Responses of Rat and Guinea Pig Hearts to Glucagon
Lack of Evidence for a Dissociation between Changes in Myocardial Cyclic 3',5'-Adenosine Monophosphate and Contractility

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SUMMARY Previous studies have suggested that the inotropic effects of glucagon on the guinea pig heart, but not on the rat heart, could be dissociated from its effects on cyclic AMP formation. We compared the effects of glucagon on working rat and guinea pig hearts to reinvestigate this proposed dissociation. When administered to spontaneously beating preparations, glucagon produced similar increases in rate, but different increases in contractility, of rat and guinea pig hearts. The inotropic effects of glucagon on the rat heart were greater in magnitude than those on the guinea pig heart. Glucagon (10^{-9} to 3 \times 10^{-7} M) increased left ventricular pressure (LVP) and the rate of pressure development (dP/dt), and reduced the time-to-peak pressure (TPP) of both rat and guinea pig spontaneously beating hearts. Studies in which the chronotropic responses to glucagon (10^{-7} M) were duplicated electronically showed that the inotropic effects on the spontaneously beating guinea pig heart were entirely frequency-dependent, whereas those on the rat heart were not. In addition, glucagon (10^{-7} M) markedly increased LVP, dP/dt, and ventricular cyclic AMP levels of the paced rat heart, but had no effect on any of these variables in the paced guinea pig heart. The results do not support the proposed dissociation between cyclic AMP generation and positive inotropic responses of the guinea pig heart to glucagon. They suggest further that the guinea pig heart exhibits regional differences in sensitivity to glucagon.

MANY studies of the effects of glucagon on the heart support the second messenger hypothesis (Sutherland et al., 1968) that an enhancement of cyclic AMP generation is required for the full expression of the inotropic response. Positive inotropic and metabolic effects of glucagon in some species, including the rat, are qualitatively similar to the effects of catecholamines (Farah and Tuttle, 1960; Kreisberg and Williamson, 1964; Glick et al., 1968), but are mediated through the activation of glucagon-specific receptors (Birnbaumer et al., 1974). Glucagon, like the catecholamines, increases myocardial adenylate cyclase and phosphorylase \(\alpha\) activities (La Rain et al., 1968; Murad and Vaughan, 1969) and increases ventricular cyclic AMP levels (Mayer et al., 1970). The glucagon-induced increases in cyclic AMP concentrations precede or coincide with the positive inotropic response (Brunt and McNeill, 1978).

A few studies have suggested that the positive inotropic and cyclic AMP-generating effects of glucagon can be dissociated (Oye and Langslet, 1972; Henry et al., 1975; Wildenthal et al., 1976). In one such study, Henry et al. (1975) found that glucagon produced dose-dependent increases in contractility of isolated, paced rat and guinea pig hearts, but elevated adenylate cyclase activity and cyclic AMP levels only in the rat heart. They hypothesized that inotropic and cyclic AMP-generating effects were dissociated in the guinea pig and, by inference, merely coincidental in the rat. Previous studies have shown, however, that agonists that increase myocardial cyclic AMP levels also produce inotropic responses similar to the effects of catecholamines, e.g., abbreviated systole and enhanced contraction and relaxation rates at constant frequency of stimulation (Epstein et al., 1971; Tsien, 1977). Thus, catecholamine-like inotropic responses provide good presumptive evidence for cyclic AMP-dependent mechanisms. In forming their hypothesis, Henry and coworkers did not specifically demonstrate that the inotropic effects of glucagon on guinea pig hearts were both quantitatively and qualitatively similar to the effects on the rat heart. Preliminary evidence from our laboratory suggested that the chronotropic effects of glucagon on rat and guinea pig hearts were similar but that the inotropic effects of glucagon on rat ventricular muscle preparations were markedly different from the...
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Effects on guinea pig ventricle (MacLeod et al., 1979). We therefore carried out detailed comparative studies of the inotropic, chronotropic, and cyclic AMP-generating effects of glucagon on rat and guinea pig working hearts to reinvestigate the proposed dissociation between positive inotropy and cyclic AMP formation in the guinea pig.

Methods

Working Heart Preparation

Hartley guinea pigs (250-350 g) and Sprague-Dawley rats (200-300 g) of either sex were injected ip with heparin (1000 IU/kg) and stunned by a blow to the head 5-10 minutes later. Their hearts were excised quickly and attached to a stainless steel cannula via the aortic remnant, and perfused in a retrograde fashion with a modified, oxygenated, Chenoweth-Koelle solution at a pressure of 45 cm H2O. A 2-inch length of bevelled PE 90 tubing then was inserted through the left atrium via the remnant of the pulmonary veins, into the left ventricle, and out the apex of the heart, leaving approximately 0.5 cm of the tubing inside the left ventricular chamber. The left atrium then was connected to a filling reservoir system by attaching a second stainless steel cannula to the remnant of the pulmonary veins. After verification of uninterrupted inflow, a pressure transducer was attached to the PE 90 tubing. This transducer was used to record changes in left ventricular pressure. The system then was switched from a retrograde perfusion to a working preparation by initiating inflow from the filling reservoir system and redirecting outflow into an external resistance of predetermined pressure-flow characteristics. The working preparation was based, with substantial modifications, on the method of Neely and Rovetto (1975).

Preload (left atrial filling pressure) and afterload (ventricular input impedance) were controlled as follows. Filling pressure was adjusted by varying the height of the inflow reservoir relative to the heart, and maintained constant by adjusting inflow into the reservoirs slightly in excess of the cardiac output and drawing off the excess with suction. In later experiments, inflow was controlled by feedback regulator valves. The resistance system consisted of small-diameter glass tubing interconnected by latex tubing, and could be adjusted by varying the combinations of parallel or series arrangements of the glass tubing. The latex tubing provided enough capacitance to restrict aortic outflow pulse pressures to 36.9 ± 1.2 mm Hg (rat) and 30.2 ± 1.3 mm Hg (guinea pig) at paced rates of 300/minute (values are mean ± SE, n = 12). These pressures were monitored with a second pressure transducer connected to the outflow tubing.

Preliminary studies revealed that preload and afterload requirements were different for the two species. Optimum filling pressures were selected as those which resulted in approximately ½ maximum left ventricular pressure development at a given rate and peripheral resistance. Optimum resistance was selected as that which exceeded the resistance of the coronary vascular bed (determined during retrograde perfusion). On this basis, the filling pressures and resistance characteristics used in the study were as follows: rat, 15 cm H2O and 0.18 ml/min per mm Hg; guinea pig, 12.5 cm H2O and 0.22 ml/min per mm Hg (at a paced rate of 300 beats/min).

Hearts either were allowed to beat spontaneously or were paced at 300-320 beats/min. Pacing was achieved by positioning bipolar platinum point electrodes on the left atrium and on the apex of the heart. Each electrode was connected to a stimulator (Grass model SD9). The stimulators were connected such that each atrial pulse triggered a separate ventricular pulse 4-6 msec later. Both atrial and ventricular pulses were 5 msec in duration and 50% above threshold (threshold voltage was usually between 0.4 and 0.6 V).

The modified Chenoweth-Koelle solution consisted of 120 mM NaCl, 5.6 mM KCl, 2.4 mM CaCl2, 2.2 mM MgCl2, 0.2 mM EDTA, and 10 mM glucose. The bicarbonate (NaHCO3) concentration was varied according to the experimental conditions. In most cases, the bicarbonate concentration was the same for rat and guinea pig hearts (15 mM). However, it was found that the rat hearts were relatively more sensitive to the pH of the solution, requiring more alkaline conditions to achieve stable rates. Therefore, spontaneously beating rat hearts were perfused with buffer containing 25 mM bicarbonate. Continuous gassing with 95% O2/5% CO2 (37°C) resulted in a pH of 7.38 at 15 mM HCO3- or 7.58 at 25 mM HCO3-. Perfusate temperature was maintained at 37.0°C, and ambient air temperature immediately surrounding the heart was controlled thermostatically between extremes of 36.5 and 37.5°C.

The following measurements were recorded or calculated: heart rate (HR), left ventricular pulse pressure as the difference between left ventricular systolic and diastolic pressures (LVPP = LVSP - LVDP), rates of left ventricular pressure development and decline (+ and -dP/dt, respectively), and time-to-peak pressure (TPP). Changes in dP/dt were recorded continuously with a differentiating preamplifier, but the values reported in the study were obtained manually from the recordings of the LVP curves at rapid chart speed (100 mm/sec). TPP was obtained by measuring the distance between the initial point of upward deflection of the dP/dt tracing and the point at which the tracing again intersected the midline (zero slope). Aortic outflow pressures were recorded but were not reported in the present study.

Glucagon Administration

Glucagon was administered to the hearts by perfusion. Prior to drug administration, each heart was...
allowed to stabilize for 20–30 minutes. During this stabilization period, the hearts were perfused with drug-free buffer solution through two filling reservoir systems simultaneously. At the end of this stabilization period, inflow from the first reservoir system was allowed to continue and flow from the second was interrupted. Glucagon then was added to the second system from a concentrated stock solution. Perfusion of the heart was then switched abruptly from the drug-free to the glucagon-containing solution. This procedure minimized the effect of temperature differences that result from the transfer from flowing to static solutions, and allowed washout after drug perfusion when necessary. Cumulative dose-response curves were obtained by adding increasing amounts of glucagon to the perfusate without interruption of inflow. The lyophilized glucagon (Eli Lilly Co.) was dissolved in 1 ml of 1 mM Tris buffer and diluted in distilled water before being added to the buffer solution. In calculating the doses of glucagon during cumulative administrations, we took into account the contributions of prior doses and reductions in buffer volume due to cardiac output.

Electronic Resolution of Interval-Dependent and Interval-Independent Inotropic Effects of Glucagon

In one series of experiments, the influence of changes in the interval between beats on the inotropic response of spontaneously beating hearts to glucagon was analyzed by duplicating the chronotropic response electronically. Each heart was exposed to a single perfusion of glucagon (10⁻⁷ M, 3–5 min); this dose of glucagon had been determined to be maximally chronotropic in both species. Left ventricular pulsations were recorded on magnetic tape (Hewlett-Packard model 3960) several minutes prior to, and during perfusion of, glucagon. At the end of the perfusion period, the drug was washed out, and the hearts were allowed to restabilize. We then duplicated the chronotropic response electronically by playing back the recorded pulses through a discriminating circuit (designed and built by David Harris and Roland Burton of this Faculty). The circuit was used to trigger the pacing stimulators in response to each recorded pulsation. Atrial-ventricular pacing allowed the stimulation of the heart at rates which were identical to the spontaneous rates prior to drug infusion. Subsequent changes in paced heart rate, in response to changes in the frequency of recorded pulsations, exactly duplicated the previous chronotropic response to the glucagon perfusion. Thus any changes in +dP/dt, −dP/dt, or LVP resulting from this procedure (henceforth referred to as the recorded chronotropic response) were due entirely to the chronotropic component of the drug effect. These changes then could be subtracted from the previous response in the same heart to detect any interval-independent inotropic effects of a maximally chronotropic dose of glucagon on spontaneously beating hearts. This procedure was employed to avoid the use of rapid pacing and its attendant alterations in basal conditions, thus eliminating any influences these alterations might have on the subsequent inotropic response to glucagon.

Effects of Glucagon on Coronary Flow Rates

For coronary flow studies, rat and guinea pig hearts were perfused via the aortic remnant at a constant pressure of 45 cm H₂O, and paced at 320 beats/min. The working heart was not used because of artifactually high coronary flow estimations, secondary to Thebesian flow, interatrial leakage, etc. (Neely and Rovetto, 1975). The pulmonary artery was cannulated with polyethylene tubing, and the outflow rate was determined volumetrically by recording the time in tenths of seconds per 2.00 ml outflow. To allow for Thebesian drainage, a 2-inch length of PE90 tubing was inserted into the left ventricle as described above. Right and left atria were tied off as thoroughly as possible. The estimate of coronary flow (CFR) was expressed as ml/min per gram wet weight.

Glucagon (10⁻⁸ M) or the vehicle (0.3 mM Tris) was infused 5 cm above the aortic remnant at 0.1 ml/min. The final dose of glucagon depended upon the total equilibrated flow rate during infusion, and varied slightly between hearts. Assuming complete mixing, the doses of glucagon administered to rat and guinea pig hearts were calculated to be 0.28 ± 0.03 and 0.17 ± 0.03 μM, respectively (n = 4). Glucagon or the vehicle was infused for 5 minutes. Flow rates during infusion reached equilibrium between 1.5 and 2.5 minutes.

Radioimmuno assay of Ventricular Cyclic AMP Concentrations

At various times after initiation of glucagon perfusion (10⁻⁷ or 10⁻⁸ M) of working hearts, the apex of each heart was frozen quickly with clamps cooled in liquid nitrogen. Portions of the frozen tissue were weighed, homogenized in ice-cold 6% trichloroacetic acid, and centrifuged at 5000 rpm for 20 minutes. The supernatant and standard solutions were extracted three times in five volumes of water-saturated ether (Harper and Brooker, 1975). Cyclic AMP concentrations were determined using the Beckton-Dickinson radioimmunoassay kit, and expressed as femtomoles cyclic AMP per milligram tissue (wet weight).

Statistics

Sample means were compared using Student’s t-test for either paired or unpaired samples. Differences were considered to be significant if P < 0.05.
Results

Chronotropic Effects of Glucagon

Glucagon caused concentration-dependent (10^-9 to 3 x 10^-7 M) increases in the rate of spontaneously beating rat and guinea pig hearts (Fig. 1). The pD2 values (rat, 8.29 ± 1.0; guinea pig, 8.28 ± 0.06) were not significantly different, and the maximal dose (10^-7 M) was found to be the same in the two species. Therefore, the chronotropic effects of glucagon in rat and guinea pig hearts were similar. Comparison of the maximum responses to cumulative administration with those of single maximal concentrations did not reveal any evidence of chronotropic desensitization to glucagon. The maximum response of the guinea pig heart to cumulative additions of glucagon was 53.7% over the basal rate, and the response to single maximal doses was 53.1% over control. Similarly, maximum responses of the rat heart to cumulative and single doses were 34.7 and 30.1%, respectively.

Inotropic Effects of Glucagon on Spontaneously Beating Hearts

Glucagon produced concentration-dependent (3 x 10^-9 to 3 x 10^-7 M) increases in contractility (+dP/dt) of spontaneously beating rat and guinea pig hearts (Fig. 2). The maximum inotropic concentration (10^-7 M) was the same in both species. However, the inotropic response of the rat heart to the maximum concentration of glucagon was approximately 4-fold greater than that of the guinea pig heart.

Comparison of responses to single and cumulative concentrations showed that desensitization occurred during cumulative administration of glucagon to the rat heart, but not to the guinea pig heart (Fig. 2). A low concentration of glucagon (10^-8 M) produced the same response in the rat heart whether administered alone or cumulatively. However, the responses to higher single concentrations (10^-7 and 3 x 10^-7 M) were greater than the responses to the same cumulative concentrations. In contrast, the highest single and cumulative concentrations (3 x 10^-7 M) produced similar responses in the guinea pig. Increasing the concentration of glucagon up to 3 x 10^-6 M did not cause any further increases in +dP/dt of the spontaneously beating guinea pig heart (not shown). Thus, although the chronotropic effects of glucagon on the spontaneously beating rat and guinea pig hearts were similar, the inotropic effects were markedly different.

Separation of Interval-Dependent and Interval-Independent Inotropic Effects of Glucagon

The marked differences in magnitude of the responses of rat and guinea pig hearts suggested that the inotropic response of the guinea pig heart may have been, at least in part, secondary to changes in the interval between contractions. The interval-dependent and interval-independent inotropic effects of glucagon on rat and guinea pig hearts therefore were resolved according to the following procedure (for details, see Methods). Each heart was perfused for 3-5 minutes with glucagon (10^-7 M) and the response was recorded on magnetic tape. After washout, the same heart then was paced by playing back the recorded chronotropic response. Thus the mechanical changes resulting from glucagon perfusion, and those which were secondary only to its chronotropic effects, could be separated and compared. Representative recordings of the effects of glucagon perfusion (10^-7 M) and of the recorded...
chronotropic response on LVP, dP/dt, and HR of spontaneously beating rat and guinea pig hearts are shown in Figure 3. The effects of glucagon perfusions are shown on the left, and the effects of the recorded chronotropic response on the same heart are shown on the right.

Glucagon produced marked and sustained increases in LVP, +dP/dt, -dP/dt, and HR of the working rat heart (Fig. 3, top left). The recorded chronotropic response changed the increase in LVP to a decrease. However, small increases in +dP/dt and -dP/dt occurred during the early phase of the recorded chronotropic response (Fig. 3, top right). These increases returned to baseline as the recorded chronotropic response reached its maximum. Thus, in the rat, responses to glucagon perfusion and to the recorded chronotropic response were markedly different.

In the guinea pig heart, however, glucagon perfusion caused a decrease in LVP, an increase in +dP/dt, a small and transient increase in -dP/dt, and a sustained increase in HR (Fig. 3, bottom left). The recorded chronotropic response virtually duplicated these effects (Fig. 3, bottom right). Slight temperature variations caused by the changing of buffer solutions probably account for the small differences between glucagon perfusion and the recorded chronotropic response in the illustrated example.

The effects of glucagon and the recorded chronotropic response on TPP of rat and guinea pig hearts (Table 1) further illustrate the specific differences with respect to interval dependence of the effect. Glucagon perfusion significantly reduced TPP in both species. The recorded chronotropic response duplicated this effect in the guinea pig heart but had no effect on the rat heart. Thus, the glucagon-induced reduction in TPP of the guinea pig heart was much greater than that of the rat heart.

The effects of glucagon and the recorded chronotropic response on TPR of rat and guinea pig hearts (Table 2) further illustrate the specific differences with respect to interval dependence of the effect. Glucagon perfusion significantly reduced TPR in both species. The recorded chronotropic response virtually duplicated these effects (Fig. 3, bottom right). Slight temperature variations caused by the changing of buffer solutions probably account for the small differences between glucagon perfusion and the recorded chronotropic response in the illustrated example.

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pig heart could be attributed entirely to the increase in frequency, but in the rat heart appeared to be a direct drug effect.

Changes in +dP/dt vs. changes in HR in response to glucagon and to the recorded chronotropic response are compared in Figure 4. For a given increase in HR, glucagon perfusion increased contractility of rat hearts much more than did the recorded chronotropic response. However, the inotropic responses of the guinea pig heart to glucagon and to the recorded chronotropic response were not statistically different. These results show that the inotropic response of the guinea pig heart to a maximally chronotropic dose of glucagon was entirely the result of the concomitant change in the interval between beats. The results also demonstrate the substantial interval-independent inotropic effects of glucagon in the rat heart.

### Effect of Glucagon on Contractility and Ventricular Cyclic AMP Levels of Paced Rat and Guinea Pig Hearts

Perfusions of glucagon (10^{-7} M) increased +dP/dt, −dP/dt, LVP, and cyclic AMP levels of the paced (300 beats/min) rat heart (Fig. 5). The increase in rat ventricle cyclic AMP preceded the increases in LVP and +dP/dt, although all measurements reached the maximum within 60 seconds, and remained elevated throughout the 3-minute perfusion period. However, glucagon (10^{-7} M) had no significant effect on dP/dt, LVP, or cyclic AMP levels of the guinea pig heart up to 180 seconds of perfusion.

The possibility that a higher dose of glucagon might affect one or more of these measurements in the paced guinea pig heart was tested by perfusing the hearts with glucagon at a dose of 10^{-5} M. The results are summarised in Table 2. Perfusion of 10^{-5} M glucagon slightly increased +dP/dt and −dP/dt, reaching a maximum at or before 120 seconds. At this time point, glucagon had no effect on LVP, TPP, or ventricular cyclic AMP levels.

A comparison of the inotropic responses of paced rat and guinea pig hearts to the maximum effective doses of glucagon in each species is illustrated in Figure 6. Glucagon increased the mechanical activity of the rat heart to a much greater extent than that of the guinea pig, even though the rat heart required 100-fold less glucagon to obtain a maximum response (Fig. 6, A and B). Isoproterenol perfusion resulted in a marked dose-dependent increase in contractility (Fig. 6C), suggesting that the relatively small response of the guinea pig heart was not a consequence of a general insensitivity of this preparation to the inotropic effects of drugs. The effect of isoproterenol on the guinea pig heart was similar in magnitude to the effect of glucagon on the rat heart.

### Effects of Glucagon on Coronary Flow Rates of Paced Rat and Guinea Pig Hearts

Glucagon (2–3 × 10^{-7} M) significantly increased coronary flow rates (CFR) of both rat and guinea pig Langendorff-perfused hearts (Table 3). Infusion of the vehicle alone caused a slight but significant vasoconstriction in both rat and guinea pig hearts. The overall effect of glucagon therefore was determined as the difference between the effect of the
A. GLUCAGON (10⁻⁵Μ) - Guinea Pig -

B. GLUCAGON (10⁻⁷Μ) - Rat -

C. ISOPROTERENOL - Guinea Pig -

FIGURE 5  Effect of a single 3-minute perfusion of 10⁻⁷ M glucagon on left ventricular dP/dt (top panel), and ventricular cyclic AMP levels (lower panel) of paced rat and guinea pig hearts. Hearts were paced at 300-320 beats/min. The circles show the responses of the rat hearts, and the squares show the lack of corresponding responses of the guinea pig hearts (n = 4-6). In the upper panel, the filled and unfilled symbols denote + and −dP/dt, respectively.

The effects of glucagon on paced (5 Hz) isovolumic heart preparations were also tested by infusing the drug for 5 minutes into two hearts from each species at a final concentration of 6 × 10⁻⁶ M. The aortas were perfused with Chenoweth-Koelle buffer at a constant flow sufficient to generate a pressure of 70 mm Hg. Basal +dP/dt values were between 800 and 1000 mm Hg/sec. Glucagon increased LVP and +dP/dt of both rat hearts by more than 2-fold, but had no effect on either of the guinea pig hearts.

**Table 2**  Effects of Glucagon (10⁻⁵ M, 60 sec) on Left Ventricular Pressure (LVP), Rate of Pressure Development (+dP/dt), Time-to-Peak Pressure, and Cyclic AMP Levels of the Working Guinea Pig Heart

<table>
<thead>
<tr>
<th>ALVP(%)</th>
<th>Δ+dP/dt(%)</th>
<th>ATPP(msec)</th>
<th>cAMP(fmoles/mg)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>9.2 ± 3.6*</td>
<td>7.4 ± 2.6*</td>
<td>−2.0 ± 2.0</td>
<td>296 ± 8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 5

* Significant change (P < 0.05), paired t-test.
† Control values were obtained from untreated hearts. Glucagon had no significant effect on cyclic AMP levels (unpaired t-test).

**Discussion**

The likelihood that a cardiostimulatory agent will increase cyclic AMP levels can be predicted on the basis of the characteristics of the inotropic response. Both cyclic AMP-associated and cyclic AMP-independent agonists increase maximum tension development and rates of tension development and decline at constant temperature and frequency of contraction (Tsien, 1977). However, agonists that increase cyclic AMP levels have been reported to reduce time to peak tension or pressure (Edmands et al., 1969; Osnes et al., 1978), whereas inotropic agents that do not elevate cyclic AMP concentrations do not shorten the duration of systole, regardless of the magnitude of the increase in maximum tension (Ledda et al., 1975; Ebner and Reiter, 1979).

Among previous studies suggesting a lack of correlation between cyclic AMP levels and contractile...
The lack of any detectable inotropic response of guinea pig working hearts to a maximally chronotropic dose of glucagon \((10^{-7} \text{ M})\) could not be attributed to impaired drug delivery to the ventricles. Glucagon, at a dose exceeding \(10^{-7} \text{ M}\), significantly increased coronary flow rates (CFR) of both rat and guinea pig hearts (Table 3). The increase in CFR of the rat heart may have been, at least in part, secondary to the increased ventricular performance. Glucagon-induced increases in canine and human CFR have been characterized as being secondary to the concomitant increases in cardiac output, metabolic activity, and \(O_2\) consumption (Manchester et al., 1970; Moir and Nayler, 1970; Simaan and Fawaz, 1976). However, this cannot explain the effects of glucagon on guinea pig CFR, because glucagon did not affect contractility (Fig. 5) or phosphorylase \(a\) activity (Henry et al., 1975) in this species. Therefore, glucagon seems to produce a direct vasodilatory effect on the guinea pig coronary vasculature. A similar direct effect of subinotropic doses on the rat heart would partially explain the reported protection against hypoxia (Busuttil et al., 1976).

The majority of studies concerned with the role of cyclic AMP in the inotropic response to glucagon support the second messenger hypothesis (for review, see Tsien, 1977), but some investigations have suggested that the two events may be dissociated. La Raia et al. (1968) found that both glucagon and isoproterenol increased rat ventricular pressures but that only isoproterenol increased cyclic AMP levels. However, the inotropic response to glucagon was smaller than the response to isoproterenol, so that subtle increases in cyclic AMP levels may have escaped detection. Recent studies have demonstrated discrete pools of cyclic AMP generation which may or may not accompany inotropic changes (Hayes et al., 1980). Mayer et al. (1970) and Øye and Langwed (1972) did not observe increases in cyclic AMP concentrations or in phosphorylase \(a\) activity of the rat heart before the inotropic effects of glucagon took place. However, Brunt and McNeill (1978) did detect increases in

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**Table 3** Effects of Glucagon \((2-3 \times 10^{-7} \text{ M})\) on Coronary Flow Rates (CFR) of Paced (320/min) Rat and Guinea Pig Langendorff-Perfused Hearts

<table>
<thead>
<tr>
<th>Species</th>
<th>CFR (ml/min per g)</th>
<th>Vehicle</th>
<th>Glucagon</th>
<th>Δ (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Infusion</td>
<td>Control Infusion</td>
<td>Δ (%)*</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>3.2 ± 0.3 3.0 ± 0.3</td>
<td>3.1 ± 0.2 3.4 ± 0.2</td>
<td>+17.2 ± 3.9†</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>5.5 ± 0.9 5.0 ± 0.8</td>
<td>5.4 ± 0.7 5.7 ± 0.8</td>
<td>+13.4 ± 1.6†</td>
<td></td>
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</tbody>
</table>

Glucagon \((10^{-4} \text{ M})\) or the vehicle alone \((0.3 \text{ mM Tris}\) was infused at 0.1 ml/min just above the aortic root. Based on the total flow rates (at a constant pressure head of 45 cm H2O), and assuming complete mixing, the doses of glucagon administered to rat and guinea pig hearts were 0.28 ± 0.03 and 0.17 ± 0.03 \(\mu M\), respectively. Time of infusion required to reach stable levels varied from 1.5 to 2.5 minutes. Flow rates were determined volumetrically from pulmonary arterial outflow. Values given are the means ± SEM, \(n = 4\).

* Effect of glucagon as the mean of the differences between the effects of the vehicle and the drug solution on each heart.
† Significant change \((P < 0.5)\), paired \(t\)-test.

Responses to cardiostimulatory agents, some have shown an increase in one of these variables without an associated increase in the other. In one such study, Henry et al. (1975) compared the responses of paced, isovolumic rat and guinea pig hearts to infusions of glucagon, and found that glucagon produced increases in contractility of apparently similar magnitude in each species, but increased ventricular cyclic AMP levels only in the rat heart. Their hypothesis, that positive inotropy and cyclic AMP generation was dissociated in the guinea pig heart, rested on the implicit assumption that glucagon exerted both quantitatively and qualitatively different effects on the hearts of both species. However, these workers restricted their criteria of inotropy to peak ventricular pressure and rate of ventricular pressure development \(+dP/dt\), two variables which can be influenced either by cyclic AMP-associated or by cyclic AMP-independent mechanisms.

The results of the present study show that the inotropic effects of glucagon on rat and guinea pig hearts differed markedly from each other. At constant preload (filling pressure), afterload (peripheral resistance), and frequency of stimulation, glucagon \((10^{-7} \text{ M})\) produced marked increases in \(+dP/dt\), \(-dP/dt\), and LVP, and significantly decreased TPP of the working rat heart (Figs. 5 and 6; Table 2). Collectively, these effects are characteristic of cyclic AMP-dependent responses, and in fact were associated with increases in cyclic AMP levels (Fig. 5). However, under identical conditions, the same dose of glucagon had no effect on any of these measurements in the guinea pig heart (Fig. 5). Increasing the dose of glucagon 100-fold produced only moderate increases in LVP, \(+dP/dt\), and \(-dP/dt\) without altering TPP (Fig. 6 and Table 2). Therefore, the inotropic effects of glucagon on the guinea pig heart differed both quantitatively and qualitatively from those on the rat heart. As may be expected, even this very high dose of glucagon had no effect on guinea pig ventricular cyclic AMP levels (Table 2).
the former measurements which preceded the inotropic response of the rat heart. These results were confirmed in the present study (Fig. 4). Glucagon increased maximum tension and rate of tension development of fetal mouse hearts, but had no effect on adenylate cyclase activity (Wildenthal et al., 1975). In the latter study, however, glucagon also had no effect on total duration of contraction or on the time to peak tension. Therefore, it seems that a demonstration of glucagon-induced inotropic responses which can be clearly dissociated from cyclic AMP generation remains to be established.

The marked differences between the results of Henry et al. (1975) and those of the present study cannot be explained easily. One possibility, that the two studies employed different experimental preparations (isovolumic vs. working hearts), does not seem likely. Glucagon produced nearly identical responses in the isovolumic and working rat hearts. Further, the working rat and guinea pig hearts responded similarly to the chronotropic actions of glucagon (Fig. 1). An alternative possibility, that the working guinea pig heart is relatively insensitive to the inotropic effects of drugs, also seems unlikely. Isoproterenol produced a response in the guinea pig heart that resembled the inotropic effect of glucagon on the rat heart (Fig. 6), confirming that the working guinea pig heart is capable of responding to cyclic AMP-generating agonists in the characteristic fashion (Flynn et al., 1978; Rodgers et al., 1979). Also, the guinea pig preparation was sufficiently sensitive to allow the resolution of subtle mechanical changes which were dependent upon small variations in frequency of contraction (Fig. 4). In addition, neither the rat nor the guinea pig heart showed any evidence of chronotropic desensitization to glucagon, whereas only the rat heart exhibited an inotropic desensitization to the drug.

Finally, in the present study, glucagon infusion (6 $\times$ $10^{-8}$ M) had no effect on isovolumic guinea pig hearts, but doubled both LVP and +LVdP/dt of isovolumic rat hearts. These results reaffirm the hypothesis that the inotropic effects of glucagon on the rat ventricle, which are largely independent of the frequency of contraction, are distinct from its inotropic effects on the guinea pig ventricle, which are almost completely dependent upon the changes in heart rate.

Previous studies have not clearly demonstrated a consistent positive inotropic response of guinea pig ventricular muscle to glucagon. Kobayashi et al. (1971) reported that a single injection of glucagon (3 $\mu$g) increased maximum tension development of the paced, Langendorff-perfused guinea pig heart. However, these effects were neither consistent nor quantified. Prasad (1975) found that glucagon had no effect on guinea pig papillary muscles at concentrations up to 10 $\mu$g/ml ($3 \times 10^{-8}$ M), but indicated that glucagon increased tension of guinea pig atria.

Glucagon, unlike isoproterenol, failed to restore contractions of potassium-depolarized guinea pig hearts, and did not affect ventricular cyclic AMP levels. The observation that a maximally chronotropic dose of glucagon exerted no direct inotropic effects (Fig. 4) lends support to the suggestion (Prasad, 1975) that the guinea pig heart exhibits regional differences in sensitivity to glucagon.

Much evidence indicates that rat ventricular muscle preparations, unlike those of other mammalian species, show negative force-frequency relationships (Benforado, 1958; Hoffman and Kelley, 1959; Koch-Weser and Blinks, 1963). When the chronotropic effects of glucagon were separated from its inotropic effects, it was found that the increase in frequency alone was associated with increases in +dP/dt of both rat and guinea pig hearts (Figs. 3 and 4), albeit to a lesser extent in the rat heart. Henry (1975) reported a positive staircase effect in the isovolumic rat heart, which was similar in magnitude to the effect shown in Figure 4 over the appropriate frequency range. Henry also reversed the negative staircase of rat papillary muscle to a positive one by increasing the glucose concentration in the medium, and hypothesized that isolated rat ventricular muscle preparations required relatively greater metabolic support than corresponding preparations from other species. These findings also suggest that both the working and isovolumic rat heart preparations meet these nutritional requirements more effectively than papillary muscle or ventricular strip preparations.

Increasing the frequency of stimulation also reduced TPP of the guinea pig, but not of the rat heart (Table 1). These effects are not associated with changes in cyclic AMP levels (Dobson et al., 1976). Similar species differences in the effects of frequency on characteristics of ventricular muscle contraction have been attributed to variations in the relative dependence on sarcoplasmic reticular vs. sarcotomal calcium stores for beat-to-beat regulation (Langer, 1965; Reiter, 1966; Henderson et al., 1969; Boden and Sonnenblick, 1975).

In summary, the chronotropic effects of glucagon on rat and guinea pig working hearts were found to be similar, but the inotropic effects were not. Glucagon produced inotropic responses of the rat heart which were much greater in magnitude than the effects on the guinea pig heart, and only increased rat heart cyclic AMP levels. The results therefore do not support the proposed dissociation between positive inotropy and cyclic AMP generation in the guinea pig heart. They further suggest that the guinea pig heart, unlike the rat heart, exhibits regional differences in sensitivity to the effects of glucagon.

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Responses of rat and guinea pig hearts to glucagon. Lack of evidence for a dissociation between changes in myocardial cyclic 3’5’-adenosine monophosphate and contractility.

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