**Glucagon**

**ITS ENHANCEMENT OF CARDIAC PERFORMANCE IN THE CAT AND DOG AND PERSISTENCE OF ITS INOTROPIC ACTION DESPITE BETA-RECEPTOR BLOCKADE WITH PROPRANOLOL**

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**ABSTRACT**

The action of glucagon on cardiac performance was studied in 21 isolated cat papillary muscle preparations, in 13 spontaneously beating cat atria, in 15 intact dog hearts, and in 4 isolated perfused dog hindlimbs. In each papillary muscle preparation, addition of glucagon produced marked increases in maximal developed tension, averaging $36 \pm 4.2\% \text{ (SEM)} \ (P < 0.01)$, and shifted the force-velocity curve upwards and to the right, indicating that contractility was augmented. Glucagon always increased the rate of the spontaneously beating atrium, the rise averaging $28.8 \pm 5.5 \text{ contractions/min} \ (P < 0.01)$. In the dog, myocardial performance was markedly augmented by the administration of glucagon, $50 \mu g/kg$ iv, as indicated by an average increase of $72.2 \pm 18.4\% \ (P < 0.01)$ in the left ventricular peak $dP/dt$ and of $58.9 \pm 12.8\% \ (P < 0.01)$ in force recorded by a strain gauge arch, despite an average decrease of $3.8 \pm 1.2 \text{ cm H}_2\text{O} \ (P < 0.02)$ in left ventricular end-diastolic pressure. Heart rate rose an average of $38.7 \pm 10.9 \text{ beats/min} \ (P < 0.02)$. Small but significant decreases in peripheral vascular resistance were produced. Single intravenous injections produced effects lasting 15 to 20 minutes. Propranolol did not prevent the inotropic responses in either the cat or dog preparations but markedly decreased the chronotropic effects.

**ADDITIONAL KEY WORDS**
treatment of heart failure cyclic AMP theory of beta-receptors comparison with catecholamines adenyl cyclase effects on vascular bed

As a result of extensive and elegant biochemical research carried on during the past ten years, it now seems clear that many of the effects of stimulation of beta-receptors result from activation of the adenyl cyclase enzyme system, which catalyzes the reaction leading to the production of adenosine 3', 5'-monophosphate (cyclic AMP) from ATP (1). Studies in a variety of isolated tissues have demonstrated that one of the most powerful stimulants of this adenyl cyclase system is glucagon, which appears to have effects that may be even greater than those produced by epinephrine (1, 2). Furthermore, it has been postulated that the adenyl cyclase system is, in fact, the beta-receptor (3). Since it is generally agreed that the positive inotropic and chronotropic effects produced by catecholamines and nerve stimulation are the result of stimulation of beta-receptors, we studied the effects of glucagon on cardiac performance.

Because they believed that some of the reported cardiac effects demonstrated by insulin could be accounted for by glucagon that was present as a contaminant, Farah and Tuttle examined the action of glucagon on the heart of the intact dog and also in
the heart-lung preparation (4). In the intact dog, they observed no cardiac effects, but in the isolated heart-lung preparation they noted that administration of glucagon caused an increase in cardiac output, together with a decrease in right atrial pressure. This effect of glucagon seemed most apparent after the heart had been depressed by barbiturates. Regan and his coworkers studied the effects of glucagon in closed-chest dogs by infusing the drug directly into the left coronary artery (5); they found that the maximum rate of pressure rise in the left ventricle increased. Whitehouse and James studied the chronotropic effect of glucagon by infusing the agent directly into the sinus node artery of dogs and found that it significantly increased heart rate (6). All these investigators reported that the cardiac effects of glucagon could be virtually abolished by treatment with either of the beta-receptor blocking agents, dichlo roisoproterenol and pronethalol (4-6).

In the present study, the effects of glucagon on cardiovascular function were assessed in several experimental preparations including the cat papillary muscle, the isolated cat atrium, the intact dog heart and the isolated perfused canine hindlimb. In addition, the effects of beta-receptor blockade produced by propranolol on the actions of glucagon were investigated.

Methods

Right ventricular papillary muscles were obtained from 14 normal cats and from 7 cats in which the endogenous catecholamine stores had been depleted by the administration of reserpine, 3 mg/kg ip, on each of the two days preceding the study (7). The muscles, which had an average cross-sectional area of 1.37 ± 0.16 mm² (SEM), were suspended in a myograph filled with Krebs bicarbonate solution that was bubbled with 95% O₂-5% CO₂ and that was held at a constant temperature of 30°C. Field stimulation was carried out at a rate of 12/min with platinum electrodes placed parallel to the muscle, using pulses of 5-msec duration and voltages 10% above threshold. The effects of glucagon on maximal actively developed tension, on the rate of tension development (dF/dt), and on the time required to reach peak tension were studied with the muscle maintained at the peak of its length-active tension curve. In 7 of the muscles obtained from normal cats, concentration-response curves to glucagon were obtained over a range of concentrations extending from 1.45 X 10⁻⁹ to 14.5 X 10⁻⁴ M (0.05 to 50 μg/ml) by adding small aliquots (0.1 ml) of increasing concentrations of glucagon. In 5 of these experiments the response to glucagon was determined before and after beta-receptor blockade had been produced by dl-propranolol in a concentration of 10⁻⁴ M (8), and in the remaining 2 experiments the effects of glucagon were observed before and after glycopyrrolate had been blocked by the addition of iodoacetic acid, 5 X 10⁻⁴ M (9). One of the muscles that had been subjected to beta-receptor blockade was further treated with phenoxybenzamine, 10⁻⁵ g/ml, and the effects of glucagon were again determined. The 7 muscles obtained from cats treated with reserpine and the remaining 7 muscles obtained from normal cats were tested with a dose of 5.7 X 10⁻⁶ M (20 μg/ml) glucagon, and 3 of the muscles treated with reserpine were also treated with dl-propranolol, 10⁻⁴ M. Force-velocity curves were obtained from 8 of the normal papillary muscles and from 8 of the reserpine-treated muscles, with a preload of 0.5 g. The data are reported in terms of the velocity at 0.5 g, although the same qualitative results would be observed using the velocity obtained by extrapolating the force-velocity curve back to zero load.

The direct chronotropic effect of glucagon was investigated in 13 spontaneously beating cat atria that were suspended in a myograph and attached to a force transducer. Nine of these atria were obtained from normal cats and 4 from cats that had been treated with reserpine as described above. Two of the normal atria and 2 of the reserpine-treated atria were also treated with dl-propranolol, 10⁻⁴ M, prior to testing with glucagon.

The cardiovascular effects of glucagon were studied in 15 open-chest dogs that had been anesthetized with pentobarbital, 30 mg/kg iv. Two of these animals had been treated with reserpine in a total dose of 0.5 mg/kg iv, administered in two equally divided doses on the two days preceding the experimental study, and analysis of left atrial and left ventricular tissue samples confirmed that complete catecholamine depletion had been produced. They were all artificially ventilated by a Harvard respirator. A Walton-Brodie strain gauge arch was sewn to the right ventricle in 12 of the dogs and it was attached to the left ventricle in the remaining 3; contractile force was not calibrated in absolute terms but was assessed in terms of millimeters of deflection.

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Left ventricular pressure was measured by a Statham P23Db manometer attached directly to a wide-bore metal cannula that was inserted into the left ventricle through the apical dimple. The signal from the manometer was recorded at low and high sensitivity and was also differentiated to give the instantaneous dP/dt. Arterial pressure was recorded from the femoral artery, and drugs were injected through a catheter inserted into the femoral vein.

The 15 dogs studied were divided into two groups. In group 1, composed of 10 animals, responses to three doses of glucagon (0.5, 5, and 50 µg/kg) were recorded. In 4 of these dogs glucagon was also injected after beta-receptor blockade had been produced by injection of dl-propranolol, 2 mg/kg iv; in 4 dogs glucagon was given after administration of ouabain, 0.04 to 0.06 mg/kg; in 2 dogs it was given after 10 units of regular insulin had been injected to prevent the rise in blood sugar that would ordinarily occur after glucagon; in 3 it was given after bilateral cervical vagotomy; and in 1 it was given after the heart had been depressed by a large dose of pentobarbital. Several of these procedures were sometimes carried out in a single experimental study.

In group 2, composed of 5 dogs, responses to both glucagon and to isoproterenol were recorded before and after beta-receptor blockade had been produced by propranolol, 0.068 to 0.5 mg/kg. A much smaller dose of propranolol was given to these animals than was used in those receiving propranolol in group 1, to minimize the cardiac depression produced by this beta-receptor blocking agent. The actual dose of propranolol given was determined by infusing an amount that produced a barely detectable fall in right ventricular contractile force. Doses of glucagon employed were 0.5, 5, and 50 µg/kg; doses of isoproterenol were 0.01, 0.033, and 0.1 µg/kg.

After administration of propranolol, glucagon and isoproterenol were injected alternately and the variables were allowed to return to control levels before the next injection was made. All of these dogs were vagotomized.

The direct effects of glucagon on the peripheral vascular bed were investigated in 4 dogs. In these animals, the hindlimb was perfused at a constant flow rate by a sigma-motor pump, as has been described in detail previously (10). Changes in perfusion pressure, therefore, were indicative of changes in hindlimb vascular resistance. Glucagon, 15 to 50 µg/kg, was injected into the perfusion circuit immediately proximal to the pump, and the effects on perfusion pressure were determined.

In all experiments, the diluent in which the glucagon was ordinarily dissolved was administered alone for control purposes. Statistical analysis of the results was carried out using Student's two-tailed t-test for paired data (11).

Results

Cat Papillary Muscles

There were no statistically significant differences in the responses of muscles obtained from normal cats compared to those from animals treated with reserpine. In addition, responses of the muscles exposed to a glucagon concentration of $5.7 \times 10^{-6}$ M were quantitatively similar to the responses observed in...
Effect of glucagon on the isolated cat papillary muscle. Glucagon produced significant increases in active tension (panel A) and the rate of force development (panel B) but did not significantly alter the time between the onset of contraction and the development of peak tension (panel C). The average values for the group before and after treatment are shown by the short horizontal lines on either side.

Panel A: Representative force-velocity curves determined in a cat papillary muscle preparation before and after treatment with glucagon. This muscle was obtained from a cat that had been treated with reserpine to deplete its catecholamine stores. The shift in the curve upward and to the right produced by glucagon indicates augmented contractility.

Panel B: Effects of glucagon on the velocity of contraction determined at a load of 0.5 g. An increase in the intrinsic speed of contraction, the hallmark of increased contractility, was observed in every muscle so studied. Mean values are shown by the short horizontal lines.
INOTROPIC AND CHRONOTROPIC EFFECTS OF GLUCAGON

Concentration-response curves to glucagon obtained in the cat papillary muscle preparation before and after beta-receptor blockade with propranolol show that although the response to glucagon was lessened at the lower dose levels it was not affected at the higher dose levels. Vertical lines = SEM.

As illustrated in Figure 2, panel A, the addition of glucagon caused an augmentation of maximal actively developed tension in every study, the rise averaging 36 ± 4.2% (SEM) (P < 0.01), increasing from a control value of 4.06 ± 0.24 g/mm² to 5.56 ± 0.40 g/mm². The augmentation of contractile force resulted primarily from an increase in the rate of force development, which rose an average of 42.1 ± 5.52 (P < 0.01), from a control level of 16.2 ± 1.0 g/sec/mm² to 22.9 ± 1.6 g/sec/mm² (Fig. 2, panel B); no significant changes in time to peak tension were noted (Fig. 2, panel C).

The force-velocity relation was shifted by exposure to glucagon upward and to the right in all 12 studies in which this relationship was determined, as illustrated by a representative experiment in Figure 3, panel A. The velocity of muscle shortening measured at a load of 0.5 g for all the muscles is plotted in Figure 3, panel B, and shows that velocity was increased significantly in each study (P < 0.01), rising by an average of 56.2 ± 11.7%, from a control value of 5.82 ± 0.89 to 8.46 ± 1.0 muscle lengths/sec/mm².

As shown in Figure 4, beta-receptor blockade produced by a high concentration of propranolol did not significantly change the magnitude of the augmentation in active tension produced by the large doses of glucagon, but markedly lessened the responsiveness to those muscles exposed to a glucagon concentration of $14.5 \times 10^{-6}$ M, which means that at this concentration the muscles had reached the plateau of their dose-response curve. Therefore, for the purposes of discussion, the data from these various groups of muscles have been pooled. Figure 1 shows a representative tracing from an experiment in which the effect of glucagon on actively developed tension was studied before and after beta-receptor blockade had been produced by propranolol. It is evident that marked increases in force were produced both in the control period and following beta-receptor blockade, although the effect of isoproterenol was completely blocked following propranolol.

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Comparison of the effects of glucagon and norepinephrine. Doses of these drugs were chosen that produced comparable peak effects. However, although the effects of norepinephrine lasted less than two minutes, the action of glucagon was considerably longer-lasting. Though not shown in the figure, the effects of a single injection of glucagon lasted for over 15 minutes.

SPONTANEOUSLY BEATING CAT ATRIUM
The rate of the spontaneously beating cat atrium always rose ($P < 0.01$) following exposure to $1.2 \times 10^{-6}$ M to $2.9 \times 10^{-6}$ M glucagon by an average value of $28.8 \pm 5.5$ beats/
INOTROPIC AND CHRONOTROPIC EFFECTS OF GLUCAGON

FIGURE 7

Effect of intravenously administered glucagon on the maximum rate of left ventricular pressure development (panel A), on contractile force (panel B), on left ventricular end-diastolic pressure (panel C), and on heart rate (panel D). The average values for the group before and after treatment are shown by the short horizontal lines on either side.

INTACT DOG HEART

In the 10 dogs in group 1, glucagon strikingly augmented the contractile state of the myocardium in a dose-dependent manner as shown by a typical tracing in Figure 6. The striking effects on the rate of pressure development and on contractile force produced by the intravenous administration of 50 μg/kg glucagon are plotted in Figure 7, panels A and B. Left ventricular dP/dt rose by an average of 72.2 ± 18.4% (P < 0.01), increasing from a mean level of 1424 ± 80 to 2645 ± 332 mm Hg/sec, and the force recorded by a strain gauge arch rose by an average of 58.9 ± 12.8% (P < 0.01) increasing from a control level of 18.9 ± 0.7 to 29.9 ± 2.4 mm of deflection. These increases occurred despite an average decrease in left ventricular end-diastolic pressure of 3.8 ± 1.2 cm H2O (P < 0.02) (Fig. 7, panel C). These effects on myocardial contractility occurred even when heart rate was held constant by electrical pacing. In these studies, when heart rate was not controlled, it rose an average of 38.7 ± 10.9 beats/min (P < 0.02), increasing from a mean value of 177.6 ± 12.7 to 216.3 ± 6.2 beats/min (Fig. 7, panel D). Mean arterial pressure fell significantly (P < 0.01) from an average control level of 91 ± 5 mm Hg to 81.4 ± 7.2 mm Hg, representing a decrease of 12.1 ± 3.2%. Depletion of catecholamines by treatment with reserpine did not significantly alter the cardiovascular response to glucagon. Furthermore, beta-receptor blockade did not prevent the augmentation in myocardial performance produced by glucagon. After administration of subtoxic doses of ouabain (12), glucagon still produced a significant positive inotropic effect as manifested by
Comparison of the effects of glucagon and isoproterenol before and after beta-receptor blockade produced by propranolol. Panels A and B illustrate the finding that although the effects of isoproterenol (triangles) on right ventricular contractile force and on left ventricular dP/dt were markedly decreased after treatment with propranolol, the effects of glucagon (circles) on these variables were not altered significantly. However, as shown in panel C, the positive chronotropic effects of these two drugs were strikingly lessened by administration of propranolol. Solid symbols connected by solid lines illustrate the data before beta-receptor blockade; open symbols connected by broken lines depict the data after blockade. Vertical bars = SEM.

Increases in dP/dt and in contractile force. Moreover, this positive inotropic effect was not accompanied by the production of premature contractions or arrhythmias. In two studies, glucagon abolished pulsus alternans that had developed spontaneously, and in one experiment it markedly improved the contractile state of a heart that had been intentionally depressed by the intravenous administration of pentobarbital. When the hyperglycemic effects of glucagon were prevented by treating two dogs with insulin, a positive inotropic effect comparable to that obtained in dogs not treated with insulin was still elicited by the intravenous injection of glucagon. Since the effects of glucagon were not significantly altered by treatment with either reserpine or insulin, the data from these experiments were combined with the results from untreated animals for the purpose of statistical analysis. Vagotomy did not impair the augmentation in contractile state produced by the subsequent administration of glucagon.

In the 5 dogs in group 2, direct comparisons were made between the effects of glucagon and isoproterenol in the same animal, and these data are illustrated in Figure 8. Although the effects of isoproterenol on right ventricular contractile force (P < 0.01) and on left ventricular dP/dt (P < 0.05) are significantly depressed after administration of propranolol, the effect of glucagon on these two variables is not significantly affected. However, the heart rate response to both glucagon (P < 0.02) and isoproterenol (P < 0.05) is decreased, although a positive chronotropic effect is still detectable with the higher doses of glucagon.

Tachyphylaxis to glucagon was not noted in these acute experiments. The responsiveness to glucagon remained unimpaired after as many as 8 injections and for 3 hours.

**Perfused Hindlimb**

Intra-arterial injection of glucagon, 15 to 50 μg/kg, into a limb perfused at a constant flow rate produced a decrease in vascular resistance (P < 0.05) of 10.6 ± 3.0%, as manifested by a fall in mean perfusion pressure from an average control value of 101.5 ± 14.5 mm Hg to 92.8 ± 13.1 mm Hg. This dilation occurred despite treatment with sufficient doses of propranolol to cause blocking of the responses produced by isoproterenol.
Discussion

It is apparent from the data obtained in this study that glucagon exerts marked positive inotropic and chronotropic effects as determined in the experimental preparations utilized. These results are consonant with the findings of other investigators who noted similar actions in the failing heart-lung preparation (4) and following direct intracoronary infusion (5, 6). It is of interest, however, that administration of small doses of propranolol had differential effects on the inotropic and chronotropic properties of glucagon (Fig. 8). Whereas the absolute increases in contractile force and left ventricular dP/dt were not significantly altered by beta-receptor blockade, the chronotropic action of glucagon was considerably less. This difference in the ability of propranolol to block the chronotropic versus the inotropic effects of glucagon was also apparent when percent increase, rather than absolute increase, was measured, since after beta-receptor blockade the control values were either the same or lower than before blockade. This disparity in responsiveness after beta-receptor blockade may indicate that glucagon exerts its major inotropic and chronotropic effects by different molecular mechanisms: it may produce its chronotropic effects mainly by stimulation of receptors that can be blocked by propranolol, and its inotropic action primarily by stimulation of receptors that cannot be blocked by propranolol.

From our data it is not possible to determine whether activation of the adenyl cyclase system, with consequent production of cyclic AMP, is responsible for the positive inotropic effects produced by glucagon. Although the positive inotropic effects of both glucagon and catecholamines may be mediated through cyclic AMP, definite proof of this hypothesis has not been obtained. If, however, this theory is correct, the fact that propranolol antagonizes the action of isoproterenol but does not interfere with the inotropic action of glucagon suggests the presence of different receptor types in the adenyl cyclase system, one that is activated by catecholamines and one that is stimulated by glucagon. It should be emphasized that adenyl cyclase is not a purified enzyme but is, rather, a particulate fraction of cells, the components of the fraction remaining unidentified. It is also possible that neither type of agent exerts its inotropic effects through cyclic AMP. Indeed, a recent preliminary report indicates that the tissue level of cyclic AMP does not increase in the rat heart after exposure to glucagon (13).

The fact that previous investigators demonstrated that dichloroisoproterenol blocked the inotropic action of glucagon may be the result of several factors (4, 5). First, dichloroisoproterenol has strong sympathomimetic actions. Therefore, the positive inotropic effect induced by the blocking drug may have obscured the effects of glucagon. Second, Farah and Tuttle used very large doses of dichloroisoproterenol (4), and Regan and associates injected it directly into the coronary circulation (5), so that a nonspecific type of depression may have been produced. In our experiments on cat papillary muscles, we noted that the effect of glucagon was attenuated at the lower end of the dose-response curve after administration of large doses of propranolol that depressed myocardial contractility (Fig. 4). It is possible that this type of nonspecific depression was the cause of the apparent blockade of the inotropic action of glucagon noted by these investigators. Finally, the possibility exists that dichloroisoproterenol interferes with the inotropic action of glucagon by an as yet undetermined mechanism. With regard to the chronotropic effects of glucagon, our findings are consistent with those of Whitehouse and James who observed that pronethalol decreased but did not abolish the increase in heart rate produced by glucagon (6).

Other possible mechanisms of action for glucagon were also investigated. The fact that iodoacetic acid does not block the action of glucagon indicates that its action is not dependent on a metabolic product of glycolysis beyond glyceraldehyde 3-phosphate in the Embden-Meyerhof pathway, since
formation of these products is prevented by treatment with iodoacetic acid. The effects of glucagon cannot be accounted for by its hyperglycemic action since treatment with insulin did not lessen the cardiac response in the intact dog and since the papillary muscles were studied in vitro. Similarly, it does not act by releasing catecholamines, because both in our studies and in the reports of others (4-6), treatment with reserpine does not prevent the inotropic action of glucagon. Finally, glucagon does not exert its effects through a vagolytic mechanism since it is equally effective in the vagotomized dog and since it also manifests its actions in the in vitro preparations.

The observed dissimilarities between glucagon and catecholamines may relate to the differences in their molecular weights: glucagon, 3485; isoproterenol, 211. As a result, their membrane permeabilities would probably differ, as would chemical reactions that would depend on surface phenomena. In addition, the enzymes for the metabolism of catecholamines, namely, catechol-O-methyl transferase and monoamine oxidase are widely distributed and account in large part for the short duration of action of the catecholamines; glucagon, on the other hand, is apparently inactivated in the liver through proteolytic degradation by enzyme systems that have not as yet been completely defined (14).

Glucagon may have some useful clinical applications. Its ability to decrease the left ventricular end-diastolic pressure and at the same time augment myocardial performance suggests that it may have a valuable place in the therapy of a failing heart. As stated previously, it is not possible to say from our data whether glucagon and the catecholamines work through the same final mechanism. However, certain differences are evident which may make glucagon more advantageous in the management of a patient with a cardiovascular disorder. First, the effects of glucagon may occur even in a fully digitalized preparation and are not accompanied by the production of premature contractions and arrhythmias; these side effects have been unfortunate and unavoidable consequences of therapy with the various catecholamines. Second, whereas the effects of single doses of norepinephrine that produce equivalent peak changes in myocardial performance last for less than 2 minutes, the effects of a single injection of glucagon in the dog continue for 15 to 20 minutes (Fig. 6), and tachyphalaxis to glucagon has not been noted. Third, since in the cat and dog its positive chronotropic effect can be antagonized by propranolol without decreasing its ability to augment myocardial contractility, it is possible that potentially dangerous sinus tachycardias that may be induced by the drug in man can be prevented or combated. Finally, there is the possibility that it may be useful in the treatment of acute congestive failure sometimes produced by administration of beta-receptor blocking agents.

It remains to be determined whether the increased oxygen consumption that inevitably results from the augmented myocardial contractility and increased heart rate (15) will adversely affect the clinical application of glucagon in the treatment of a failing myocardium, or whether the improved cardiac performance will reverse a progressively downhill course. The fact that glucagon produces a slight decrease in peripheral vascular resistance is probably desirable since it augments myocardial performance without, at the same time, increasing the external load against which the heart must labor.

In summary, glucagon has marked inotropic and chronotropic actions and these actions were not significantly altered by treatment with reserpine, insulin, or vagotomy. Propranolol, however, lessened the chronotropic action without affecting the inotropic action. In addition, the positive inotropic effects of glucagon were manifest despite full digitalization, and were not accompanied by the production of arrhythmias.
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References
Glucagon: Its Enhancement of Cardiac Performance in the Cat and Dog and Persistence of its Inotropic Action Despite Beta-Receptor Blockade with Propranolol

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