

Time sequence of changes in the responsiveness of glycogen breakdown to adrenergic agonists in perfused liver of rats with insulin-induced hypoglycemia

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Abstract

The time-course changes of the responsiveness of glycogen breakdown to α - and β -adrenergic agonists during insulin-induced hypoglycemia (IIH) were investigated. Blood glucose levels were decreased prior to the alteration in the hepatic responsiveness to adrenergic agonists. The activation of hepatic glucose production and glycogenolysis by phenylephrine (2 μ M) and isoproterenol (20 μ M) was decreased in IIH. The changes in the responsiveness of glycogen catabolism were first observed for isoproterenol and later for phenylephrine. Hepatic β -adrenergic receptors showed a higher degree of adrenergic desensitization than did α -receptors. Liver glycogen synthase activity, glycogen content and the catabolic effect of dibutyl cyclic AMP (the β -receptor second messenger) were not affected by IIH.

Key words

- Hypoglycemia
- Adrenergic responsiveness
- Insulin-induced hypoglycemia
- Hepatic glucose production
- Glycogenolysis

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Introduction

The effect of epinephrine is known to be mediated by either α - or β -adrenergic receptors. The response of liver glycogen breakdown to epinephrine has been reported to vary with animal species (1), being influenced by a period of fasting (2), training for a starvation schedule (3), steroid hormones (4), sex (5), adrenalectomy (6), hypothyroidism (7), and aging (8). It should be mentioned that the effects on adrenergic receptors observed *in vivo* could not be fully re-

produced in hepatocyte culture (9).

Recently, we developed a rat model to investigate hypoglycemia induced by insulin (10,11). Using this model, we have shown decreased hepatic responsiveness to phenylephrine (an α -adrenergic agonist) and isoproterenol (a β -adrenergic agonist), but not to glucagon or cyanide (12). However, all of these experiments were performed at a fixed time, i.e., 60 min after insulin injection.

For this reason, we determined the time sequence of changes in the responsiveness of

glycogen breakdown to α - and β -adrenergic agonists in perfused liver of rats with insulin-induced hypoglycemia (IIH). The hepatic responsiveness to phenylephrine and isoproterenol was examined 15, 30, 60 and 120 min after insulin administration. For this purpose, isolated liver perfused with non-recirculating medium was employed. This technique is suitable since metabolites do not accumulate, and metabolite rates can be determined directly from the metabolic concentrations in the perfusate effluent. Therefore, the release of pyruvate, L-lactate and glucose reflects almost quantitatively the glycogen breakdown. Moreover, additional experiments were performed to elucidate the mechanism by which IIH decreases hepatic responsiveness of liver glycogen breakdown to adrenergic agonists using dibutyryl cyclic AMP.

Material and Methods

Albino male Wistar fed rats weighing about 200 g were used. Hypoglycemia was induced by an intraperitoneal injection of 1 U/kg of regular insulin (IN group). Control rats were injected with the same volume of saline (CN group). Liver perfusion was performed 15, 30, 60 and 120 min after insulin or saline administration. The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg). After laparotomy, blood was collected from the vena cava for determination of glucose (13) and insulin (14). Livers were perfused *in situ* using an open system with no recirculation of the perfusion fluid as previously described (15). After 10 min of perfusion (10-min time) phenylephrine (2 μ M), isoproterenol (20 μ M) or dibutyryl cyclic AMP (30 μ M) was dissolved in the perfusion fluid and infused between the 10th and 30th min of the perfusion period.

Glucose (13), L-lactate (16) and pyruvate (17) were measured at 2-min intervals in the perfusate effluent. Assuming that L-

lactate, pyruvate and glucose released into the perfusate are the main products of carbohydrate degradation, glycogenolysis can be calculated as the sum of glucose plus the half-sum of L-lactate and pyruvate [glucose + 1/2 (L-lactate + pyruvate)]. The release of these metabolites provides a good approximation of the rate of glycogenolysis because pyruvate oxidation, pentose-monophosphate shunt and recycling of pyruvate to glucose are minimal (18).

All metabolic measurements are reported as μ mol min^{-1} g tissue $^{-1}$ fresh weight. In part of the experiments, liver samples were collected 10 min before infusion (time 0 min) of phenylephrine or isoproterenol to measure hepatic glycogen concentration (19) and glycogen synthase activity (20). A similar procedure has been used in our previous studies (10-12).

Data were analyzed statistically by the unpaired Student *t*-test or ANOVA using the Primer Biostatistics software. A 95% confidence level was accepted for all comparisons. The GraphPad Prism computer program was used to calculate the areas under the curves (AUCs) reported as μ mol/g. Data are reported as means \pm SEM.

Results

Insulin administration caused a sustained decrease of glycemia from the 15th to the 120th min (Table 1). Since at this time glycemia had returned to normal in half the animals, we divided them into recovered and not recovered groups, respectively representing rats with normoglycemia and hypoglycemia 120 min after insulin administration. In contrast, insulin injection caused a slight not significant decrease of hepatic glycogen content (Table 2).

Since hypoglycemia was established, as early as after 30 min of insulin administration, this period of time was then chosen to carry out the liver perfusion experiments. As shown in Figure 1, the infusion of phenyl-

ephrine or isoproterenol promoted a rapid and transient increase of hepatic glucose production and glycogenolysis in normoglycemic (CN group) and hypoglycemic rats (IN group). Peak values were obtained 2 min after the beginning of the infusion of both adrenergic agonists. The values of AUC calculated by subtracting the basal rates indicate that the activation of hepatic glucose production (Figure 1A) and glycogenolysis (Figure 1B) by isoproterenol was clearly decreased by hypoglycemia (IN group). On the other hand, the increment of hepatic glucose production (Figure 1C) and glycogenolysis (Figure 1D) promoted by phenylephrine did not differ between the CN and IN groups.

Since decreased responsiveness to isoproterenol was observed as early as after 30 min of insulin administration, we decided to verify if this change could be observed earlier, i.e., 15 min after insulin administration. Our results demonstrated that the increment

of hepatic glucose production and glycogenolysis promoted by isoproterenol did not differ between the CN and IN groups (data not shown).

The maximal difference between the CN and IN groups was found at 60 min after insulin administration (Figure 2). At this time, the stimulation of hepatic glucose production (Figure 2A,C) and glycogenolysis (Figure 2B,D) by α - and β -adrenergic agonists was clearly lower ($P < 0.05$).

The responsiveness of liver glycogen breakdown was investigated 120 min after insulin administration. The recovered group showed similar hepatic glucose production (Figure 3A,C) and rates of glycogenolysis (Figure 3B,D) during the infusion of either isoproterenol or phenylephrine. In contrast, the non-recovered group displayed lower adrenergic responsiveness as reflected by the decreased glucose production (Figure 4A,C) and rate of glycogenolysis (Figure 4B,D) during the infusion of isoproterenol

Table 1 - Values of glycemia (mg/dl) determined 15, 30, 60, and 120 min after saline (CN group) or insulin (IN group) administration.

The results are reported as mean \pm SEM for at least 5 rats. * $P < 0.05$, significant difference from CN group (unpaired Student t-test).

Groups	Periods of time (min)				
	15	30	60	120 recovered	120 not recovered
CN	146 \pm 5.21	135 \pm 2.23	135 \pm 3.61	142 \pm 4.80	141 \pm 3.92
IN	114 \pm 11.87	58 \pm 8.84*	40 \pm 4.2*	132 \pm 6.19	38 \pm 2.27*

Table 2 - Liver glycogen content (mg%) determined 15, 30, 60, and 120 min after injection of insulin (IN group) or saline (CN group).

Data are reported as the mean \pm SEM for at least 5 experiments.

Groups	Periods of time (min)				
	15	30	60	120 recovered	120 not recovered
CN	2.30 \pm 0.18	2.62 \pm 0.28	1.52 \pm 0.58	2.26 \pm 0.25	1.80 \pm 0.11
IN	1.92 \pm 0.21	2.19 \pm 0.20	1.37 \pm 0.55	2.18 \pm 0.17	1.38 \pm 0.16

and phenylephrine.

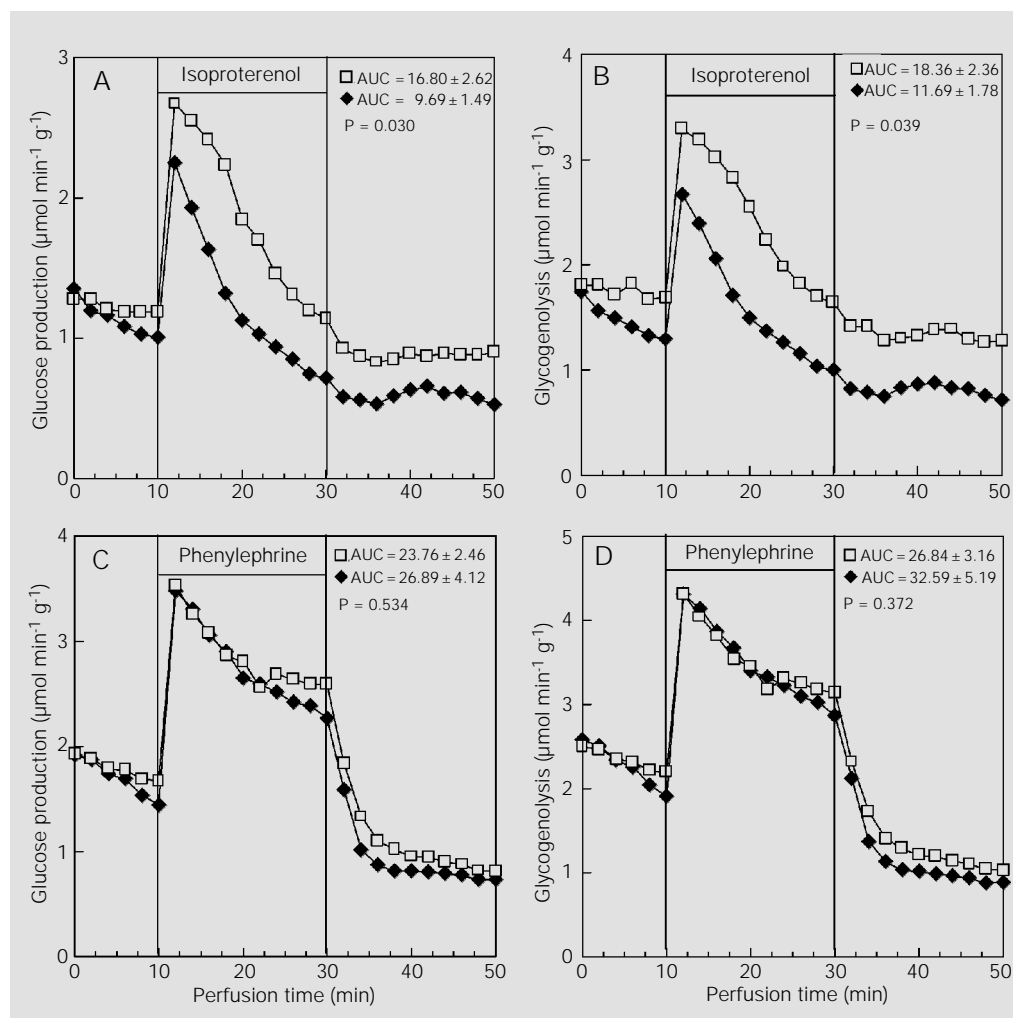
To better understand why hepatic glycogen content was not affected by hypoglycemia (Table 2), we measured glycogen synthase activity. As shown in Table 3, total synthase activity and the proportion of the active form were not influenced by IHH.

Finally, in the last set of experiments the effect of dibutyryl cyclic AMP on hepatic glucose production and glycogenolysis was investigated. The kinetics of the activation of glycogen catabolism was similar in the two groups (Figure 5). However, the responses of the liver from hypoglycemic rats were slightly less pronounced, as indicated by their glycogen content (Table 2).

Discussion

When liver is perfused in a non-recirculating system with medium containing no L-lactate, pyruvate or glucose, these products are washed out due to the large concentration gradients between cytosol and extracellular fluid. This artificial condition provides the rates of carbohydrate degradation (18). Using this technique, we demonstrated that hepatic responsiveness of glycogen catabolism to phenylephrine and isoproterenol decreased significantly 60 min after insulin administration (12). In the present study, this period of time was expanded in both directions and the time course of the changes in the responsiveness of glycogen catabolism

Figure 1 - Effect of isoproterenol (20 μ M; panels A and B) and phenylephrine (2 μ M; panels C and D) on glucose production and glycogenolysis. The data reported are the mean of 5-8 individual liver perfusion experiments performed 30 min after injection of saline (CN group, squares) or insulin (IN group, diamonds). The livers were perfused as described in Material and Methods. AUC = Area under the curve. The statistical test used was the unpaired Student t-test.



during hypoglycemia to α - and β -adrenergic agonists was investigated.

Glycemia decreased before the alteration of hepatic responsiveness. However, the responsiveness of glycogenolysis to phenylephrine and isoproterenol varied extensively along the experiment. Decreased responsiveness to phenylephrine occurred between 30 (Figure 1) and 60 min (Figure 2) after insulin administration. On the other hand, the decrease in hepatic responsiveness to isoproterenol occurred earlier, i.e., between 15 (data not shown) and 30 min (Figure 1) after insulin injection. Furthermore, during the infusion of adrenergic agonists, the IN group presented lower glycogen breakdown in response to isoproterenol (Figure 1A,B) than

during the infusion of phenylephrine (Figure 1C,D), suggesting desensitization of β -adrenergic receptors during hypoglycemia. This finding is particularly relevant due to the fact that hepatic β -adrenoreceptors play an important role in the effect of epinephrine in a large number of experimental conditions (6-9).

In spite of the findings discussed above, the kinetics of activation of glycogen breakdown promoted by both adrenergic agonists was similar, with peak values obtained 2 min after initiating the infusion of both adrenergic agonists (Figures 1-4). Stümpel et al. (21) reported similar observations, i.e., decreased hepatic glucose production within 1-2 min of continuous exposure to norepi-

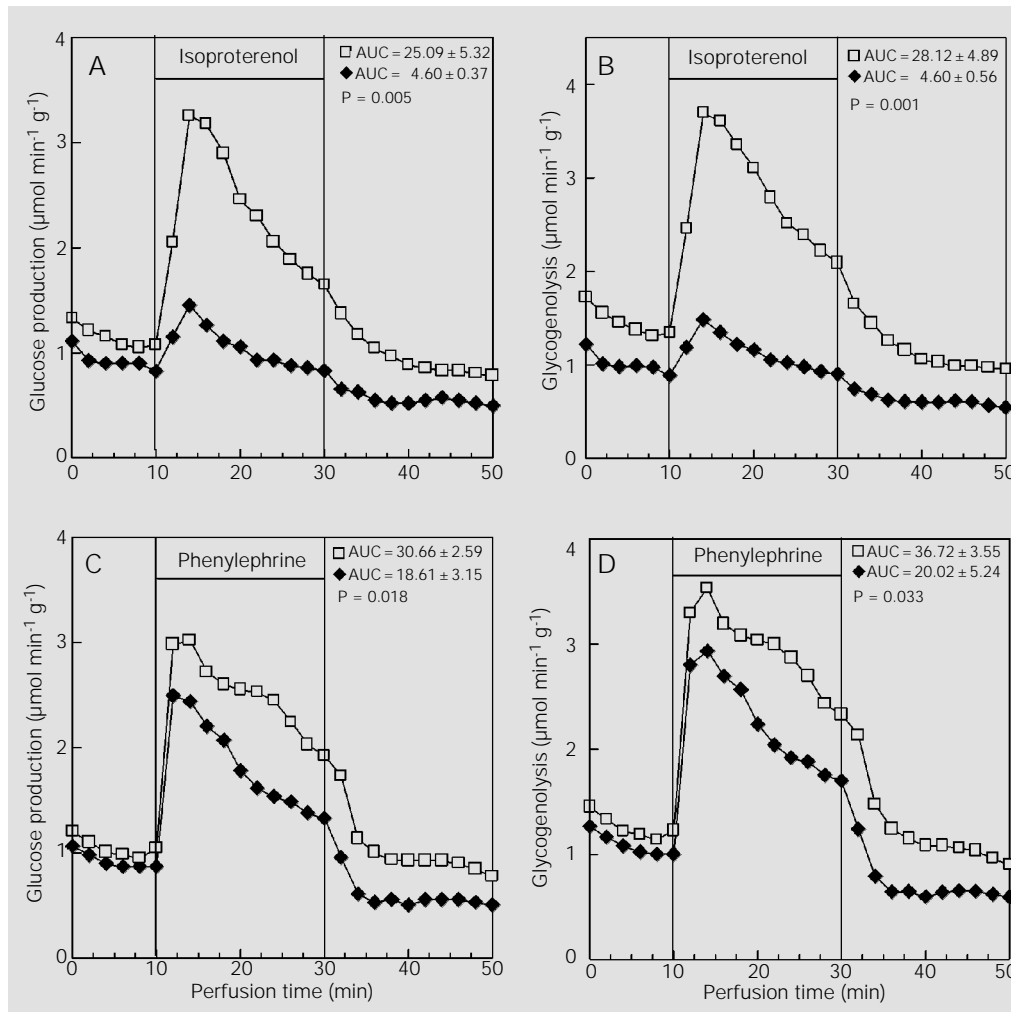
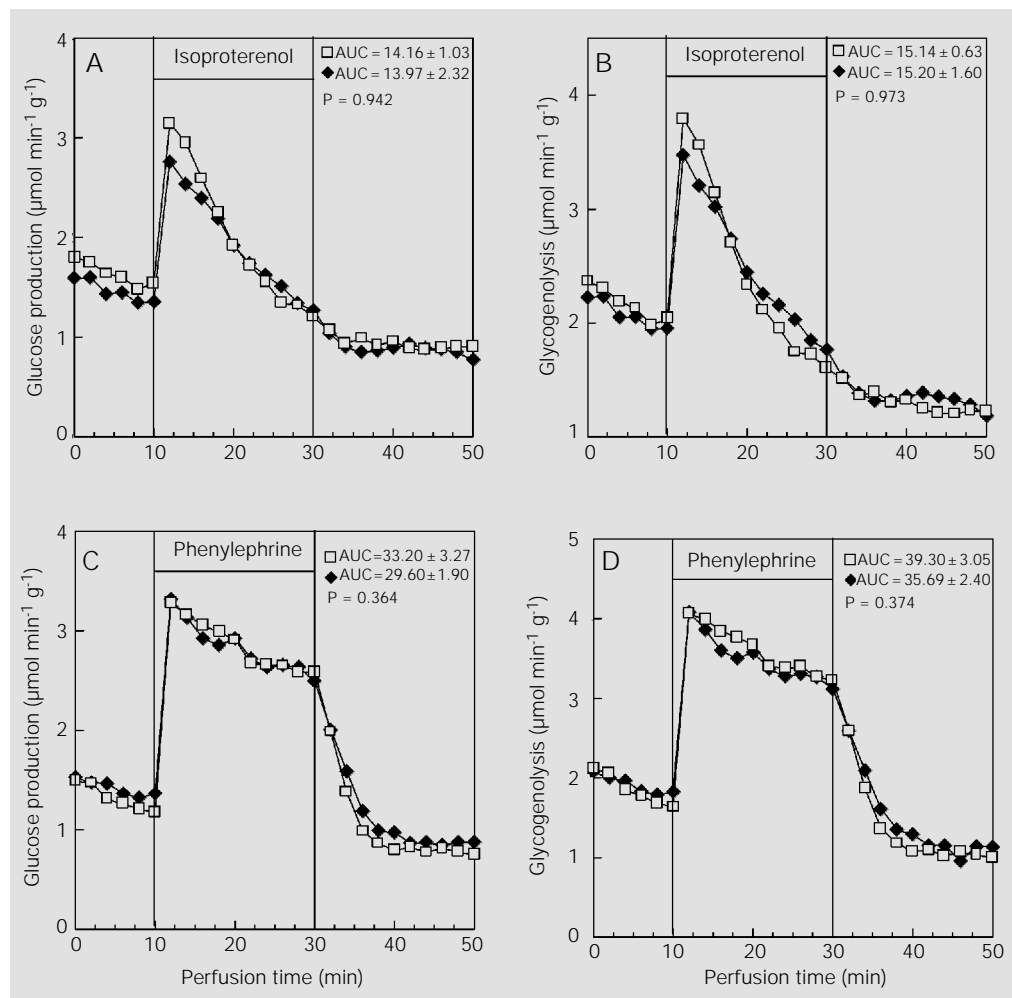


Figure 2 - Effect of isoproterenol (20 μ M; panels A and B) and phenylephrine (2 μ M; panels C and D) on glucose production and glycogenolysis. The data reported are the mean of at least 5 individual liver perfusion experiments performed 60 min after injection of saline (CN group, squares) or insulin (IN group, diamonds). The livers were perfused as described in Material and Methods. AUC = Area under the curve. The statistical test used was the unpaired Student t-test.

Figure 3 - Effect of isoproterenol (20 μ M; panels A and B) and phenylephrine (2 μ M; panels C and D) on glucose production and glycogenolysis. The data reported are the mean of 5 individual liver perfusion experiments performed 120 min after injection of saline (CN group, squares) or insulin (IN group, diamonds). The livers were perfused as described in Material and Methods. The IN group represents recovered animals, i.e., rats that showed normoglycemia after 120 min of insulin administration. AUC = Area under the curve. The statistical test used was the unpaired Student t-test.



nephrine.

Since the most pronounced changes in the hepatic responsiveness to adrenergic agonists occurred 60 min after insulin administration (Figure 2), we decided to obtain more information about the factors that control adrenergic responsiveness by carrying out the experiments at this time. For this purpose, two additional sets of experiments were performed.

First, we determined the activity of total glycogen synthase and the proportion of the active form of this enzyme. None of these parameters was affected by IIH (Table 3). As previously shown (12), glycogen phosphorylase activity was also not influenced by hypoglycemia. Taken together, these

results led us to suggest that hyperinsulinemia promoted by insulin administration ($436.0 \pm 13.2 \mu\text{U/l}$ of the IN group vs $47.6 \pm 3.8 \mu\text{U/l}$ of the CN group - results obtained 60 min after insulin injection) may prevent the effect of an increased endogenous release of counterregulatory hormones promoted by hypoglycemia (22).

Cyclic AMP is known to be the second messenger of β -adrenergic receptors (23). Thus, due to the fact that the most pronounced changes in glycogen breakdown were observed for isoproterenol, in the second set of experiments the effect of dibutyryl cyclic AMP was tested. As shown in Figure 5, the kinetics of the activation of glycogen breakdown promoted by dibutyryl cyclic

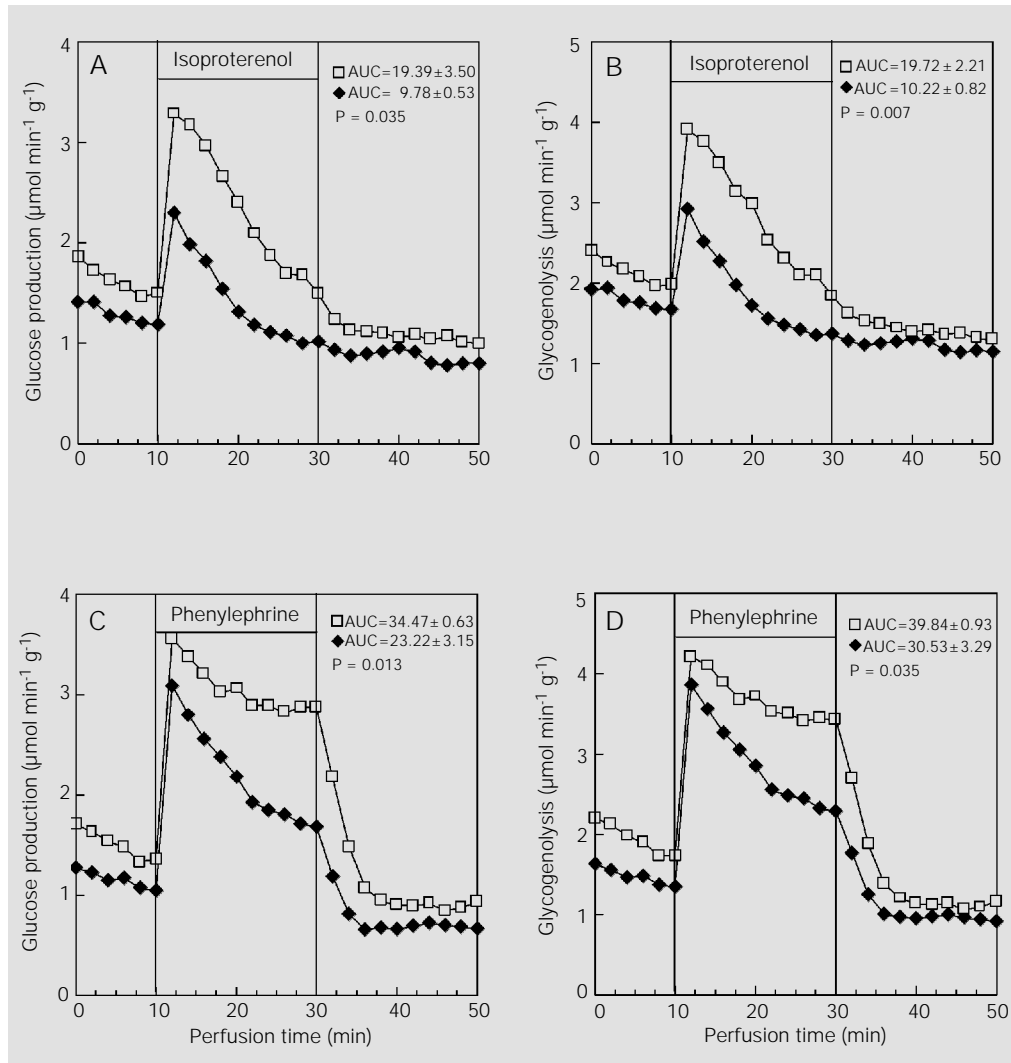


Figure 4 - Effect of isoproterenol (20 μM ; panels A and B) and phenylephrine (2 μM ; panels C and D) on glucose production and glycogenolysis. The data reported are the mean of 4 individual liver perfusion experiments performed 120 min after injection of saline (CN group, squares) or insulin (IN group, diamonds). The livers were perfused as described in Material and Methods. The IN group represents non-recovered animals, i.e., rats that showed hypoglycemia 120 min after insulin administration. AUC = Area under the curve. The statistical test used was the unpaired Student t-test.

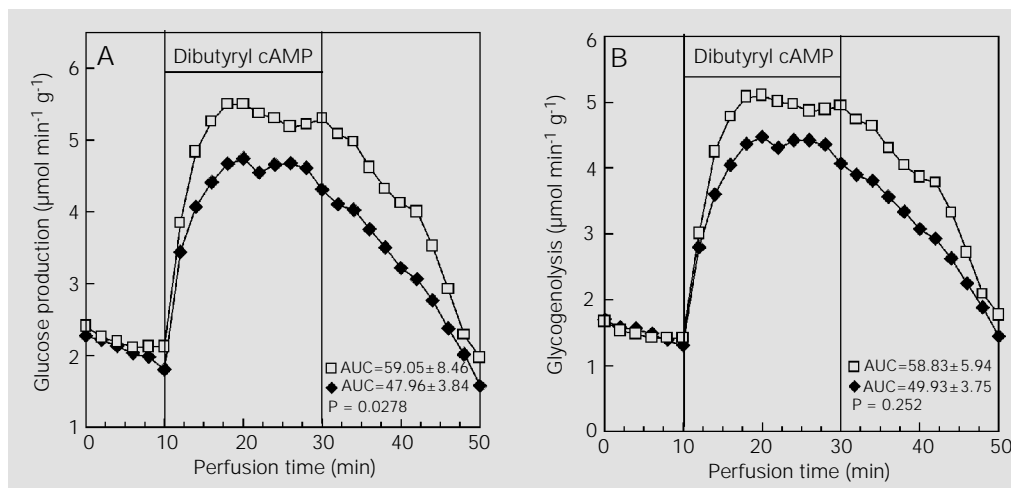


Figure 5 - Effect of dibutyryl cyclic AMP (cAMP; 30 μM) on glucose production (panel A) and glycogenolysis (panel B). The data reported are the mean of 4 individual liver perfusion experiments performed 60 min after injection of saline (CN group, squares) or insulin (IN group, diamonds). The livers were perfused as described in Material and Methods. AUC = Area under the curve. The statistical test used was the unpaired Student t-test.

Table 3 - Activities of the total and active forms of glycogen synthase and proportion of the active form (%AS) in rats which received saline (CN group, glycemia: 144.7 ± 2.4 mg/dl) or insulin (IN group, glycemia: 42.4 ± 3.6 mg/dl) 1 h before hepatic perfusion.

Glycogen synthase activity was assayed in liver samples collected 10 min before the infusion of phenylephrine or isoproterenol (time 0). Data are reported as the mean \pm SEM for 6 rats of glycogen synthase activity ($\text{nmol mg}^{-1} \text{min}^{-1}$).

Glycogen synthase activity	IN	CN
Total	16.2 ± 0.55	14.6 ± 0.55
Active	0.7 ± 0.15	0.65 ± 0.15
%AS	4.52 ± 1.16	4.10 ± 0.57

AMP was similar in both groups. Thus, we conclude that the mechanism of decreased responsiveness to isoproterenol occurred at some step preceding cyclic AMP formation. This effect can occur on receptor, G-protein or adenylate cyclase activity. Current ideas indicate that the initial event in β -adrenergic desensitization is a rapid uncoupling of the receptor from G_s -protein due to receptor phosphorylation (24). However, if this mechanism operates during IIH remains to be elucidated.

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