly modify the cardiac rate.\(^1\) In isolated cardiac Purkinje fibers, an \(\alpha\)-adrenergic slowing of spontaneous rate has been reported,\(^2\) even when \(\alpha\)-adrenoceptor stimulation does not appear to modify the pacemaker current\(^4\) that underlies the normal process of diastolic depolarization. Recent evidence, however, suggests that under pathologic conditions, i.e., during myocardial ischemia and reperfusion, \(\alpha\)-adrenergic influences may affect the cardiac rhythm, resulting in arrhythmia.\(^5\) Furthermore, \(\alpha\)-adrenergic blockade is antiarrhythmic and antifibrillatory in ischemic and/or reperfused myocardium.\(^6\)

The antiarrhythmic effect of \(\alpha\)-adrenergic blockade has been attributed to the prevention of the increase in intracellular calcium in reversibly injured cardiac tissue.\(^6\) The possible relationship between calcium overload, electrophysiologic mechanisms, and early ischemic arrhythmias has been recently discussed,\(^6\) but the role played by \(\alpha\)-adrenoceptors in the events leading to the development of the arrhythmias is still unclear.

The present experiments were carried out to study the effects of norepinephrine (NE), which stimulates both \(\alpha\)- and \(\beta\)-adrenoceptors, on postdrive automaticity in isolated cardiac Purkinje fibers superfused with normal or hypoxic glucose-free (HGF) Tyrode’s solution. Such conditions were purposely chosen because they may simulate cardiac ischemia better than the hypoxia alone\(^10\); hypoxia alone, on the other hand, does not exert pronounced effects in Purkinje fibers.\(^11\) The two major aims of this work were (1) to determine if the effect of NE on automaticity was modified during superfusion with HGF solution, and (2) to evaluate the possible contribution of \(\alpha\)-adrenergic influences on
of NE were observed during superfusion with the normal oxygenated Tyrode’s solution (PO$_2$ in the tissue bath 400 to 450 mm Hg, pH 7.3 to 7.4). (2) NE washout was followed by a 30 min period of superfusion with a solution containing no glucose and having a lower PO$_2$ (50 mm Hg) (HGF Tyrode’s solution), the solution being saturated with 97% N$_2$ and 3% CO$_2$ (pH 7.3 to 7.4). (3) The same concentration of NE was then superfused again and the exact protocol used in the control experiments and in the presence of NE in normal Tyrode’s solution was followed. This means that the same drive rate was used under control and experimental conditions, thus excluding any possible direct effect of the stimulating procedure on the observed phenomena.

In the experiments with practolol, we first superfused the preparations with normal Tyrode’s solution containing NE. The same procedure was repeated in the presence of practolol. Then the preparations were superfused with the HGF Tyrode’s solution for 30 min and the effects of NE were studied again in the continued presence of the same concentration of practolol. In the experiments with α-antagonists, the effects of NE in HGF solution were studied both in the absence and in the presence of the α-antagonist. In an additional group of preparations the effects of yohimbine on transmembrane action potential of electrically driven (0.5, 1 and 2 Hz) Purkinje fibres and on automaticity induced by 5 × 10$^{-8}$M isoprotanol were studied.

The stock solution of NE contained ascorbic acid (5.6 mM) and was prepared on the day of the experiment; it was diluted to the desired concentration just before each test. Data from all the experiments in which impalement was not maintained were discarded. All the data are presented as mean ± SEM. Comparisons involving two groups were performed by Student’s paired t test (two-tailed), whereas comparisons involving more than two groups were performed by analysis of variance. The Tukey test was used to compare individual groups.

Drugs used were as follows: norepinephrine bitartrate (Sigma Chemical Co.), practolol (ICI), prazosin (Pfizer Inc.), yohimbine hydrochloride (Sigma), isoprotanol hydrochloride (Sigma).

**Results**

**Effects of NE in normal oxygenated Tyrode’s solution.** In normal Tyrode’s solution ([K$^+$]$_i$ = 4 mM) a period of drive is followed by quiescence; in the presence of NE, the same period of drive may be followed by the induction of spontaneous activity (overdrive excitation); pacing-induced automaticity$^{17}$). The interactions between overdrive excitation and overdrive suppression are complex.$^{18}$ We observed that the induction of automaticity was influenced not only by the cycle length of the previous drive, but also by the concentration of NE. In fact, in the preparations driven at 1 Hz, automaticity was induced in 33% of the preparations by 10$^{-3}$M NE (18 experiments) and in 68.8% by 3 × 10$^{-7}$M NE (16 experiments). Qualitatively similar results were obtained in preparations paced at 2 Hz when the concentration of NE was increased.

**Effect of NE in HGF Tyrode’s solution.** After washout of NE all the preparations were superfused with the HGF solution. The effect caused by 30 min superfusion with such a solution on the action potential characteristics was evaluated in 22 preparations driven at 1

---

**LABORATORY INVESTIGATION—ARRHYTHMIA**

Vol. 73, No. 1, January 1986

---

181
Table 1

Effect of 30 min superfusion with HGF Tyrode’s solution on action potential characteristics

<table>
<thead>
<tr>
<th></th>
<th>AP (mV)</th>
<th>OS (mV)</th>
<th>MDP (mV)</th>
<th>APD$_{60}$ (msec)</th>
<th>APD$_{90}$ (msec)</th>
<th>Vmax (V/sec)</th>
<th>Slope of phase 4 (mV/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 22)</td>
<td>129.6 ± 1.3</td>
<td>40.2 ± 0.8</td>
<td>89.4 ± 0.9</td>
<td>308.0 ± 14.4</td>
<td>349.1 ± 14.7</td>
<td>549.4 ± 47.3</td>
<td>7.2 ± 0.6</td>
</tr>
<tr>
<td>HGF Tyrode’s solution (n = 22)</td>
<td>128.5 ± 1.2</td>
<td>40.7 ± 0.8</td>
<td>88.0 ± 0.9$^b$</td>
<td>303.9 ± 16.0</td>
<td>347.9 ± 16.3</td>
<td>530.6 ± 48.5$^A$</td>
<td>7.7 ± 0.5</td>
</tr>
</tbody>
</table>

Driving rate 1 Hz. Values are the mean ± SEM.

AP = action potential amplitude; OS = overshoot; MDP = maximum diastolic potential; Vmax = maximum rate of upstroke.

$^a$p < .02; $^b$p < .01 vs control.

Hz. As shown in table 1, action potential characteristics underwent only slight and apparently unimportant modifications. Qualitatively similar results were obtained in preparations driven at 2 Hz. Notwithstanding the lack of effect of hypoxia in the absence of glucose on the slope of phase 4 (diastolic depolarization), the addition of NE often caused a marked enhancement in spontaneous discharge. As shown in figure 1, the preparation was, as usual, quiescent after the drive at 1 Hz in the absence of NE (figure 1, A); in the presence of $3 \times 10^{-7}$M NE the same drive was followed by spontaneous action potentials (B). The automaticity is obviously enhanced if the same concentration of NE is repeated after superfusion with the HGF solution (C), and the preparation becomes quiescent as soon as NE is washed out (D). After superfusion with the HGF solution, exposure to $3 \times 10^{-7}$M NE significantly increased the average number of spontaneous action potentials during interruption of stimulation in six of 11 preparations (table 2). This effect was associated with a statistically significant increase in the action potential duration; the maximum diastolic potential was not significantly modified (table 2). In the five preparations in which automaticity was not enhanced by NE after superfusion with the HGF solution, the action potential duration was not affected.

Figure 1. Hypoxia in the absence of glucose enhances NE-induced automaticity. Each panel shows the last six driven action potentials (driving rate 1 Hz) and the electrical activity during the interruption of the stimulation. A, control; B, effect of NE ($3 \times 10^{-7}$M) in normal oxygenated Tyrode’s solution; C, effect of the same concentration of NE during superfusion with HGF solution; D, washout of NE.
TABLE 2
Effect of $3 \times 10^{-7}$M NE on postdrive automaticity, action potential duration, and maximum diastolic potential (MDP)

<table>
<thead>
<tr>
<th></th>
<th>Average No. of spontaneous action potentials</th>
<th>APD$_{\infty}$ (msec)</th>
<th>APD$_{90}$ (msec)</th>
<th>MDP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal solution (n = 6)</td>
<td>10.2 ± 6.2</td>
<td>284.2 ± 37.0</td>
<td>326.0 ± 40.2</td>
<td>89.1 ± 1.2</td>
</tr>
<tr>
<td>HGF solution (n = 6)</td>
<td>22.2 ± 3.5$^a$</td>
<td>324.8 ± 40.9$^b$</td>
<td>372.7 ± 44.8$^b$</td>
<td>88.8 ± 2.0</td>
</tr>
</tbody>
</table>

The number of spontaneous action potentials was measured in the first 60 sec of interruption of the stimulation. Values are the mean ± SEM.

$^a$p < .005; $^b$p < .0001 vs normal solution.

On the whole, NE induced spontaneous action potentials after perfusion with the HGF solution in 31% (9/29) of the preparations that were quiescent after NE in normal oxygenated Tyrode's solution; the average number of spontaneous action potentials was instead increased in 63.8% (23/36) of those preparations in which NE already induced automaticity in normal Tyrode's solution.

Effects of $\alpha$- and $\beta$-adrenergic blockade. Figure 2 shows the effect of $\beta$-adrenergic blockade by practolol on automaticity induced by $10^{-7}$M NE in both normal and HGF solution. It is apparent that this low concentration of NE induced spontaneous discharge in normal Tyrode's solution; oscillatory potentials preceded the spontaneous action potentials. The effect of NE is completely blocked by $10^{-7}$M practolol (figure 2, top). After 30 min of superfusion with the HGF solution in the continued presence of practolol, the same concentration of NE resulted again in induction of automaticity. Washout of NE led to quiescence as usual (figure 2, bottom).

In eight of 10 experiments, $10^{-7}$M practolol either fully blocked or prevented the appearance of automaticity induced by $10^{-7}$M NE in normal Tyrode's solu-
tion. As previously stated, superfusion with the HGF solution enhances NE-induced automaticity. In seven experiments with 10^{-7}M NE (in the absence of practolol), an increase in the average number of spontaneous action potentials from 4.3 ± 2.3 in normal Tyrode’s solution to 10.1 ± 2.4 in HGF Tyrode’s solution (p < .001) was, in fact, observed. In the presence of practolol, the average number of spontaneous action potentials rose from 2.7 ± 1.3 in normal Tyrode’s solution to 8.8 ± 2.4 (p < .005, n = 10) after superfusion with the HGF solution, suggesting that practolol was quite ineffective in antagonizing the enhancement of the NE effects in HGF solution. Again the enhancement of automaticity was associated with a statistically significant increase in action potential duration: APD_{90} increased from 314 ± 35 to 334 ± 33 msec (p < .05) and APD_{99} increased from 359 ± 38 to 381 ± 35 msec (p < .05).

The average number of spontaneous action potentials observed in HGF solution in the presence of practolol was slightly less than in its absence (10.1 ± 2.4 vs 8.8 ± 2.4; NS). This finding seems to rule out any important role for β-adrenoceptors in the observed phenomenon. However, our procedure could have partially masked the importance of the β-adrenoceptor stimulation. We did not study, in the same preparation, the effect of practolol on the enhancement of automaticity due to the HGF Tyrode’s solution since this procedure would have required successive periods of superfusion with the HGF Tyrode’s solution alternated with normoxic superfusion and it has been shown that such a procedure may cause a cumulative effect of hypoxia.19

Prazosin, which selectively blocks α_{1}-adrenoceptors, caused a slight and statistically nonsignificant reduction (from 16.7 ± 7.7 to 13.3 ± 8.5; p > .05, n = 5) in the number of spontaneous action potentials in preparations exposed to NE after superfusion with the HGF solution. Figure 3 illustrates the effects of two different concentrations of prazosin (10^{-7}M and 5 × 10^{-7}M) on automaticity induced by 10^{-7}M NE in the presence of 10^{-7}M practolol during hypoxia in the absence of glucose. It clearly appears that NE-induced automaticity was not affected by prazosin. However, yohimbine, which is claimed to preferentially block α_{2}-adrenoceptors, was able to slow or even to arrest the automaticity induced by NE (10^{-7}M and 3 × 10^{-7}M) during superfusion with the HGF solution. Results of a typical experiment are shown in figure 4. A burst of spontaneous action potentials was induced by NE (3 × 10^{-7}M) during superfusion with the HGF solution. That this activity was due to an oscillatory potential is suggested by several findings: it begins immediately after the last driven action potential, the cycle length progressively increases, and the last two spontaneous action potentials are preceded by oscillatory potentials that do not reach the threshold. Yohimbine (10^{-7}M) clearly reduced the number and rate of spontaneous action potentials; each one was now preceded by an oscillatory potential that progressively decreased in amplitude after addition of yohimbine.

Similar results were obtained in six of eight preparations driven at 1 Hz, with a reduction from 22.3 ± 5.0 to 14.6 ± 4.9 (p < .005) in the average number of spontaneous action potentials. The effects of yohimbine (10^{-7}M) on action potential characteristics are summarized in table 3. No statistically significant variations were detected in any of the measured parameters values. The effective refractory period was also unaffected by 10^{-7}M yohimbine (n = 5).

A typical record showing the effects of yohimbine on action potential configuration and on effective re-

![FIGURE 3. Prazosin does not antagonize NE-induced automaticity. Each panel shows the last driven action potentials (driving rate 1 Hz) and the electrical activity after the interruption of stimulation. The records are taken from a preparation superfused with HGF Tyrode's solution (N_{2} glucose-free) in the presence of 10^{-7}M practolol and exposed to 10^{-7}M NE. The effects of the indicated concentrations of prazosin are shown in the middle and right panels.](image-url)
fractory period is shown in Figure 5. The effects of yohimbine were not influenced by the rate of the previous drive since qualitatively similar results were obtained in a preparation driven at 0.5 (n = 7) or 2 Hz (n = 6). Finally, yohimbine did not reduce the rate of discharge of Purkinje fibers in which automaticity was induced by $5 \times 10^{-4}$M isoproterenol (n = 6), the mean rate being 45.3 ± 2.8 beats/min in the absence and 44.0 ± 1.9 beats/min in the presence of yohimbine.

**Discussion**

The present results show that (1) superfusion with HGF Tyrode's solution enhances the postdrive automaticity induced by NE (10$^{-7}$ and $3 \times 10^{-7}$M) in sheep cardiac Purkinje fibers, (2) practolol, in concentrations able to block the NE-induced automaticity under normal conditions, does not antagonize the NE effects during superfusion with the HGF Tyrode's solution, and (3) yohimbine, but not prazosin, significantly reduces the number of spontaneous action potentials induced by NE in the HGF solution and such an effect is observed with concentrations of yohimbine that do not appear to have direct membrane depressant effects.

α-Adrenergic effects have been documented for a long time in the mammalian heart; however, their role is far from clear (for review, see Benfey'). Low concentrations of adrenergic amines, as used in the present experiments, preferentially stimulate α- rather than β-

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>AP (mV)</th>
<th>OS (mV)</th>
<th>MDP (mV)</th>
<th>APD$_{90}$ (msec)</th>
<th>APD$_{99}$ (msec)</th>
<th>V$_{max}$ (V/sec)</th>
<th>AV (mV)</th>
<th>Slope of phase 4 (mV/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td>128.9 ± 2.5</td>
<td>38.4 ± 1.6</td>
<td>91.0 ± 1.7</td>
<td>257.1 ± 15.8</td>
<td>289.4 ± 17.2</td>
<td>671 ± 53</td>
<td>79.6 ± 1.4</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td>Yohimbine (n = 7)</td>
<td>126.7 ± 2.9</td>
<td>35.5 ± 2.0</td>
<td>90.0 ± 1.9</td>
<td>256.1 ± 19.0</td>
<td>289.4 ± 20.3</td>
<td>643 ± 54</td>
<td>80.8 ± 1.9</td>
<td>4.9 ± 0.4</td>
</tr>
</tbody>
</table>

Driving rate 1 Hz. AV and slope of phase 4 and relative controls were measured in seven preparations driven at 0.5 Hz. AV = activation voltage; other abbreviations are as in table 1.
adrenoceptors, especially in the presence of β-adrenergic blockade. In cardiac Purkinje fibers low concentrations of NE (up to $5 \times 10^{-7}$M) prolong the action potential duration, an effect that is typically considered an α-adrenergic mediated.

We observed that the increase in automaticity caused by low concentrations of NE in preparations superfused with HGF solution was associated with a significant increase in action potential duration, both in the absence and presence of practolol. The role of α-adrenoceptors in these events is also supported by the finding that practolol, in a concentration able to block or to prevent the NE-induced automaticity in normal Tyrode’s solution, did not prevent the NE-induced automaticity after superfusion of the preparation with the HGF solution. Even if our protocol did not allow a full evaluation of the role of β-adrenoceptor stimulation in NE-induced automaticity in HGF solution, it surely permits us to suggest a role for α-adrenoceptors.

The enhancement of the effects of NE on automaticity could be partly due to a change in the responsiveness or number of β-adrenoceptors, the concentrations of which are modified by a variety of pathophysiologic variables, or to an unmasking of a β₂-adrenergic effect in the presence of β₁-adrenoceptor blockade by practolol.

$\beta_2$-Adrenoceptors are present in the human ventricle; they are scarcely distributed in the ventricle of other species and, at present, no data are available for the sheep heart. Even if a possible contribution of $\beta_2$-adrenoceptors cannot be completely excluded, we reasoned that, if part of the observed enhancement by the HGF superfusion was due to α-adrenoceptor stimulation, it could be blocked by specific α-adrenergic-blocking drugs. And, in fact, yohimbine was able to antagonize the enhancement of the NE effects on automaticity in HGF Tyrode’s solution. It is extremely important to observe that, at the same concentration, yohimbine was completely ineffective in reducing the spontaneous rate of discharge of Purkinje fibers in which automaticity had been induced by isoproterenol, that is, by β-adrenoceptor stimulation. On the whole, our results suggest a role for α-adrenoceptors in overdrive excitation, i.e., automaticity induced by drive in the presence of NE, in cardiac Purkinje fibers superfused with HGF Tyrode’s solution. The explanation for these findings is not obvious but several hypothesis can be advanced.

Repetitive stimulation influences the passive and active ionic movements across the cell membrane. An increased rate of stimulation in Purkinje fibers has been reported to increase intracellular sodium and calcium activities and extracellular potassium activity and to activate an outward current due to an electrogenic sodium extrusion. All these events can affect postdrive automaticity. Recent evidence, however, suggests that induction of spontaneous activity by drive in the presence of NE is brought about by an oscillatory potential.

Oscillatory potentials have been recorded in the presence of cardiac steroids but also in their absence. They are due to an oscillatory inward current that has been recorded in the presence and in the absence of cardiac steroids. The oscillatory current is independent of the pacemaker current and is enhanced by all the procedures that supposedly increase cellular calcium.

Purkinje fibers are quite resistant to metabolic inhibition and in fact we observed small effects on action potential characteristics after 30 min of hypoxia in the absence of glucose. Even if we did not establish experimentally the metabolic state of our preparations, several considerations can be presented. The slope of diastolic depolarization was not affected; since spontaneous frequency in isolated Purkinje fibers eventually decreases during hypoxia, the increased likelihood of induction of automaticity during hypoxia in the absence of glucose does not appear to be due to a direct

![FIGURE 5. Results of a typical experiment showing the effects of yohimbine on action potential configuration and effective refractory period. Driving rate 1 Hz.](image-url)
effect on the normal process of diastolic depolarization. Hypoxia in glucose-free Tyrode's solution has been reported to cause an increase in cellular calcium. 10

Finally, an increased preponderance of \( \alpha \)-adrenoceptor activity, possibly related to the genesis of acute postischemic arrhythmias, appears to be present early in myocardial ischemia. 6, 7, 32 The combined effect of hypoxia in the absence of glucose and the increased \( \alpha \)-adrenergic responsiveness could cause an increase in intracellular calcium to such a level that the induction of automaticity due to oscillatory potential is facilitated. This hypothesis is supported by recent evidence showing that \( \alpha \)-adrenergic stimulation increases the slow inward current 33 and induces delayed afterdepolarizations (oscillatory afterpotentials) and triggered activity in calcium-loaded Purkinje fibers 34 and that \( \alpha \)-adrenergic blockade prevents the increase in intracellular calcium in reversibly injured cardiac tissue. 8 However, the previously described effects are generally attributed to \( \alpha_1 \)-adrenoceptors. In this connection it was quite surprising to find that yohimbine, but not prazosin, was effective in antagonizing the NE-induced automaticity under our experimental conditions. Yohimbine, however, has been shown to have antiarrhythmic properties in several models in vivo. 6, 7 Its effect appears to be specific under our experimental conditions since, at the concentration used, yohimbine did not modify the action potential characteristics or automaticity. This is in agreement with other reports showing that yohimbine exerts local anesthetic-like actions and decreases automaticity of Purkinje fibers only at concentrations higher than \( 10^{-7} \)M. 35, 36 Many studies have demonstrated the existence of postjunctional \( \alpha_1 \)-adrenoceptors in many tissues, including the heart (see Hoffman and Lefkowitz 27 and Van Zwieten and Timmermans 38 for reviews), but this appears to be the first demonstration of their presence in sheep Purkinje fibers, where they were shown to control automaticity at least under certain "pathologic" conditions.

In conclusion, our results support the view of an enhanced \( \alpha \)-adrenergic responsiveness under pathologic conditions and suggest that \( \alpha \)-adrenoceptors, possibly belonging to the \( \alpha_2 \)-subtype in sheep cardiac Purkinje fibers, might influence abnormal automaticity through an effect on oscillatory potentials.

References


Vol. 73, No. 1, January 1986
Enhancement by norepinephrine of automaticity in sheep cardiac Purkinje fibers exposed to hypoxic glucose-free Tyrode's solution: a role for alpha-adrenoceptors?

A Mugelli, S Amerini, G Piazzesi, E Cerbai and A Giotti

_Circulation_. 1986;73:180-188
doi: 10.1161/01.CIR.73.1.180

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/73/1/180

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/