Inhibition by Catecholamines of the Induction of Rat Liver Glucokinase*

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SUMMARY

Epinephrine, norepinephrine, and isoproterenol prevent the induction of rat liver glucokinase (EC 2.7.1.2) produced by the administration of glucose to animals with low levels of the enzyme. The low levels of glucokinase were obtained by feeding the animals a carbohydrate-free diet for 6 days. When epinephrine was given 4 hours after the administration of carbohydrate there was only a slight inhibition of the rate of enzyme increase, whereas when given simultaneously with the carbohydrate there was a complete blockade of the induction. The N\(^{6}\),O\(^{2′}\)-dibutyryl analogue of cyclic adenosine 3′,5′-monophosphate also inhibited glucokinase induction. Insulin counteracted about 50% of the epinephrine effect, but not the inhibition elicited by dibutyryl cyclic AMP. The data suggest that the epinephrine action on glucokinase induction may result both from a direct effect on the liver cell, possibly mediated by cyclic AMP, and a blockade of insulin secretion at the pancreas.

Normal levels of glucokinase (EC 2.7.1.2) in rat liver apparently depend on the proper interplay of dietary and hormonal factors. The enzyme activity decreases after the animals have been deprived of food (3–11) or fed a carbohydrate-free diet (5, 6, 10, 11), and is restored to normal values by the administration of carbohydrate (4–11). Insulin is essential for both the maintenance of normal levels of glucokinase and the induction of the enzyme after the depletion of carbohydrate (6, 8, 12–14). On the other hand, glucagon prevents the induction of glucokinase in normal and diabetic rats (15–17), and it has been briefly reported that epinephrine also blocks the induction (16, 18). The term "induction" is used to indicate a selective increase of glucokinase activity, due most probably to synthesis de novo of the enzyme. Considering the importance of the participation of all of these hormones in the adaptation of mammals to the avail-
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Fig. 1. Dose-effect relationship for the action of epinephrine on glucokinase induction. The procedures for decreasing rat liver glucokinase are described under "Experimental Procedure." Carbohydrate was given at the beginning of the experiment and repeated 4 hours later. A single injection of epinephrine at the doses indicated was administered by the intraperitoneal route 15 min before the first administration of carbohydrate. The animals were killed 8 hours afterward. Vertical lines on each point represent standard errors and the figures indicate number of rats.

Fig. 2. The effect of a single subcutaneous injection of epinephrine on liver glucokinase induction by glucose. The procedures for decreasing rat liver glucokinase are described under "Experimental Procedure." At the times indicated groups of animals were killed for the assay of liver glucokinase. One group of rats was killed as initial controls. The remaining animals received carbohydrate every 4 hours (arrows, d). Of these animals some were treated by the subcutaneous route with epinephrine (0.5 mg per kg of body weight) every 2 hours (O), either from the start of the experiment, or after 4 hours of carbohydrate administration, as indicated by the arrows (f). The remaining rats receiving carbohydrate were injected with NaCl solution and acted as induced controls (O). Epinephrine injections started 15 min before carbohydrate administration. The vertical lines on each point represent standard errors and the figures indicate number of rats.

Fig. 3. Inhibition by epinephrine of glucokinase induction in rat liver. Data from two separate experiments were pooled. The procedures for decreasing liver glucokinase are described under "Experimental Procedure." At the times indicated groups of animals were killed for the assay of liver glucokinase. One group of rats was killed as initial control. The remaining animals received carbohydrate every 4 hours (arrows, €). Of these animals some were treated by the subcutaneous route with epinephrine (0.5 mg per kg of body weight) every 2 hours (O), either from the start of the experiment, or after 4 hours of carbohydrate administration, as indicated by the arrows (f). The remaining rats receiving carbohydrate were injected with NaCl solution and acted as induced controls (O). Epinephrine injections started 15 min before carbohydrate administration. The vertical lines on each point represent standard errors and the figures indicate number of rats.

10% homogenate was prepared in a Potter-Elvehjem apparatus provided with a Teflon pestle, in a medium containing 100 mM KCl, 25 mM Tris-HCl buffer (pH 7.5), 6 mM EDTA, and 6 mM MgCl₂. The homogenates were centrifuged at 105,000 x g for 20 min in a Spinco preparative ultracentrifuge and the supernatant fluids were collected carefully. The extracts were often frozen and kept at -20°C for 16 to 24 hours before being thawed and used to measure enzyme activity.

Enzyme Assay—ATP-hexose phosphotransferase activity was assayed by measuring spectrophotometrically the production of glucose 6-phosphate through the reduction of NADP in the presence of glucose 6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase (7, 8, 10). A Gilford spectrophotometer, provided with a thermostated cell to operate at 30°C, was used to follow changes in absorbance in 1-ml cuvettes. The final composition of the assay medium was: 10 mM KCl, 85 mM Tris-HCl buffer (pH 7.5), 12.6 mM MgCl₂, 3.3 mM ATP, 1.6 mM EDTA, 0.5 mM NADP, an excess (about 0.15 unit) of each of the dehydrogenases, 100 mM or 0.5 mM glucose, and 0.1 ml of liver extract (supernatant fluid diluted 3-fold in the homogenizing medium). In the presence of an excess of 6-phosphogluconic dehydrogenase, 2 molecules of NADP are reduced for each molecule of glucose (20). The higher concentration of glucose was used to measure total phosphorylating activity, and the lower concentration was used for hexokinases. The difference between the values obtained with the two concentrations gives an approximate estimation of glucokinase (7). The reaction was
Inhibition by norepinephrine and isoproterenol of glucokinase induction in rat liver

The procedures for decreasing liver glucokinase and for enzyme induction are described under "Experimental Procedure." Animals received carbohydrate every 3 hours and were killed after 6 hours of induction, with the exception of the two control groups. The catecholamines were dissolved in 0.9% NaCl solution and injected by the subcutaneous route every 2 hours at the doses indicated, the first dose 15 min before carbohydrate administration. Figures indicate means ± standard errors.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Drug</th>
<th>Dose (mg/kg body weight)</th>
<th>Glucokinase units/100 g body weight</th>
<th>Glycogen mg/100 g liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>None</td>
<td>0.60 ± 0.08</td>
<td>3.0 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>1.51 ± 0.47</td>
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<td>4</td>
<td>Norepinephrine 0.25</td>
<td>2.42 ± 0.29a</td>
<td>4.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Norepinephrine 0.50</td>
<td>2.27 ± 0.39a</td>
<td>4.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Isoproterenol 1.00</td>
<td>1.99 ± 0.34a</td>
<td>3.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
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<td>2.25 ± 0.68a</td>
<td>4.1 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Isoproterenol 0.50</td>
<td>2.41 ± 0.26a</td>
<td>3.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Isoproterenol 1.00</td>
<td>1.68 ± 0.22a</td>
<td>2.3 ± 0.6</td>
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</tr>
</tbody>
</table>

a Control rats, killed at zero time.

b Control animals that received water instead of carbohydrate and were killed at the end of the experimental period.

c Differs from noninduced controls receiving water with p < 0.01.

d Differs from induced controls with p < 0.01.

Effect of Epinephrine on Glucokinase Induction

The injection of epinephrine either by subcutaneous or by intraperitoneal route inhibited the glucose-mediated induction of glucokinase in rat liver, the effect depending on the dose (Fig. 1). Most of the experiments were performed with repeated doses of epinephrine.

Effect of dibutyryl cyclic AMP and 5'-AMP on glucokinase induction and glycogen deposition in liver

The procedure for decreasing liver glucokinase and for enzyme induction is described under "Experimental Procedure." Animals received carbohydrate every 4 hours and were killed after 8 hours of induction. Only the initial controls did not receive carbohydrate. Drugs were injected by the intraperitoneal route every 4 hours, starting 15 min before carbohydrate administration. Figures indicate means ± standard errors.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Drug</th>
<th>Dose (mg/kg body weight)</th>
<th>Glucokinase units/100 g body weight</th>
<th>Glycogen mg/100 g liver</th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>None</td>
<td>0.36 ± 0.13</td>
<td>2.6 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>4.01 ± 0.27</td>
<td>5.7 ± 0.3</td>
<td></td>
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<tr>
<td>3</td>
<td>Dibutyryl cyclic AMP 25</td>
<td>2.39 ± 0.09b</td>
<td>3.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Dibutyryl cyclic AMP 50</td>
<td>1.56 ± 0.38b</td>
<td>1.9 ± 0.3</td>
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<td>3</td>
<td>Dibutyryl cyclic AMP 100</td>
<td>0.84 ± 0.11c</td>
<td>0.6 ± 0.1</td>
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<tr>
<td>4</td>
<td>5'-AMP 50</td>
<td>3.13 ± 0.47c</td>
<td>7.0 ± 0.7</td>
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</tr>
<tr>
<td>3</td>
<td>Epinephrine 0.25</td>
<td>0.79 ± 0.13c</td>
<td>4.1 ± 0.5</td>
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</tr>
</tbody>
</table>

a Control rats killed at zero time.

b Differs from induced controls with p < 0.01.

c Does not differ significantly from induced controls (p < 0.1).

RESULTS

Effect of Epinephrine on Glucokinase Induction

The injection of epinephrine either by subcutaneous or by intraperitoneal route inhibited the glucose-mediated induction of glucokinase in rat liver, the effect depending on the dose (Fig. 1). Most of the experiments were performed with repeated doses of epinephrine.

in view of the well known biological and chemical instability of the catecholamines. However, a single dose was also sufficient to block almost completely glucokinase induction for 8 hours (Figs. 1 and 2). Even the highest doses used did not modify glucokinase activity in extracts prepared from rats fed a balanced diet and receiving epinephrine at various intervals before...
**TABLE III**

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Epinephrine</th>
<th>Insulin</th>
<th>Glucokinase</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>units/100 g body weight</td>
<td>mg/100 mg liver</td>
<td>units/100 g body weight</td>
</tr>
<tr>
<td>2a</td>
<td>-</td>
<td>1.26 ± 0.42</td>
<td>3.0 ± 0.1</td>
<td>5.73 ± 0.51</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>1.51 ± 0.33</td>
<td>1.9 ± 0.2</td>
<td>2.18 ± 0.35</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>3.70 ± 0.60</td>
<td>4.2 ± 0.5</td>
<td>3.74 ± 0.36</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>2.5</td>
<td>4.6 ± 0.4</td>
<td>3.67 ± 0.43</td>
</tr>
</tbody>
</table>

*Control rats killed at zero time.*
*Control animals that received water instead of carbohydrate solution, and were killed at the end of the experimental period.*
*Different from noninduced controls receiving water with p < 0.01.*
*Different from rats receiving only carbohydrate with p < 0.05.*
*Different from animals receiving only carbohydrate with p < 0.02.*
*Different from rats receiving carbohydrate and epinephrine with p < 0.01.*
*Different from animals receiving only carbohydrate with p < 0.01.*
*Different from rats receiving carbohydrate and epinephrine with p < 0.02.*

**TABLE IV**

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Drug</th>
<th>Insulin</th>
<th>Glucokinase</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>units/100 g body weight</td>
<td>mg/100 mg liver</td>
<td>units/100 g body weight</td>
</tr>
<tr>
<td>2a</td>
<td>None</td>
<td>-</td>
<td>0.50 ± 0.18</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>3a</td>
<td>None</td>
<td>-</td>
<td>0.58 ± 0.29</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>-</td>
<td>5.50 ± 0.06</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>Epinephrine</td>
<td>-</td>
<td>1.75 ± 0.20</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>Epinephrine</td>
<td>+</td>
<td>2.77 ± 0.41</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>Dibutyryl cyclic AMP</td>
<td>+</td>
<td>1.83 ± 0.27</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>Dibutyryl cyclic AMP</td>
<td>+</td>
<td>1.75 ± 0.15</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

*Control rats killed at zero time.*
*Control animals that received water instead of carbohydrate solution, and were killed at the end of the experimental period.*
*Differs from rats receiving only carbohydrate with p < 0.001.*
*Differs from rats receiving carbohydrate and epinephrine with p < 0.05.*

The effective doses were similar to those used by others (27, 28) in studies on enzyme induction in the whole animal. 5'-AMP had a small but not significant effect at a dose that would be clearly effective for dibutyryl cyclic AMP (Table II).

**Glucogen Analysis**—Liver glucogen was measured as an index of the effectiveness of the drugs on another sensitive system. Doses of catecholamines that markedly inhibited glucokinase induction had marginal effects on glucogen deposition (Tables I to IV). Only the highest doses used prevented completely the increase in liver glucogen associated with the load of carbohydrate. In contradistinction, the administration of dibutyryl cyclic AMP resulted in net glycogenolysis at doses partially effective as inhibitory of glucokinase induction (Table II). 5'-AMP did not show any effect on glucogen deposition (Table II).

**Partial Reversal of Epinephrine Effect by Insulin**—The administration of insulin partially counteracted the inhibition produced by epinephrine on glucokinase induction (Table III). However, even the highest doses used (5 units of insulin every 4 hours) did not antagonize completely the catecholamine effect. Insulin also appeared effective occasionally in counteracting the effect of epinephrine on glucogen deposition. On the other hand, the inhibition caused by dibutyryl cyclic AMP was not reversed by a dose of insulin which was active in partially antagonizing the epinephrine action (Table IV).

**DISCUSSION**

Sutherland et al., have postulated that cyclic AMP acts as a second messenger which mediates the effects of a number of hormones (22–24). Hormones such as glucagon and catecholamines stimulate adenyl cyclase activity in the cell membrane of the liver (29–32) and other tissues. The resulting in-
crease in cyclic AMP concentration would be responsible for the activation of some key enzymes in various metabolic pathways (22, 24). From the work of several investigators it appears now that, in addition to this role in regulating enzyme activity, glucagon, epinephrine, or both can induce several liver enzymes (27, 28, 32, 39). The dibutyryl derivative of cyclic AMP mimics the hormone induction in all cases studied (27, 28, 40, 41). The possibility of explaining part of the actions of the cyclic nucleotide as a derepression of DNA brought about through the phosphorylation of histones has been pointed out (42). It is interesting to note that cyclic AMP also enhances induction of some enzymes in bacteria (43, 44). The inhibition of glucose-mediated glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44). The inhibition of glucokinase induction by glucagon, catecholamines, and cyclic AMP constitutes perhaps the first example of induction of some enzymes in bacteria (43, 44).

In the intact animal the interpretation of the actions of hormones on certain metabolic parameters of a given tissue is complicated by their effects on the secretion of interfering hormones and on the metabolism of organs other than those under study. This situation has been discussed previously in relation to glucokinase induction and the participation of glucose, insulin, and hyperglycemic-glycogenolytic hormones (16). The secretion of insulin is under the control of blood concentrations of glucose, epinephrine, and glucagon. Thus, epinephrine under normal conditions inhibits insulin secretion (56–59). As insulin is an obligatory requirement for glucokinase induction, it could be postulated that part of the repressive effect of catecholamines is mediated through a mechanism involving a blockade of insulin secretion. The partial counteraction elicited by insulin of the epinephrine inhibition of glucokinase induction could be the result of the replacement of exogenous insulin for the endogenous hormone. Results obtained with α-adrenergic blocking agents, that will be reported elsewhere, give some support to this hypothesis.

If catecholamines and glucagon, on the one hand, and insulin on the other, interact at the liver cell to regulate the synthesis of specific proteins through modifications in the concentration of cyclic AMP, it is puzzling that insulin does not counteract the inhibition of glucokinase induction by glucagon (15, 17) and does not reverse completely the inhibition by epinephrine. We do not know whether a more delicate interaction of doses and time of administration of the antagonistic hormones exists that has escaped our exploration. Actions of the hormones independent of their effects on cyclic AMP levels may be alternatively postulated. What appears clear is that insulin is unable to antagonize the action of cyclic AMP as has been postulated in other cases (41, 49, 54).

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