POTENTIATION OF INSULIN SECRETORY RESPONSE OF RATS BY BCG INJECTION

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Abstract—Effects of BCG cells on blood insulin, glucose, glycerol and free fatty acid (FFA), and liver glycogen were examined in rats in vivo. Freund’s complete adjuvant (FCA) was used as a solution containing BCG cells. Insulin secretion and lactate production by isoproterenol were enhanced by FCA injection. Hyperglycemia induced by epinephrine was somewhat attenuated by treating rats with FCA. The increment of plasma FFA by isoproterenol was smaller in FCA-injected rats than that in control rats. The amount of glycogen in liver was increased by FCA treatment. Anti-insulin antiserum (AIS) did not modify the lactate producing action of isoproterenol. FFA release stimulated by isoproterenol was enhanced by AIS. Liver glycogen content in FCA-injected rats was decreased by AIS injection. These results suggest that some component(s) of BCG cells may enhance insulin release stimulated by secretagogues, and insulin thus released attenuates some metabolic effects of the agonist.

It has been known that epinephrine-induced hyperglycemia is markedly attenuated in pertussis-sensitized rats (1-3). This phenomenon has been explained by several investigators as a result of a selective $\beta$-adrenergic receptor blockade by the pertussis vaccine (3-5). Recently Ui and his colleagues have shown that insulin secretory responses to various secretagogues were enhanced in pertussis-sensitized rats, and this potentiation resulted from an activation of $\beta$-adrenergic receptor-mediated functions (6). Moreover, they purified a protein, termed islet-activating protein (IAP), from the culture media of Bordetella pertussis (strain Tohama, phase I) and showed that the protein, when injected at a dose as low as 0.02 to 0.1 $\mu$g per rat, doubled the insulin secretory response to glucose loading (7). Bordetella pertussis cells are known to have adjuvant activity. BCG is a constituent of Freund’s complete adjuvant (FCA). Therefore, it is of interest to study the effects of BCG cells on insulin secretory responses and on glucose metabolism in rats. To clarify this point, we injected BCG cells as FCA into rats. Insulin secretory responses to the secretagogues tested were enhanced in the FCA-injected rats.

MATERIALS AND METHODS

Animals: Male rats of the Donryu strain weighing 200 to 300 g were employed for these studies. Animals were given food and water ad libitum.

Injections, fasting and sacrifice: Rats were injected i.p. with FCA (1 ml/100 g), then fasted for 24 hr and used for experiments. In some experiments, hydrocortisone suspended in sterile saline was injected s.c. immediately prior to FCA injection and then 3 hr prior to the experiment at a dose of
25 mg/kg. Tolbutamine was injected (50 mg/kg) s.c., and glucose was administered p.o. as a 20% solution (1 ml/100 g). Control animals received sterile saline alone. Anti-insulin antiserum (AIS) was injected i.v. (0.6 ml/100 g) in some experiments. To determine the content of glycogen in liver, animals were sacrificed under pentobarbital anesthesia, and the liver was quickly removed, frozen with liquid N₂, and used for glycogen assay.

Determination: The insulin concentration in plasma was determined by the radioimmunochemical method of Herbert et al. (8). Blood glucose, lactate, and glycerol levels were determined by the methods of Bergmeyer and Bernt (9), Barker and Summerson (10), and Pinter et al. (11), respectively. Free fatty acid (FFA) in plasma was determined by the method of Tokumitsu et al. (12). Liver glycogen was assayed by the anthrone method (13).

Sources of reagents: Sources of reagents are as follows: FCA and Freund’s incomplete adjuvant (FIA), from latron Inc.; BCG cells, a gift from latron Inc.; hydrocortisone acetate, epinephrine, isoproterenol, phenylephrine, glucose oxidase and peroxidase, from the Sigma Chemical Co.; propranolol, a gift from the Ohtsuka Pharmaceutical Co.; tolbutamide, a gift from the Yamanouchi Pharmaceutical Co., Ltd. Anti-insulin antiserum (AIS) was prepared by immunizing guinea pigs (14).

RESULTS

Insulin secretory responses to various secretagogues in FCA-injected rats. Isoproterenol known as a typical β-adrenergic agonist shows an increasing-effect on the insulin secretion in normal rats (Fig. 1A). In FCA-injected rats, this drug very markedly increased the plasma level of insulin. When rats were injected with β-adrenergic blocking agent, propranolol, prior to injection of isoproterenol, the stimulatory effect of isoproterenol was diminished even in FCA-injected rats (Fig. 1A). Although epinephrine, a mixed type of agonist, usually inhibits the release of insulin from the pancreas in vitro, it did not decrease the plasma level of insulin in normal rats in vivo (Fig. 1B). However, it caused a small increase in the plasma level of insulin in FCA-injected rats. Phenylephrine, an α-adrenergic agonist, also failed to decrease the normal level of insulin in vivo and had no effect on the level of insulin even in FCA-treated rats (Fig. 1C). These results suggest that the plasma insulin response to the β-adrenergic agonist was augmented in FCA-treated rats.

The effects of glucose and tolbutamide on the plasma insulin level were then examined in the control and FCA-treated rats. Following administration of each of them, there was a larger increase in the plasma insulin level in
FCA-treated rats than in control rats (Fig. 2A and 2B). These data suggest further that the B-cells of the pancreas from FCA-treated rats are hypersensitive.

Effects of glucose, epinephrine and isoproterenol on the blood glucose level in FCA-injected rats: In FCA-treated rats, the extent of epinephrine-induced hyperglycemia was less than that in normal rats as shown in Fig. 3A. Attenuation of hyperglycemia induced by glucose loading was also seen in FCA-injected rats when 0.2 g/100 g body wt. of glucose was administered p.o. (Fig. 3B). However, isoproterenol which has little effect on the blood glucose level of normal rats elevated the blood level of glucose slightly but significantly in FCA-treated rats (Fig. 3C). A marked increase in plasma insulin induced by isoproterenol was not associated with suppression of the blood glucose level.

Effects of isoproterenol on the blood level of lactate, glycerol and FFA, and liver glycogen content in FCA-injected rats: The effects of isoproterenol on blood levels of lactate, glycerol and FFA, and liver glycogen content as additional indices of \( \beta \)-adrenergic responses were examined in FCA-treated rats. Isoproterenol-induced hyperlactacidemia was markedly enhanced by FCA-injection, suggesting that \( \beta \)-adrenergic receptors were hypersensitive in FCA-injected rats (Fig. 4). On the other hand, hepatic glycogen level was higher in FCA-treated rats than in normal rats as shown in Table 1. Isoproterenol, a
β-adrenergic agonist, did not show its glycogenolytic effect in both normal and FCA-treated rats, whereas epinephrine caused glycogenolysis in normal rats, but not in FCA-injected rats. Concentrations of glycerol and FFA in the blood were increased by the injection of isoproterenol. Glycerol levels increased by isoproterenol in FCA-injected rats were the same as that increased by the drug in normal rats (Fig. 5A), whereas the FFA level in FCA-treated rats was lower than that in normal rats (Fig. 5B). Lipolysis itself seems not to be affected by treating animals with FCA.

Effect of injection of AIS on blood level of glucose, lactate and FFA, and liver glycogen content: To study the role of endogenous insulin in controlling the concentrations of blood glucose, lactate and FFA, and liver glycogen level, AIS was injected i.v. into normal and FCA-treated rats. The prompt neutralization of circulating insulin with AIS resulted in an upward trend of the blood glucose level in normal rats; in FCA-treated rats, the trend was extremely enhanced, showing that endogenous insulin was more active in controlling the blood glucose level in FCA-treated than in normal rats (Fig. 6).

As already shown in Fig. 4, isoproterenol injected into FCA-treated rats markedly increased the blood level of lactate. This increment was not attenuated with AIS injected i.v. (Fig. 7). This shows that the increment in the blood level of lactate was

![Fig. 4. Effect of isoproterenol on blood level of lactate. Isoproterenol (200 μg/kg) was injected s.c. at 0-time. Mean±S.E.M. from 4 observations. ○—○: normal rats; ●—●: FCA-injected rats.](image)

![Fig. 5. Effect of isoproterenol on lipolysis in normal and FCA-injected rats. Isoproterenol (200 μg/kg) was injected s.c. at 0-time. Mean±S.E.M. from 4 observations. ○—○: normal rats; ●—●: FCA-injected rats.](image)

Table 1. Glycogenolytic effect of epinephrine and isoproterenol in normal and FCA-injected rats. Livers were excised 30 min after the drug injection (i.p.). Number of observations is shown in parentheses

| Drugs               | Normal (mg/g) | FCA-injected (mg/g) |
|---------------------|---------------|---------------------|
| None                | 1.4±0.3 (4)   | 4.9±1.3 (4)         |
| Epinephrine (100 μg/kg) | 0.6±0.1 (4)* | 4.5±0.4 (4)*       |
| Isoproterenol (200 μg/kg) | 2.0±0.6 (4)  | 5.6±0.9 (4)*       |

*P(＜0.05) for the difference from none. **P(＜0.01) for the difference from normal.
not due to insulin released by isoproterenol, but due to the action of the drug itself as a \( \beta \)-adrenergic agonist.

Although the rate of glycerol release stimulated by isoproterenol in FCA-treated rats was the same as that in normal rats, the blood level of FFA in the former was not increased by isoproterenol (Fig. 5A and 5B). If failure of isoproterenol to increase the blood FFA level in FCA-treated rats reflects insulin-stimulated reesterification of FFA, isoproterenol would become effective after AIS even in FCA-treated rats. In fact, after AIS, isoproterenol increased the blood level of FFA in FCA-treated rats as in normal rats (Fig. 7B).

As shown above, the liver glycogen content in FCA-treated rats was higher than that in control rats, and the glycogenolytic action of epinephrine and isoproterenol were not seen in FCA-treated rats. However, epinephrine showed its glycogenolytic effect in normal rats (Table 1). This suggests that increased insulin activity caused by FCA-injection attenuated the glycogenolytic action of epinephrine and isoproterenol. In fact, AIS lowered the liver glycogen level even in FCA-treated rats (Table 2).

Effect of hydrocortisone on insulin secretion stimulated by isoproterenol in FCA-

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**Table 2.** Effect of AIS on liver glycogen level in normal and FCA-injected rats. Livers were excised 30 min after the injection of normal serum or AIS (0.6 ml/100 g). Number of observations is shown in parentheses.

| Glycogen Level (mg/g) | Normal | FCA-injected |
|-----------------------|--------|--------------|
| AIS                   |        |              |
| -                     | 2.2±0.3 (4) | 6.8±0.5 (3)* |
| +                     | 1.1±0.2 (4)* | 4.2±0.6 (3)* |

\( *P(0.05) \) for the difference from “minus AIS”; \( *P(0.01) \) for the difference from normal.
treated rats: It has been known that pretreatment of normal rats with hydrocortisone raised the blood level of insulin (15). Moreover, it was demonstrated that hyperinsulinemia induced by pertussis vaccine was augmented by the superimposition of hydrocortisone therapy (6). Therefore, the effect of hydrocortisone on insulin secretion stimulated by the prior treatment of rats with FCA was studied (Fig. 8). The blood level of insulin in hydrocortisone-treated rats was as high as in FCA-treated rats when they were injected with isoproterenol. However, hyperinsulinemia caused by isoproterenol in FCA-injected rats was likewise augmented by the superimposition of hydrocortisone therapy.

Effect of FIA and BCG cells on insulin-releasing action of isoproterenol: FCA used in these experiments is composed of FIA and BCG cells. FIA consists of mineral oil and surface reagents. We examined the effect of FIA or BCG cells alone on the insulin-releasing action of isoproterenol (Fig. 9). When BCG cells suspended in saline at the concentration of 2.5 mg/ml was preinjected i.p. (1 ml/100 g), the increment caused by isoproterenol was about ten times as much as in the control. BCG cells injected i.v. into rats as an emulsion also enhanced the insulin-increasing action of isoproterenol (data not shown).

DISCUSSION

The findings presented in this paper show that some components in BCG cells potentiate insulin secretion mediated by the stimulation of β-adrenergic functions. Isoproterenol elevated the plasma level of insulin more than epinephrine in FCA-treated rats (Fig. 1A and 1B). Phenylephrine scarcely
stimulated insulin secretion (Fig. 1C). The order of effectiveness of catecholamines used is the same as the order of potency of these drugs as $\beta$-stimulants. It is also known that insulin secretion in response to glucose and tolbutamide is dependent on changes in the cyclic AMP level in pancreatic B-cells in rats (16–18). Therefore, the findings that the insulin-releasing actions of glucose and tolbutamide were also enhanced in FCA-treated rats (Fig. 2) could be explained by the same mechanism, that is, $\beta$-adrenergic functions are predominant over $\alpha$-adrenergic functions in pancreatic B-cells in FCA-treated rats.

Other $\beta$-adrenergic responses such as lactate production, glycogenolysis, or lipolysis seem to be more sensitive to $\beta$-agonists in FCA-treated rats. In fact, lactate production was more stimulated by isoproterenol in FCA-treated rats (Fig. 4). However, pre-treatment of animals with AIS had no effect on the rate of isoproterenol-stimulated lactate production (Fig. 7A), suggesting that the enhanced lactate production does not depend on the insulin level. On the other hand, glycogenolysis and lipolysis were not enhanced further by isoproterenol in FCA-injected rats (Fig. 5A and Table 1). This may be due to variations in responsiveness of each parameter to isoproterenol and to FCA or due to the different degree of attenuation by insulin. Judging from the results shown in Figs. 6, 7A and 7B, insulin released by isoproterenol seems to attenuate differently the other $\beta$-adrenergic actions of isoproterenol. It is reasonable that epinephrine which does not increase the release of insulin in normal rats (Fig. 1B) could show its glycogenolytic action (Table 1), whereas isoproterenol which increases the release of insulin did not show its glycogenolytic action.

On the other hand, injection of AIS into rats allowed isoproterenol to increase the blood level of FFA even in FCA-injected rats (Fig. 7B). This suggests that reesterification of the FFA released through isoproterenol-stimulated lipolysis was enhanced by insulin released by isoproterenol in FCA-treated rats, i.e. insulin released in response to isoproterenol seems to be responsible for an abolition of isoproterenol-induced glycogenolysis and hyperlipidemia (FFA) in FCA-treated rats.

A large dose of hydrocortisone injected into rats enhanced the isoproterenol-stimulated insulin release (Fig. 8) (15). It has been postulated that glucocorticoids enhance the $\beta$-actions of catecholamines by activating the processes following the formation of cyclic AMP (19). However, it is not clear now whether FCA acts at the receptor level or on the events following the formation of cyclic AMP.

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