Responsiveness of the 4-Day-Old Embryonic Chick Heart to Catecholamines

In a recent paper (Circ Res 31:932-952, 1972) Shigenobu and Sperelakis reported that they were unable to demonstrate a significant effect of either epinephrine or isoproterenol in concentrations as high as $1 \times 10^{-5} \text{M}$ on electrical or mechanical activity of driven ventricular muscle from young chick embryos (3-5 days old). Similarly, Hollman and Green (Fed Proc 32:711 [abstr.], 1973) could not obtain a positive inotropic response to isoproterenol from the electrically driven (Green, personal communication) myocardium of 4-day-old chick embryo. On the other hand, McCarty et al. (J Pharmacol Exp Ther 129:315-321, 1960) showed that epinephrine at a concentration as low as $5.47 \times 10^{-10} \text{M}$ exerts both positive inotropic and chronotropic effects in the isolated, spontaneously beating 4-day-old embryonic chick heart.

Because of my interest in using the 4-day-old embryonic chick heart as a model to study certain characteristics of the adrenergic receptor, it was necessary for me to establish beyond reasonable doubt that such receptors exist and are functional at this stage of development. Accordingly, I have used isolated, spontaneously beating hearts from embryos of this age and determined their responsiveness to the four catecholamines, l-isoproterenol, l-epinephrine, l-norepinephrine, and dopamine. The method originally described by McCarty et al. (J Pharmacol Exp Ther 129:315-321, 1960) and subsequently modified and used in our laboratory (LeLorier, Ph.D. Thesis, University of Minnesota, 1972) was employed to measure effects of these drugs on rate and contractility. Hearts with rates between 150 and 180 beats/min. were mounted for recording in the modified Tyrode's solution used by McCarty et al. Drugs were added after a constant rate and amplitude of contraction had been attained. All responses to drugs were expressed as the maximal percent change compared with the rate or the amplitude existing just prior to the addition of the drug. No heart was exposed to more than one drug or one concentration of that drug.

Figure 1 clearly illustrates that isoproterenol can enhance both rate and contractility of the 4-day-old embryonic chick heart. Furthermore, within certain ranges of concentration, a linear relationship exists between the drug concentration and the effect on rate and contractility. Although not illustrated, these effects can be prevented by prior addition to the bathing medium of propranolol in appropriate concentration.

Similar dose-response relationships exist for l-epinephrine, l-norepinephrine, and dopamine. No attempt was made to determine precisely the relative potency of the various amines, but the data in Table 1 indicate their relative order of effectiveness in enhancing rate and contractility in this preparation.

It could be argued that the changes in contractile amplitude are secondary to the effects of the catecholamines on the rate, i.e., no inotropic receptors for the drugs are involved in the contractile response. However, this finding would not seem to be the case, since I have observed that 4-day-old embryonic hearts electrically driven at rates as much as 80% higher than those existing in the spontaneously beating preparation exhibit no significant change in contractile amplitude.

The inability of Shigenobu and Sperelakis and of Hollman and Green to demonstrate a positive inotropic response to catecholamines in the 4-day-old chicken heart may have been the result of one or more differences between their studies and my study. Most apparent is that both of the former used electrically driven preparations. Shigenobu and Sperelakis utilized only the ventricles, but it is not clear whether Hollman and Green used the en-


## Table I

**Effect of Various Catecholamines on the 4-Day-Old Embryonic Chick Heart**

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Drug</th>
<th>Rate</th>
<th>Contractility</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3 \times 10^{-9}$</td>
<td>$\alpha$-Isoproterenol</td>
<td>$23.2 \pm 3.0$</td>
<td>$32.9 \pm 1.4$</td>
</tr>
<tr>
<td>$5 \times 10^{-9}$</td>
<td>$\alpha$-Norepinephrine</td>
<td>$24.6 \pm 5.0$</td>
<td>$20.4 \pm 3.6$</td>
</tr>
<tr>
<td>$5 \times 10^{-8}$</td>
<td>$\alpha$-Epinephrine</td>
<td>$31.3 \pm 4.9$</td>
<td>$23.7 \pm 3.5$</td>
</tr>
<tr>
<td>$2.5 \times 10^{-5}$</td>
<td>Dopamine</td>
<td>$18.3 \pm 2.3$</td>
<td>$31.5 \pm 2.0$</td>
</tr>
</tbody>
</table>

All values are means ± SE.

*Based on data from 5-7 hearts.

tire heart or only a portion of it. In general, Shigenobu and Sperelakis used concentrations of catecholamines much higher than the concentrations that we have found will produce maximal responses. Although Hollman and Green used a much lower concentration of calcium (0.359 mM) in their medium than we did, the concentration used by Shigenobu and Sperelakis (2 mM) was similar to ours (2.4 mM). Regardless of the possible explanations for the inability of these investigators to demonstrate effects of the catecholamines on preparations of the 4-day-old embryonic chick heart, our data indicate that the spontaneously beating whole heart is responsive to these substances. Whether or not full responsiveness exists at this stage of development cannot be stated.

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**REPLY TO THE ABOVE LETTER**

Although the question raised by Shideman concerns only a relatively small part of my work and in no way affects the major conclusions, I wish to reply to his letter to the editor. The issue raised by Shideman concerns whether young chick embryonic hearts, 2-4 days old, exhibit a positive inotropic response to catecholamines. I do agree that some of these young hearts show a significant positive chronotropic response to catecholamines (p 943, Circ Res 31: 932-952, 1972, and this letter). For clarification, I must point out two erroneous statements in Shideman's letter. (1) I did not electrically drive the young hearts, because they generally had a high rate of spontaneous activity, and (2) I did not use isolated ventricles for the young hearts because of their small size. These facts were clearly stated in my paper.

Because of the extremely small size of the young hearts, it is difficult to obtain reliable mechanical data. This problem has been nicely approached by Faber (Am J Physiol 214:475-481, 1968) using a micro-pressure-transducer system. Shideman used a micromirror method to record the movements of isolated hearts. His method, as with the others, may be subject to some error, and I have reservations about his ability to make accurate measurements of about a 33% increase in contractility. Note that in Figure 1, the contractility curve actually flattens out between $3 \times 10^{-9}$M and $1 \times 10^{-8}$M isoproterenol. In his letter, he unfortunately neglected to state the time course of the effect.

I chose to use a simple piezoelectric method with a phonograph cartridge. In 3- and 4-day-old hearts, a metal pin hook connected to the piezoelectric cartridge was positioned at the middle of the loop of the tubular heart; the hook also was used to apply stretch to the heart. During systole, the tube shortens and decreases in diameter and exerts tension on the metal pin. Because of the nature of the piezoelectric crystal, this method is more reliable in recording $dT/dt$ than it is in recording the peak tension (T) developed. But this method should be very sensitive to a positive inotropic effect of catecholamines because of the well-known increase in $dT/dt$ which accompanies the response. Contractions of 3-5-day-old hearts recorded by this method did not significantly change even 15 minutes after the addition of $1 \times 10^{-8}$M isoproterenol, as clearly shown in Figure 12 of my published paper. However, to be certain, I repeated these experiments for this reply; the results are illustrated in Figure 2A-I. Again, isoproterenol, in concentrations of $10^{-6}$ M, $10^{-5}$ M, and $10^{-4}$ M, did not increase the amplitude of the mechanogram recorded by the piezoelectric method in 3-5-day-old hearts in situ. To demonstrate that this method can record increased contractions, I have included examples showing that increasing the rate of stimulation of a 5-day-old heart, which has lost automaticity, produces a positive staircase effect (Fig. 2G-H).
Letters to the Editor
F. E. SHIDEMAN

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