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ISOPROTERENOL AND GLUCAGON EFFECTS IN PERFUSED HEARTS FROM SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS*

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Abstract—The metabolic effects of isoproterenol and glucagon were compared in perfused hearts from spontaneously hypertensive (SH) Wistar rats and normotensive (NT) Wistar controls. Animals were used when either 13–15 weeks of age or when 40–44 weeks of age. Isoproterenol (3×10^{-8} M) or glucagon (10^{-7} M) was infused over a 3-min period of time. The hearts were subjected to freeze-clamping and emersion in liquid nitrogen and the powdered tissue was assayed for cyclic AMP, phosphorylase activity and glycogen. Glycerol release into the perfusate was also measured. The alterations in these parameters produced by isoproterenol were the same in both the SH and NT hearts. However, the hearts from the SH rats were less responsive to glucagon than those from the NT animals. Infusion of glucagon in isolated hearts of SH rats resulted in increases in cyclic AMP levels and phosphorylase activation which were only 25–50 per cent of those observed with the hearts from the NT rats. Glycerol release from the hearts of the SH rats was delayed after glucagon infusion and reached its maximum response 1 min after the maximum response was achieved in the hearts from the NT rats (45 sec). Infusion of glucagon resulted in a 30 per cent decrease in glycogen content of the hearts from NT rats but produced no significant change in the glycogen content of the hearts from SH rats. Cyclic AMP phosphodiesterase activity was found to be the same in the hearts of the two types of animals. These data demonstrate a diminished response to glucagon in hearts from SH rats possibly due to an alteration in the glucagon receptors.

THE METABOLIC and mechanical response in the rat heart to epinephrine and glucagon has been shown to be mediated by different receptors on the plasma membrane.[1] Also, stimulation of rat heart adenylate cyclase activity by epinephrine is blocked by dichloroisoproterenol and propranolol, but activation of the enzyme by glucagon is not influenced by these agents.[2] The adrenergic and glucagon receptors in the heart appear to form at different stages of development. In terms of adenylate cyclase stimulation and glycogenolysis, the epinephrine response is functional in the fetal rat heart while the glucagon response does not appear until about 4 weeks after birth.[3]

The present experiments were conducted to test for possible differences between the adrenergic and glucagon responses in perfused hearts from normotensive Wistar (NT) and the genetically hypertensive Wistar rats called the spontaneously hypertensive

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(SH) rats.[4] The rapid rise in systolic blood pressure in the SH animals occurs between 3–8 weeks of age or at the same period when the glucagon response is first apparent in the heart. Also, cardiac hypertrophy secondary to elevated systolic blood pressure has been found to begin at about 20 weeks of age in the SH rat. Therefore, hearts were obtained from animals 13–15 and 40–44 weeks old and the inotropic response, cyclic AMP content, phosphorylase activity, glycogen content and glycerol release were measured in perfused hearts before and after infusion of D,L-isoproterenol (IPR) or glucagon. Phosphodiesterase activity was also compared between NT and SH hearts.

METHODS

Fed, male SH or NT Wistar rats, 13–15 or 40–44 weeks old, were decapitated and their hearts quickly removed and perfused by a modified Langendorf procedure.[5] Blood pressure was measured in unanesthetized animals using a tail plethysmographic method[6] no more than 2 days before each experiment. Pressures of 150 mm of Hg were taken as evidence of hypertension. Isolated hearts were perfused with a Krebs–Henseleit bicarbonate solution containing the following concentrations: NaCl, 120 mM; KCl, 5·6 mM; $CaCl_2$, 1·22 mM; $MgSO_4$, 1·34 mM; NaH_2PO_4, 1·21 mM; glucose, 5·5 mM and $NaHCO_3$, 25·37 mM. The perfusing fluid was equilibrated with 95% O_2, 5% CO_2 and perfusate temperature maintained by 37°. The flow rate was maintained at 10 ml/min. A non-recirculating system was used and the perfusate collected for glycerol analysis.

The hearts were electrically paced at 300 beats/min and a constant force was placed on the hearts by passing a silk thread through the apex and attaching it to the lever arm of a Grass strain gauge, then adjusting to 10 *g* diastolic force as described previously.[7] D,L-Isoproterenol (IPR) and glucagon were dissolved in saline and infused into the perfusing buffer using the Harvard infusion pump. The final concentrations of IPR (3×10^{-8} M) and glucagon (1×10^{-7} M) entering the perfused hearts represented approximately half-maximal doses for the cardiac changes that were studied. Phosphorylase and cyclic AMP in these hearts were assayed after maximal levels had been achieved with a 3-min infusion of either glucagon or IPR.

Hearts were perfused for 30 min in order that the high initial release of glycerol could return to stable basal levels. IPR or glucagon was then infused for 3 min after which the hearts were clamp-frozen with aluminum blocks cooled in liquid nitrogen.[8]

Biochemical measurements. Hearts were powdered in a stainless steel mortar and pestle previously cooled with dry ice. Powdered tissues were stored at −70°. Phosphorylase *a* and total phosphorylase were assayed as described by Cori and Illingsworth as modified by Mayer *et al.*[9] Glycerol was determined enzymatically using a fluorometric assay.[10] Enzymes used in the assay were purchased from Boehringer Mannheim. Cyclic AMP was assayed by the methods of Gilman[11] and/or Steiner *et al.*[12] with materials for the latter method purchased from Collaborative Research, Inc., Waltham, Mass. Glycogen was assayed by an anthrone procedure.[13] Cyclic AMP phosphodiesterase activity in heart homogenates was assayed by the method described by Huang and Kemp.[14]

Statistical analysis. Data are presented as means ± S. E. Statistical analysis was performed by the Student *t*-test with $P<0.05$ considered significant between two groups of data.

RESULTS

Systolic blood pressure in SH rats rises progressively in the young animals[15] and is 55 per cent higher than the pressure found in the NT group in animals at 13–15 weeks (Table 1). A similar difference in blood pressure was found in animals 40–44 weeks of age between SH (195±5·0 mm of Hg) and NT (125±3·1 mm of Hg) animals. Body weights were greater in NT rats compared to SH animals of the same age group. There was no significant difference in heart weight in SH and NT animals at 13–15 weeks, while SH rats 40–44 weeks old exhibited cardiac hypertrophy when compared to the NT group. This resulted in a higher heart weight/body weight ratio in the older SH animals compared to NT controls.

TABLE 1. BLOOD PRESSURE, BODY WEIGHT AND HEART WEIGHT DIFFERENCES BETWEEN SH AND NT RATS IN THE TWO EXPERIMENTAL AGE GROUPS

Rats	13–15 weeks	40–44 weeks
Normotensive		
Blood pressure	121±3·5	125±3·1
Body wt	306±10·6	477±8·8
Heart wt	1·43±0·03	1·60±0·04
Heart wt/body wt	0·0047	0·0034
Spontaneously hypertensive		
Blood pressure	188±3·4	195±5·0
Body wt	274±4·6	389±8·0
Heart wt	1·46±0·02	1·91±0·04
Heart wt/body wt	0·0053	0·0049

The effects of submaximal doses of IPR or glucagon[7] on the inotropic response are summarized in Table 2. IPR infusion resulted in an inotropic response in both the SH and NT hearts which did not differ in either per cent of increase in magnitude (20 per cent) or time to peak response (12 sec). After glucagon infusion, the peak inotropic response occurred within 20 sec in both types of hearts; however, the per cent of increase in magnitude of the response in the SH hearts was less than one-half of that in the NT hearts.

The decreased inotropic response found in SH hearts after glucagon infusion suggested that certain metabolic alterations might also occur. Cyclic AMP was assayed

TABLE 2. INOTROPIC EFFECT OF ISOPROTERENOL AND GLUCAGON IN SH AND NT RAT HEARTS

	IPR 3×10^{-8} M	Glucagon 1×10^{-7} M	Initial tension (*g*)	Peak tension (*g*)	Increase in force (%)
Normotensive	+	−	11·12±0·55	13·64±0·67	23
SH	+	−	10·01±0·55	11·98±0·98	20
Normotensive	−	+	13·40±0·71	18·81±0·81	35
SH	−	+	12·55±1·01	14·50±1·68	15

in the perfused hearts from both SH and NT rats and similar basal levels of cyclic AMP were found (Fig. 1). Infusion of glucagon (1×10^{-7} M) for 3 min resulted in a significantly smaller elevation in cyclic AMP levels in SH hearts than in NT hearts. However, infusion of IPR for 3 min produced the same elevation in cyclic AMP levels in both groups of hearts.

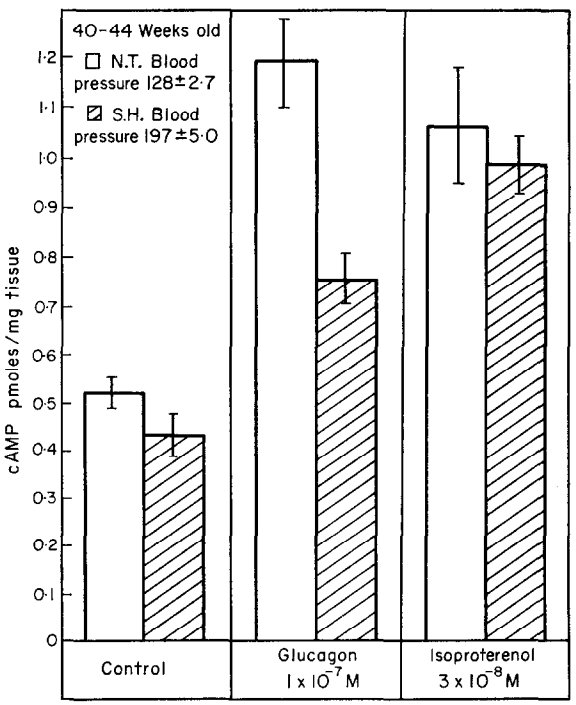

FIG. 1. Cyclic AMP levels after infusion of glucagon or isoproterenol for 3 min in perfused normotensive (NT) and sponteneously hypertensive (SH) rat hearts. Cyclic AMP was assayed by the method of Steiner *et al.*[12] Glucagon-stimulated hearts exhibited significant differences ($P < 0{\cdot}02$) while no significant difference between isoproterenol-stimulated hearts or between controls was observed.

Cyclic AMP was assayed in hearts from animals 13–15 weeks old (before the occurrence of cardiac hypertrophy in the SH animals) to determine if hypertrophy was a factor in the decreased glucagon response in hearts from older SH animals (Fig. 2). In these animals, however, systolic blood pressure had reached adult levels. The control levels for cyclic AMP were the same in both SH and NT groups at 0·26 pmoles/mg of tissue. With the infusion of glucagon, for 3 min, there was a 75 per cent increase in cyclic AMP in the NT hearts, but there was no significant increase in cyclic AMP from control values in SH hearts. With the infusion of IPR, there was a significant increase in cyclic AMP in both SH and NT hearts. It is apparent that cardiomegaly, *per se*, does not cause the decreased glucagon response since the same observations were made in hearts from younger SH animals in which cardiac hypertrophy was not evident.

Cyclic AMP phosphodiesterase activity was assayed in homogenates from both NT

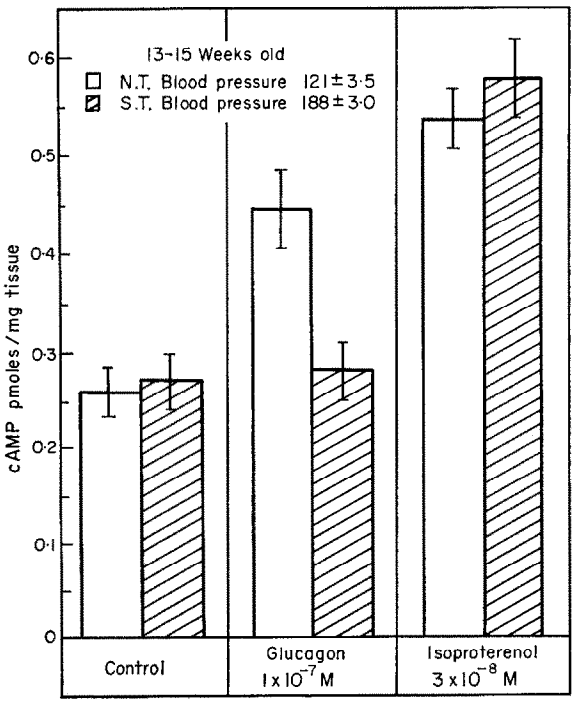

FIG. 2. Cyclic AMP levels after infusion of glucagon or isoproterenol for 3 min in perfused normotensive (NT) or spontaneously hypertensive (SH) rat hearts have been recorded. Cyclic AMP was assayed by the method of Gilman.[11] No significant difference between isoproterenol-stimulated hearts or between controls was observed while differences between glucagon-stimulated hearts proved significant at a $P<0{\cdot}01$ level.

and SH hearts. Both high and low K_m enzymic activity was measured[16] and revealed no significant difference between hearts from NT or SH animals (Table 3).

Since increased cyclic AMP levels in heart muscle are presumably related to the activation of glycogen phosphorylase,[17] this enzyme was assayed to follow IPR and

TABLE 3. CYCLIC AMP PHOSPHODIESTERASE ACTIVITY IN HOMOGENATES OF NORMOTENSIVE AND HYPERTENSIVE RAT HEARTS*

	Normotensive heart	Hypertensive heart
Cyclic AMP phosphodiesterase activity (cAMP=1 mM)		
nmoles/min/g wet wt	559·00±32·00	574·00±47·00
nmoles/min/mg protein	3·23± 0·33	3·22± 0·34
Cyclic AMP phosphodiesterase activity (cAMP=1 μM)		
nmoles/min/g wet wt	14·200±0·4	16·000±1·4
nmoles/min/mg protein	0·082±0·007	0·090±0·010

* Using the Student t-test, there is no significant difference between the values for normotensive and hypertensive rat hearts; $n=6$ for each group.

glucagon effects in SH and NT hearts. Figure 3 shows that the per cent of phosphorylase *a* was 59 per cent in NT hearts and 53·5 per cent in SH hearts when infused with IPR for 3 min. However, infusion of glucagon produced significantly less phosphorylase activation in SH hearts, 39·5 per cent phosphorylase *a*, compared to 55·5 per cent phosphorylase *a* in NT hearts. The degree of phosphorylase activation seemed to reflect the cyclic AMP elevations seen in Fig. 1 in the same hearts.

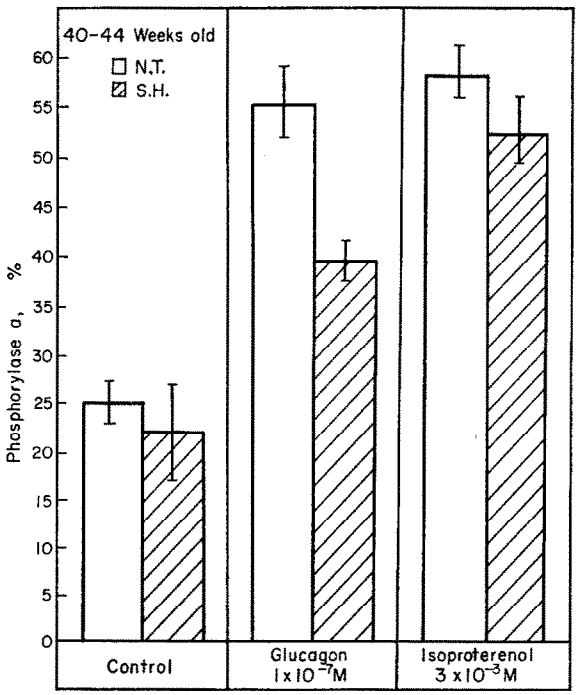

FIG. 3. Activation of glycogen phosphorylase after infusion of either glucagon or isoproterenol for 3 min is summarized. No significant difference between isoproterenol-stimulated hearts or between controls was observed while glucagon produced significant ($P < 0{\cdot}02$) differences between the SH and NT hearts.

With the decreased phosphorylase activation in SH hearts after glucagon infusion, it would be expected that glycogen might be higher in these hearts than in NT hearts since the phosphorylase step is the rate-limiting reaction in glycogenolysis in the heart. Control hearts after 30 min of perfusion had $2{\cdot}75 \pm 0{\cdot}20$ mg of glycogen/g of tissue in NT hearts and $2{\cdot}80 \pm 0{\cdot}35$ mg/g of tissue in SH hearts (Fig. 4). Infusion of glucagon for 3 min produced a significant decrease in glycogen to $1{\cdot}91 \pm 0{\cdot}10$ mg/g of tissue in NT hearts, but produced an insignificant decrease to $2{\cdot}50 \pm 0{\cdot}12$ mg/g of tissue in SH hearts. Infusion of IPR for 3 min produced a significant decrease in glycogen content in both SH and NT hearts (Fig. 4). Thus, the glycogen content after infusion of glucagon or IPR into these hearts reflects the amount of phosphorylase activation which has occurred. Glucagon had a greater glycogenolytic effect in NT hearts than in SH hearts. However, IPR infused for 3 min had essentially the same response in both NT and SH hearts in terms of its glycogen-lowering capacity.

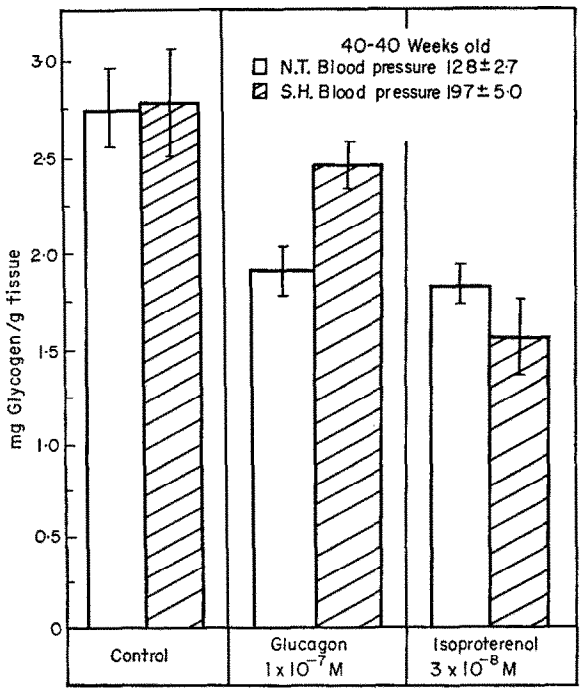

FIG. 4. Glycogen content in normotensive (NT) and spontaneously hypertensive (SH) perfused rat hearts after infusion of either glucagon or isoproterenol for 3 min is summarized. Significant differences between NT control and glucagon-stimulated ($P < 0{\cdot}02$) and between controls and isoproterenol-stimulated for both SH and NT hearts ($P < 0{\cdot}01$) were observed. No significant difference between SH control and glucagon-stimulated was found.

Another source of stored endogenous fuel in the heart is triglyceride.[18] Hydrolysis of cardiac triglyceride has been shown to be initiated by catecholamines such as IPR as well as by glucagon.[7] Compared with liver, the heart as well as adipose tissue has very little glycerokinase activity[19] and glycerol release from the heart can be utilized as an index of lipolysis. Infusion of IPR produced the same pattern of glycerol release into the perfusate in either the NT or SH hearts (Fig. 5). Peak glycerol release was reached 70 sec after the start of IPR infusion with no significant difference between the SH and NT hearts. Infusion of glucagon, however, did produce a difference in the pattern of glycerol release between NT and SH hearts (Fig. 5). The glycerol release from SH hearts rose much more slowly after glucagon infusion and did not reach a peak until 110 sec after beginning of infusion while peak glycerol release in NT hearts occurred at 45 sec. Whether IPR or glucagon was infused, both NT and SH hearts resulted in the same peak of glycerol release in terms of millimicromoles per milliliter of perfusate per gram of tissue. Only a lag in glycerol release was evident from SH hearts compared to NT controls after glucagon infusion.

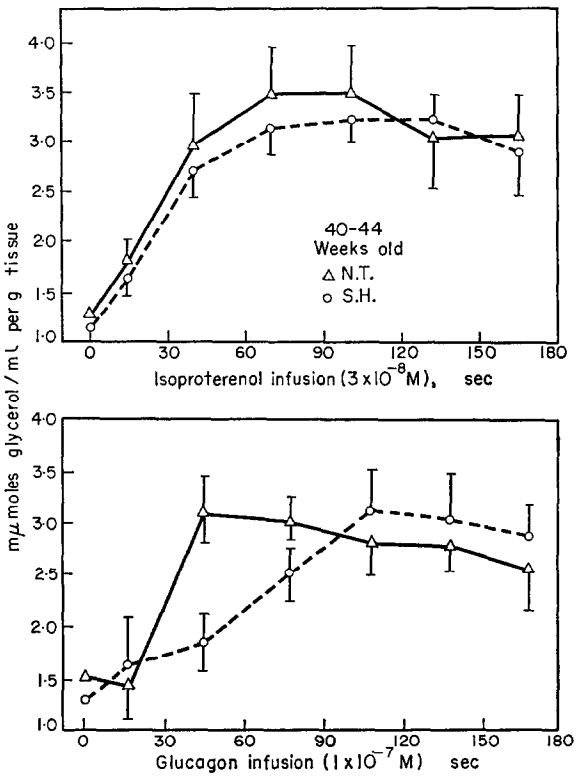

FIG. 5. Glycerol released from normotensive (NT) and spontaneously hypertensive (SH) hearts after infusion of either isoproterenol (top) or glucagon (bottom) has been plotted as a function of time. The only points that varied significantly occurred at 45 sec ($P<0{\cdot}01$) and 75 sec ($P<0{\cdot}05$) after glucagon infusion.

DISCUSSION

The hearts from SH animals have a decreased response to glucagon and yet the same response to IPR. This may reflect an alteration of the glucagon receptor on the plasma membrane of the cardiac cell. Previous studies indicate that glucagon and epinephrine do not produce their effects in the heart through the same receptor site, since propranolol blocks the catecholamine but not the glucagon response.[1] However, it is believed that epinephrine and glucagon activate a single myocardial adenylate cyclase enzyme, since no additive effect on adenylate cyclase activity is observed when maximal concentrations of these two hormones are incubated together.[20]

The glucagon receptor has been shown to become functional in rat heart at a different time in the development of the animal than the epinephrine or beta-adrenergic receptor. The beta receptor is present in the fetal heart, while the glucagon receptor is not functional until approximately 4 weeks of age.[3] During or shortly after weaning of the SH rat is when the rapid increase in systolic blood pressure appears. Whether or not the inducement of high blood pressure has an effect on the formation of the glucagon receptor is still open to speculation, but it may prove to be a factor in the reduced glucagon response found in SH hearts.

A decreased or abolished response to glucagon in heart has been shown under other conditions. The positive inotropic response to glucagon in man suggested a possible role of this hormone in cardiovascular therapeutics.[21] However, many patients with chronic cardiac decompensation demonstrated little or no cardiac response to glucagon.[22,23] Later work with experimental cardiac failure in cats demonstrated that in papillary muscles from these hearts the inotropic response to glucagon was absent while the inotropic response to norepinephrine was not affected.[20] Also, a particulate preparation of heart adenylate cyclase from cats with chronic congestive heart failure had an undiminished response to norepinephrine, compared to normal controls, while the glucagon response was abolished.[24] Since chronic heart failure does not alter the response of cardiac adenylate cyclase to norepinephrine, but does abolish the response to glucagon, the defect is most likely in the receptor at the cell membrane. Whether a similar defect is present in SH rat hearts is still uncertain at this time. In any case, the glucagon receptor in heart appears to be more "labile" in nature than the beta-adrenergic receptor.

The cyclic AMP levels in hearts from animals 13–15 weeks and 40–44 weeks old revealed the same decreased glucagon response while identical elevations in cyclic AMP with IPR infusion were found in both groups of hearts. It is apparent that cardiomegaly, *per se*, does not cause the decreased glucagon response, since the decreased response is observed at a time when blood pressure was elevated but cardiac hypertrophy was not evident.

Elevation of phosphodiesterase activity in SH hearts could lead to lower cyclic AMP levels. However, phosphodiesterase was assayed and revealed no significant difference in activity between NT and SH hearts. The decreased cyclic AMP level in SH hearts after glucagon administration is presumably not due to an increase in phosphodiesterase activity, but more likely due to a decreased ability of glucagon to stimulate cyclic AMP synthesis.

The rise in cyclic AMP produced by glucagon and epinephrine has been shown to be concurrent or to precede the rise in phosphorylase *a* activity in the rat heart.[1,25] We have observed a similar pattern in our experiments in that larger increases in cyclic AMP due to glucagon infusion into NT hearts produced a greater increase in phosphorylase *a*. In SH hearts, which had a lower cyclic AMP elevation in response to glucagon, there was also a decreased activation of phosphorylase *a* compared to NT controls.

The activation of lipase in cardiac tissue is probably controlled by a protein kinase system similar to that for activation of phosphorylase.[26] However, such a system has not been demonstrated and the mechanism of activation of cardiac lipase remains obscure. Whether the lipase system is a cyclic AMP-dependent process in cardiac tissue is also unclear; however, a cyclic AMP-stimulated protein kinase in adipose tissue has been described which activates lipolysis.[27] With glucagon infusion into SH and NT hearts, there was a lag in glycerol release from SH hearts, compared to NT controls (Fig. 5). This may be a reflection of a decrease in lipase activation in these hearts due to a decreased elevation of cyclic AMP. On the other hand, IPR infusion appeared to produce the same elevation of cyclic AMP in both sets of hearts as well as the same rate of glycerol release from these hearts.

Recent evidence has suggested that multiple strains of Wistar NT rats must be used to insure adequate controls. It was observed that differences in sensitivity of alpha-

adrenergic receptors in aortic strips to norepinephrine were found in two strains of NT Wistar rats.[28] However, in our present studies it was observed that there was an identical response to IPR in inotropic effects, cyclic AMP elevation, phosphorylase activation, glycogen depletion and glycerol release. On the other hand, glucagon did not produce parallel response in the SH rats. This may reflect a specific alteration in the glucagon receptor as a result of the hypertension. That the glucagon receptor in rat heart develops after birth during the time of development of hypertension, while the adrenergic beta-receptor develops in the fetal heart before onset of hypertension, also supports the concept that the glucagon receptor may be altered by the hypertensive condition.

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