Effect of Epinephrine on the Mechanical and Phosphorylase Activity of Normo- and Hypothermic Hearts

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With the technical assistance of Janet Howells

The use of hypothermia in surgery has renewed interest in several aspects of cardiac physiology and attention is again being paid to the mechanisms whereby excitability and mechanical activity is maintained in the hearts of hibernators and poikilotherms at low temperatures.1-8 The present investigation was carried out to record and compare the response of normo- and hypothermic hearts to epinephrine; hearts isolated from rats (homeothermic) and from toads (poikilothermic) were used. In addition, the ability of epinephrine to affect a change in the percentage of the enzyme phosphorylase present in the α form, was studied under various conditions.

Methods

PREPARATIONS

(a) Rat (Homeothermic) Series

Hearts from unselected rats of either sex were perfused at 40 cm H2O pressure by the standard Langendorff technique using modified Tyrode solution of the following composition (in mm): NaCl, 130; KCl, 5.6; CaCl2, 2.16; NaHCO3, 25.0; sucrose, 13.1; glucose, 11.1; Na2HPO4, 9.1; aerated with 95% O2 + 5% CO2. Unless otherwise stated the isolated hearts beat spontaneously.

After forty minutes perfusion at either 35°C or 25°C control hearts were frozen and analysed for phosphorylase activity (see below). The phosphorylase activity of other hearts initially perfused for five minutes at 35°C was determined after thirty-five minutes perfusion at 12°C or 2°C. Phosphorylase activity was similarly determined five minutes after 10 μg epinephrine (as l-epinephrine tartrate, Hermette) was injected as a single dose into the inflow cannula of hearts which had been perfused at either 35°C, 25°C or 12°C during the preceding thirty-five minutes. During these procedures isotonic contractions were recorded on a standard kymograph operating at a speed of 3 cm/min. When chronotropic changes were studied the kymograph was operated at a speed of 9 cm/min.

(b) Toad (Poikilothermic) Series

Hearts removed from unselected non-hibernating toads, (Bufo marinus) of either sex were perfused with a modified Tyrode solution of the following composition (in mm): NaCl, 115; NaHCO3, 20.6; NaH2PO4, 3.0; MgSO4, 1.2; glucose, 16.5; KCl, 3.2; CaCl2, 1.3; aerated with 95% O2 + 5% CO2. Inflow and outflow cannulae were placed in the inferior vena cava and right systemic arch respectively, and unless otherwise stated, the preparations beat spontaneously.

After forty minutes perfusion at 22°C, 12°C, or 2°C control hearts were frozen and assayed for phosphorylase activity. Other hearts were perfused at 8°C for forty minutes prior to assay for phosphorylase activity. In the experimental series three groups of hearts were perfused for forty minutes at 22°C, 12°C, and 2°C as above, but 30 μg epinephrine was added five minutes before the end of each perfusion. Five minutes later these hearts were frozen and assayed for phosphorylase activity.

The phosphorylase activity of electrically stimulated toad hearts was determined following forty minutes perfusion at 2°C. In these preparations the pacemaker on the surface of the atrium was destroyed by cauterization and the hearts stimulated with just suprathreshold (less than 5 v) rectangular pulses of 10 msec duration delivered from a Tektronix square wave generator at the rate of six pulses per minute, a rate which approximated that of spontaneously beating hearts when perfused at this temperature.

Other toads were injected intraperitoneally with 1 mg/kg reserpine (Serpasil CIBA) on three consecutive days. On the fourth day the hearts were isolated and perfused at 2°C for...
forty minutes before being assayed for phosphorylase activity. During perfusion the hearts were stimulated as above. 2 to 5 µg/ml of dichloroisoproterenol (DCI) was initially added to other stimulated 2°C preparations and the phosphorylase activity determined after forty minutes perfusion. The DCI remaining in the perfusion fluid throughout the entire forty minutes. Isotonic contractions were recorded either on a standard kymograph or on a direct ink writing Evershed and Vignoles recorder as previously described. Under these latter conditions a Straub perfusion was employed in contrast to the recycling preparation described above.

Phosphorylase Determination. At the end of each experiment the hearts were immediately removed from the cannula and frozen by means of forceps which previously had been immersed in a dry-ice isopentane mixture. The frozen hearts were weighed and then pulverized with a chilled crushe and each 100 mg tissue extracted with 5 ml of ice-cold 0.02 M NaF containing 0.001 M disodium ethylenediamine tetracetic acid, pH 7.0. After centrifugation at 0°C tissue extract was added to 0.5 ml of substrate mixture with and without adenosine-5-phosphate (AMP) to provide duplicate tissue-extract concentrations of 1/100, 1/150, and 1/200. The substrate mixture contained 1% glycogen, 0.016 M glucose-1-phosphate and for total phosphorylase determinations, 0.001 M adenosine-5-phosphate. The pH was adjusted to 6.1.

Inorganic phosphate was determined by the method of Lowry and Lopez before and after five minutes incubation at 30°C. Phosphorylase activity was calculated as described by Belford and Feinleib. Thus the ratio:

\[
\frac{\text{inorganic } P \text{ formed without AMP}}{\text{inorganic } P \text{ formed with AMP}} \times \frac{100}{1}
\]

is designated in the present paper as the per cent of active phosphorylase a.

The results were tested for significance by the students "t" test; a P value of 0.05 was accepted as the limit of significance.

Phosphorylase a Determination. In these experiments estimations of the phosphorylase a activity were made using duplicate tissue-extract dilutions of 1/100, 1/150, and 1/200. Those estimations of activity which were based on 1/100 tissue-extract dilutions were significantly higher than those based on the use of 1/150 or 1/200 dilutions but the changes in activity evoked by the various experimental procedures were similar at the three different dilutions. Since the use of tissue dilutions in excess of 1/150, e.g., 1/200, yielded phosphorylase a activities of the same magnitude as those found when 1/150 dilutions were used, only the results obtained during the use of 1/150 tissue dilutions have been listed below.

Results

(a) Rat (Homeothermic) Series

Effect of Temperature on Inotropic Activity of Epinephrine. In figure 1 (A-C), where isotonic contractions of a rat heart perfused at 35°C, 25°C, and 12°C are shown before and after the addition of epinephrine, it can be seen that as the perfusion temperature was lowered the amplitude of contraction declined. This decline was associated with a steady progressive bradycardia; cardiac arrest, which was not reversed by electrical stimulation, occurred at perfusion temperatures below 10°C. Spontaneous tachycardia was not encountered during perfusion at 35°C, 25°C, or 12°C; similarly, spontaneous cardiac arrest did not occur in any of the preparations perfused at temperatures above 12°C. As is shown in figure 1 (D and E) rewarming those hearts which had been arrested by perfusion at temperatures below 10°C resulted in the resumption of spontaneous activity.

In figure 1A the typical response recorded following the addition of 10 µg epinephrine to rat hearts perfused at 35°C is shown, the response recorded following the similar addition of epinephrine to the same heart when perfused at 25°C being displayed in figure 1B. In figure 1C it can be seen that although this same amount of epinephrine, when added to this same heart perfused at or below 12°C, failed to increase the amplitude of contraction it did evoke a positive chronotropic response. Rewarming the cold 2°C arrested hearts to 35°C restored the positive inotropic activity of epinephrine, as is shown in figure 1 (D and E).

Effect of Temperature on Phosphorylase a Activity. In table 1 it is shown that the percentage of the phosphorylase enzyme present in the a form declined significantly when the perfusion temperature was reduced from 35°C to 25°C, and from 35°C to 12°C. The percentage of the enzyme present in the a form in hearts perfused at 25°C was significantly (P < 0.01) different from that of other hearts perfused at 12°C. The proportion of the phosphorylase present in the a form in freshly isolated hearts was not significantly different from that of the control hearts perfused at 35°C.
different from that in other hearts which had been perfused for forty minutes. Electrical stimulation (5 v, 10 msec duration 180 to 200 pulses per minute) did not affect a significant change in percentage of phosphorylase $a$ in such hearts when perfused at either 2°C or 12°C.

**Epinephrine and Phosphorylase Activity.** In table 1, where the effect exerted by 10 µg epinephrine on the percentage of the phosphorylase enzyme present in the $a$ form in hearts perfused at 35°C, 25°C, and 12°C is shown, it can be seen that five minutes after the addition of epinephrine to the inflow cannula of hearts perfused at these temperatures there was a significant increase in the percentage of phosphorylase enzyme present in the $a$ form. In the 12°C epinephrine series, however, the percentage of the enzyme present in the $a$ form after epinephrine administration was significantly less ($P < 0.01$) than that in either the 25°C or 35°C epinephrine series.

**Toad (Poikilothermic) Series**

**Effect of Temperature on Inotropic Activity of Epinephrine.** In figure 2 (A-C) typical isotonic contractions of a toad heart perfused at 22°C, 12°C, and 2°C are shown before and
Isotonic contractions of an isolated spontaneously beating toad heart perfused at 22°C, 12°C, and 2°C as indicated. At arrow 30 μg of epinephrine was added. A and B were recorded at constant amplifier gain; in C the amplifier gain was reduced by 40%. Read from left to right. When perfused with Tyrode solution at 22°C, 12°C, and 2°C this heart contracted at the rate of 48, 35, and 8 beats per minute respectively. In the presence of 30 μg epinephrine it contracted at the rate of 70, 42, and 8 beats per minute, at 22°C, 12°C, and 2°C respectively.

**TABLE 1**

**Effect of Temperature and Epinephrine on the Percentage of Phosphorylase Enzyme Present in the a Form in Rat Hearts**

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>No. exp.</th>
<th>% Phosphorylase a</th>
<th>No. exp.</th>
<th>% Phosphorylase a</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>6</td>
<td>52 ± 2</td>
<td>6</td>
<td>66 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P* &lt; 0.001</td>
<td></td>
<td>P* = 0.01</td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td>27 ± 1.5</td>
<td>6</td>
<td>64 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P* &lt; 0.01</td>
<td></td>
<td>P* &lt; 0.01</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>18 ± 1.2</td>
<td>6</td>
<td>42 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P* &lt; 0.01</td>
<td></td>
<td>P* &lt; 0.01</td>
</tr>
</tbody>
</table>

Mean values, and standard errors of the mean values, of percentage phosphorylase enzyme present in the α form in isolated spontaneously beating rat hearts, (A) after forty minutes perfusion in Tyrode solution, and (B) five minutes after 10 μg epinephrine was added to hearts previously perfused for thirty-five minutes with Tyrode solution. P is the statistical significance of the differences between the percentage phosphorylase α activities.

*At the temperatures shown.
1In the presence and absence of epinephrine.

after the addition of epinephrine and it can be seen that reducing the perfusion temperature from 22°C to 12°C enhanced the amplitude of contraction and caused slight brady-cardia. At 2°C the bradycardia was more pronounced and the amplitude of contractions well maintained. The time interval throughout which spontaneous activity was main-
### Table 2

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Experiment</th>
<th>No. expt</th>
<th>A</th>
<th>B</th>
</tr>
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<tbody>
<tr>
<td>22</td>
<td>Elec. stim.</td>
<td>6</td>
<td>78 ± 1.5</td>
<td>92 ± 2.5 P* &lt; 0.05</td>
</tr>
<tr>
<td>22</td>
<td>Spontaneous</td>
<td>6</td>
<td>55 ± 1.5</td>
<td>69 ± 1.5 P* &lt; 0.05</td>
</tr>
<tr>
<td>12</td>
<td>Spontaneous</td>
<td>6</td>
<td>72 ± 3.0</td>
<td>98 ± 1.0 P* &lt; 0.05</td>
</tr>
<tr>
<td>8</td>
<td>Spontaneous</td>
<td>6</td>
<td>68 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Spontaneous</td>
<td>6</td>
<td>67 ± 2.0</td>
<td>76 ± 3.0 P* = 0.05</td>
</tr>
<tr>
<td>2</td>
<td>Elec. stim.</td>
<td>6</td>
<td>95 ± 0.5</td>
<td>P* &lt; 0.001</td>
</tr>
<tr>
<td>2</td>
<td>Elec. stim. + reserpine</td>
<td>4</td>
<td>50 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Spontaneous + reserpine</td>
<td>4</td>
<td>56 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Elec. stim. + DCI</td>
<td>4</td>
<td>49 ± 1.5</td>
<td>P* &lt; 0.001</td>
</tr>
<tr>
<td>2</td>
<td>Spontaneous + DCI</td>
<td>4</td>
<td>49 ± 1.0</td>
<td>P* &lt; 0.01</td>
</tr>
<tr>
<td>2</td>
<td>Spontaneous</td>
<td>6</td>
<td>67 ± 2.0</td>
<td></td>
</tr>
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</table>

Mean values and standard errors of the mean values, of percentage of phosphorylase enzyme present in the a form in isolated toad hearts. (A) after forty minutes perfusion in Tyrode solution, and (B) five minutes after 30 μg epinephrine was added to hearts previously perfused for thirty-five minutes with Tyrode solution. P is the statistical significance of the differences between the percentage phosphorylase a activities.

*At the temperatures as shown.

1. In the presence and absence of epinephrine.

2. Spontaneous = spontaneously beating.

3. Elec. stim. = electrically stimulated (see text).

4. Reserpine = hearts removed from reserpinised toads (see text).

5. DCI = 2 to 5 μg/ml DCI present throughout forty minutes of perfusion.

Mean values and standard errors of the mean values, of percentage of phosphorylase enzyme present in the a form in isolated toad hearts. (A) after forty minutes perfusion in Tyrode solution, and (B) five minutes after 30 μg epinephrine was added to hearts previously perfused for thirty-five minutes with Tyrode solution. P is the statistical significance of the differences between the percentage phosphorylase a activities.

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epinephrine. 100 μg epinephrine consistently depressed the isotonic contractions of 2°C hearts and 5 μg did not exert any effect. When 30 μg epinephrine was added to hearts which recently had been rewarmed to 22°C after thirty minutes perfusion at 2°C to 5°C, it failed to evoke positive chronotropic or inotropic responses similar to those which were consistently noted following the addition of 30 μg epinephrine to other hearts perfused throughout at 22°C.

**Effect of Temperature on Phosphorylase a Activity.** The percentage of phosphorylase activity present in the a form in toad hearts varied according to the perfusion temperature. In table 2 it is shown that the percentage of phosphorylase a activity in hearts perfused at 22°C was significantly less (P < 0.05) than that in other hearts which were perfused at 12°C, 8°C, or 2°C. The phosphorylase a activity of hearts which were perfused at 12°C, however, was not significantly different from that of those hearts which were perfused at 8°C or 2°C. These preparations were all beating spontaneously. While some spontaneously beating hearts remained mechanically active throughout the forty-minute perfusion at 2°C, others ceased beating after only twenty minutes. To overcome this difficulty another series of phosphorylase determinations was made using hearts which were perfused at 2°C and which were continuously stimulated at the rate of six beats per minute with 5 v pulses of 10 msec duration. In table 2 it is shown that this procedure caused a significant increase in the percentage of the phosphorylase activity present in the a form. Electrical stimulation with 5 v pulses at the rate of 60 beats per minute caused a small but significant increase in the percentage phosphorylase a activity in other hearts perfused at 22°C. Electrical stimulation as above, however, failed to cause a significant change in the percentage of phosphorylase in the a form in hearts which were either isolated from reserpinised toads or were perfused at 2°C in the presence of DCI, as is shown in table 2.

**Effect of Epinephrine on Phosphorylase a Activity.** In table 2 the effect of 30 μg epinephrine on the percentage of phosphorylase a in spontaneously beating toad hearts which were perfused at 22°C, 12°C, and 2°C is shown, and it can be seen that 30 μg of epinephrine evoked a significant increase in the percentage of the phosphorylase present in the a form in hearts perfused at either 22°C or 12°C but caused only a small increase of doubtful significance in the phosphorylase a activity of those hearts which were perfused at 2°C.

**Discussion**

These experiments demonstrate that the response of isolated rat (homeothermic) hearts to cold per se differs significantly from that displayed under similar conditions by isolated toad (poikilothermic) hearts. Whereas spontaneous activity in the isolated rat hearts ceased when the perfusion temperatures fell below 10°C spontaneous activity was maintained for sixty or more minutes in isolated toad hearts at temperatures as low as 2°C. The cold arrested rat hearts failed to respond to electrical stimulation at temperatures below 10°C whilst toad hearts whose spontaneous activity was arrested following prolonged perfusion at 2°C or whose pacemaker had been destroyed continued to contract indefinitely at this temperature in response to mechanical or electrical stimulation. In the isolated toad hearts low temperatures induced a pronounced positive inotropic response which was accompanied by bradycardia and a small but significant increase in the proportion of the phosphorylase activity present in the a form. When the temperature at which rat hearts were perfused was lowered from the normal to 25°C or 12°C, however, they displayed a progressive negative inotropic response and a decline in the proportion of the phosphorylase activity which was in the a form.

The ability of isolated toad hearts to maintain their mechanical and electrical excitability even at low temperatures may be related to the relatively high percentage of their phosphorylase enzyme which is in the a form. Comparison of the data shown in tables 1
and 2 indicates that the percentage of the enzyme present in the α form in toad hearts perfused at 22°C was significantly higher than that in rat hearts perfused at 25°C. This difference between the percentage of the enzyme which was in the α form in the two species was consistently encountered throughout these investigations, and became more pronounced during perfusion at reduced temperatures. It is necessary to emphasise the finding that rat heart phosphorylase α activities differed from those of toad hearts during both normo- and hypothermia despite the use of several tissue-extract dilutions in excess of 1/150. These results indicate that the observed differences in activities are probably real and not due to interference from tissue AMP.  

The present investigations may be interpreted to mean that toad hearts respond to cold by increasing the percentage of their phosphorylase enzyme which is in the α form, possibly by the release of their stored catecholamines. Other evidence suggests that catecholamines may be released from their storage sites by a series of supramaximal impulses. Although in the present experiments care was taken during electrical stimulation to avoid the use of impulses of excessive amplitude, the toad hearts responded, even at 2°C, by increasing the percentage of the enzyme which was in the α form. Pretreatment of the 2°C toads with reserpine or the presence of DCI abolished this response to stimulation suggesting that catecholamines were involved.  

There is a considerable amount of data which suggests that the transformation of phosphorylase b to the α form may be involved in the process whereby the sympathomimetic amines enhance the force of cardiac contraction. It seems possible therefore that the increased amplitude of contractions recorded from the toad hearts when perfused at low temperatures may be related to the increased percentage of the phosphorylase enzyme which was in the α state. The failure of epinephrine to exert a positive inotropic effect on isolated toad hearts perfused at 2°C may be related to the near maximal contractions displayed by these hearts when perfused at such temperatures in the absence of any added epinephrine.  

The amplitude of isotonic contractions recorded from isolated rat hearts perfused at the various temperatures closely paralleled the percentage of the phosphorylase activity which was present in the α form in the absence of any added epinephrine. At 12°C epinephrine did increase the percentage of phosphorylase α activity but exerted only a chronotropic effect, a result which suggests that the mechanism whereby epinephrine influences the contractile amplitude of cardiac muscle differs from that whereby it evokes a change in rhythmicity. The failure of epinephrine to evoke a positive inotropic response under these conditions confirms Booker’s findings and may be related to the low level of phosphorylase α activity (42%) found in these rat hearts when perfused in the presence of epinephrine at 12°C. By contrast, hearts which were similarly perfused in the presence of epinephrine at either 25°C or 35°C and which responded to epinephrine with positive inotropic response yielded phosphorylase α activities of 64% and 66% respectively.  

Recently Uchida and Mommaerts concluded that “changing levels in cyclic AMP are responsible for the variations in contractile strength occurring physiologically in the myocardium.” The association between the percentage of the phosphorylase enzyme present in the α form, and the amplitude of contractions noted throughout these experiments appears to substantiate the conclusion of Uchida and Mommaerts.  

**Summary**  

The effect of temperature on the action of epinephrine and on the percentage of the enzyme phosphorylase present in the α form in isolated rat (homeothermic) and toad (poikilothermic) hearts was investigated. Rat hearts responded to cold per se with reduced amplitude of contraction and bradycardia. The percentage of the phosphorylase enzyme which was in the α form declined as
the perfusion temperature fell. Mechanical activity ceased when rat hearts were perfused at temperatures below 10°C. Epinephrine failed to exert a positive inotropic effect when added to rat hearts perfused at 12°C; at this temperature epinephrine increased the phosphorylase \( a \) activity to only 42% compared with 66% at 35°C.

The response of toad hearts to cold per se differed from that of rat hearts. At 2°C the contractile amplitude of toad hearts was maximal and the phosphorylase \( a \) activity was well maintained. Stimulation enhanced, DCI and preliminary reserpine reduced the proportion of the enzyme which was in the \( a \) form in 2°C toad hearts.

It is suggested that poikilothermic hearts may respond to cold by releasing their endogenous catecholamines and that this may be indirectly responsible for the ability of poikilothermic cardiac tissue to withstand low temperatures.

References
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doi: 10.1161/01.RES.13.3.199

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