Biochemical Pharmacology, Vol. 30, No. 24, pp. 3305–3309, 1981.
Printed in Great Britain.

0006-2952/81/243305-05 $02.00/0

INVOLVEMENT OF ENDOGENOUS EPINEPHRINE IN HISTAMINE-INDUCED HYPERINSULINEMIA AND HYPOGLYCEMIA IN RATS TREATED WITH ISLET-ACTIVATING PROTEIN

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(*Received* 16 *January* 1981; *accepted* 18 *May* 1981)

Abstract—Islet-activating protein (IAP), purified from the culture medium of *Bordetella pertussis*, was injected i.v. into rats at a dose of 5 μg/kg. While the injection of histamine caused hyperglycemia in normal rats, it caused marked hypoglycemia associated with hyperinsulinemia in IAP-treated rats. No hypoglycemia developed after histamine if the IAP-treated rats had been adrenodemedullated or injected with a β-adrenergic antagonist or with anti-insulin serum. Histamine-induced hyperinsulinemia in IAP-treated rats was also abolished by adrenodemedullation or a β-adrenergic antagonist. Ether anesthesia, which provokes the release of epinephrine from the adrenal medullae, mimicked the action of histamine in both normal and IAP-treated rats. Histamine did not influence insulin secretion when it was added directly to the incubation medium of islets isolated from IAP-treated rats. It is concluded that epinephrine released from the adrenal medullae in response to histamine challenge enhances insulin secretion via the β-adrenergic receptors, thereby causing severe hypoglycemia in IAP-treated rats.

Islet-activating protein (IAP), recently purified from the supernatant fraction of the culture medium of *Bordetella pertussis*, potentiates the insulin secretory responses of the pancreatic B-cells to nutritional and hormonal stimuli when it is injected at a dose as low as 0.5 to 1.0 μg/kg into animals [1–6] or man [7], or added directly to the culture medium of rat pancreatic islets [4]. Moreover, IAP causes attenuation of epinephrine hyperglycemia and hypersensitivity to histamine in experimental animals [2]. It was shown that the attenuation of epinephrine hyperglycemia resulted from the hypoglycemic action of insulin secreted in excess in response to epinephrine challenge. There are a number of reports [8–12] that histamine-induced hyperglycemia was suppressed in pertussis-sensitized animals. The mechanism by which histamine-induced hyperglycemia is attenuated is not fully understood, although an involvement of the impaired action of endogenous catecholamines has been suggested [10, 13]. The present work was undertaken to examine the effect of histamine on the plasma concentrations of glucose and insulin in IAP-treated rats *in vivo* and on insulin release from islets isolated from these rats *in vitro*. It is concluded that the altered response of IAP-treated rats to histamine is accounted for by an IAP-induced modification of the action of epinephrine released from the adrenal medullae following histamine challenge.

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MATERIALS AND METHODS

In vivo *experiments*. Male Wistar rats, weighing 120–130 g, were used after a 20-hr fast. IAP was injected intravenously at a dose of 5 μg/kg body weight, and the rats were used 3 days later. In some experiments, rats had been bilaterally adrenodemedullated or sham operated (as control) under ether anesthesia 5 days prior to the IAP injection. After the experiments, the removal of adrenal medullae was confirmed histologically. Blood samples were withdrawn from the tail vein and analyzed for concentrations of glucose and insulin. Blood glucose was determined by the glucose oxidase method [14] and the plasma insulin by the double antibody method using rat insulin as standard [15]. Drug dosages injected refer to the salts.

In vitro *experiments*. Islets were isolated from IAP-treated (5 μg/kg, 3 days before) or normal (saline injected) rats by the method of Lacy and Kostianovsky [16] with a minor modification [4]. The islets (usually two islets per flask) were first incubated in 200 μl of Krebs–Ringer bicarbonate buffer fortified with 2% bovine serum albumin and 200 kallikrein inhibitory units/ml aprotinin ("basal medium"). Glucose was added in a final concentration of 3.3 mM. After this first incubation for 60 min, islets were further incubated with either 3.3 mM or 16.7 mM glucose in the presence or absence of 0.2 or 1.0 mM histamine for 60 min. Both incubations were carried out with shaking at 120 strokes/min at 37° under a gas mixture of 95% O_2/5% CO_2. After the incubation of the islets, an aliquot (10 μl) of the incubation medium was taken and assayed for the insulin content.

Materials. IAP was purified from the 48-hr culture supernatant fraction of *B. pertussis* cells according to the procedure described elsewhere [1]. The anti-insulin serum used in Fig. 6 was obtained from guinea pigs immunized with bovine crystalline insulin. The anti-insulin serum, having such a potency as to neutralize 2 units of insulin per ml, was injected into the rats at a volume of 0.4 ml/100 g body weight. Propranolol was provided by Professor M. Ui, Hokkaido University. Commercial sources of other chemicals were: epinephrine bitartrate, Nakarai Chemical Co. (Kyoto, Japan); histamine dihydrochloride, Wako Pure Chemical Co. (Tokyo, Japan); [^{125}I]insulin and anti-insulin serum for radioimmunoassay, Dainabot Radioisotope laboratories (Tokyo); aprotinin, Hoechst Japan Ltd. (Tokyo); bovine serum albumin (Fraction V), Sigma Chemical Co. (St. Louis, MO); collagenase (type IV), Worthington Biochemical Co. (Freehold, NJ). Other reagents used were of analytical grade.

Statistics. Statistical analyses of differences between mean values were carried out using Student's *t*-test. Differences occurring with a probability of ≤ 0.05 were considered significant.

RESULTS

Histamine-induced hypoglycemia in IAP-treated rats. The changes in the concentration of blood glucose following the subcutaneous injection of various amounts of histamine into normal and IAP-treated rats are shown in Fig. 1. The lowest dose (1 mg/kg) of histamine was without effect in both types of rats. The injection of histamine at doses of 10 or 100 mg/kg into normal rats caused a highly significant increase in the concentration of blood glucose. No hypoglycemia was elicited by histamine in normal rats. In sharp contrast, smaller amounts of histamine (5 mg or 10 mg/kg) induced severe hypoglycemia in IAP-treated rats.

Effect of adrenodemedullation on glycemic action of histamine. Earlier reports [10] showed that the injection of histamine into normal mice resulted in the release of endogenous catecholamines from the adrenal medullae, thereby causing hyperglycemia. Therefore, the glycemic action of histamine was studied. Figure 2 shows the concurrent change in blood glucose and plasma insulin concentrations induced by histamine. The injection of histamine into rats with intact adrenal medullae caused much greater hyperinsulinemia in IAP-treated rats than in untreated rats. The hyperinsulinemia was associated with hypoglycemia. After adrenodemedullation, however, neither hyperinsulinemia nor hypoglycemia was caused by histamine in IAP-treated rats. Likewise, histamine-induced hyperglycemia was almost abolished by adrenodemedullation in nontreated rats. Thus, the adrenal medullae may play an indispensable role in histamine-induced hyperinsulinemia in IAP-treated rats as well as hyperglycemia in normal rats. In Fig. 3, the action of histamine in normal and IAP-treated rats is compared with that of epinephrine. Epinephrine (200 μg/kg), just like histamine (5 mg/kg), caused marked hyperinsulinemia in IAP-treated rats and hyperglycemia in untreated rats. The hyperinsulinemia was associated with hypoglycemia or the attenuation of hyperglycemia in IAP-treated rats. In Fig. 4, the effect of ether anesthesia is shown, since ether vapour, like

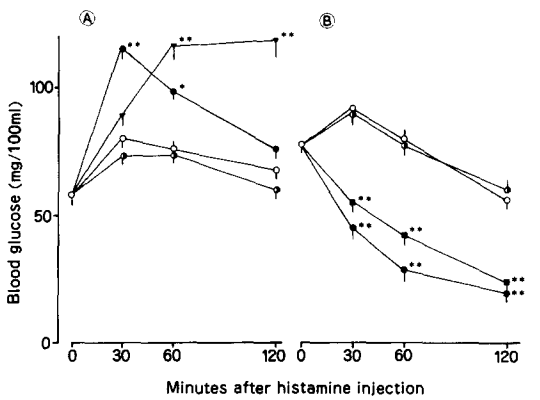

Fig. 1. Effect of histamine on the blood glucose concentration in normal (A) and IAP-treated rats (B). Rats that had been injected with saline or IAP (5 μg/kg, i.v.) 3 days before were injected s.c. with the following doses of histamine (as salts, per kg body wt); (○) 0 mg (saline), (◑) 1 mg, (■) 5 mg, (●) 10 mg, and (▼) 100 mg. Time 0 indicates the time of injection of histamine. Each point in this and the following figures shows the mean ± S.E.M. The number of observations was three. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

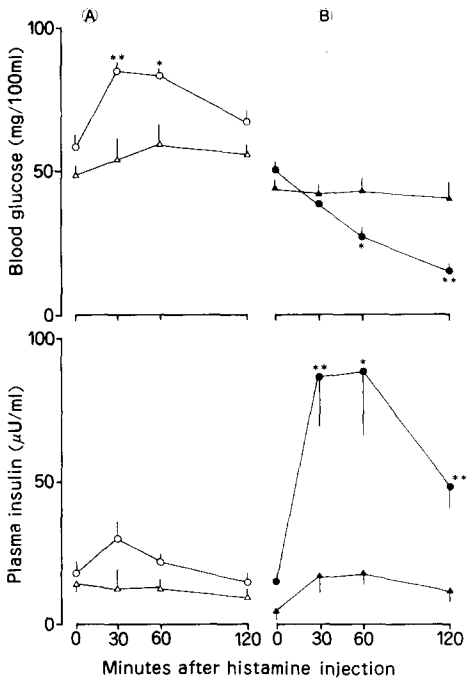

Fig. 2. Effect of adrenodemedullation on the action of histamine in normal (A) and IAP-treated rats (B). Rats had been adrenodemedullated bilaterally (△, ▲) or sham-operated (○, ●) 5 days before the injection with saline (○, △) or IAP (5 μg/kg, ●, ▲); histamine (5 mg/kg) was injected s.c. 3 days later. The number of observations was three to four. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

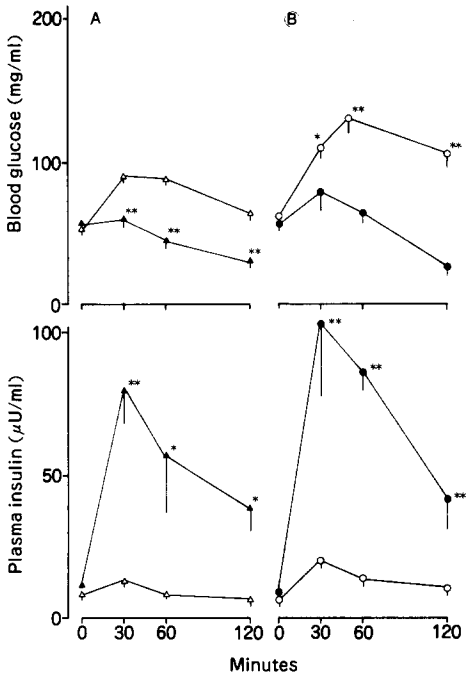

Fig. 3. Comparison of hypoglycemic and hyperinsulinemic effects of histamine (A) with those of epinephrine (B). Rats that had been injected i.v. with saline (△, ○) or IAP (5 μg/kg, ▲, ●) 3 days before were injected s.c. with histamine (5 mg/kg) or epinephrine (200 μg/kg) at time 0. The number of observations was three to four. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

histamine, is known to elicit the release of catecholamines from the adrenal medullae. Exposure of normal rats to concentrated ether vapour caused hyperglycemia followed by a slight increase in the concentration of plasma insulin. In sharp contrast, marked hyperinsulinemia accompanied by gradual hypoglycemia was observed in IAP-treated rats. Thus, the glycemic and insulinemic effects of ether anesthesia were very similar to those of histamine in IAP-treated and untreated rats.

Effect of a β-adrenergic antagonist and anti-insulin serum on the action of histamine in IAP-treated rats. Propranolol, a β-adrenergic antagonist, completely

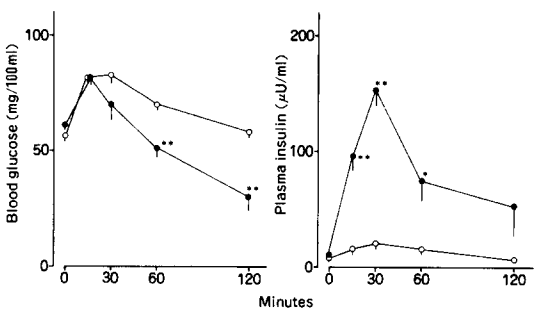

Fig. 4. Effect of ether anesthesia on concentration of blood glucose and plasma insulin. Rats that had been injected i.v. with saline (○) or IAP (5 μg/kg, ●) 3 days before were exposed to ether vapour for 2 min (time 0 represents the start of exposure). The number of observations was four. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

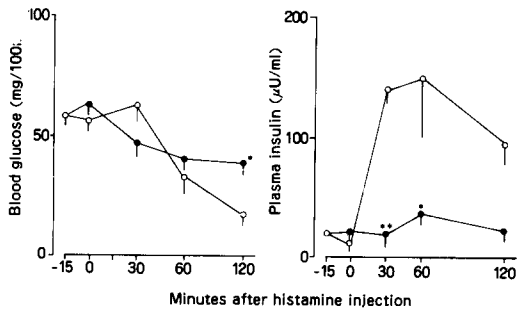

Fig. 5. Effect of a β-adrenergic antagonist on hypoglycemic and hyperinsulinemic actions of histamine in IAP-treated rats. Rats that had been injected i.v. with IAP (5 μg/kg) 3 days before were injected s.c. with propranolol (20 mg/kg, ●) or saline (○) 15 min before s.c. injection of histamine (5 mg/kg) at time. The number of observations was four. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

abolished the histamine-induced increase in the concentration of plasma insulin in IAP-treated rats (Fig. 5). Moreover, no significant hypoglycemia was observed after β-adrenergic blockade. A possibility that histamine-induced hypoglycemia in IAP-treated rats was due to secreted insulin under these conditions was further supported by the data in Fig. 6, where histamine failed to elicit hypoglycemia in IAP-treated rats when circulating insulin was neutralized by anti-insulin serum. Hyperglycemia developed instead under these conditions in IAP-treated rats, as observed even without the antiserum in rats untreated with IAP.

Effect of histamine on the secretion of insulin from isolated islets. A direct effect of histamine on insulin secretion was studied by incubating islets from normal or IAP-treated rats with histamine (Fig. 7). A much greater amount of insulin was secreted from islets of IAP-treated rats than from normal islets in response to 16.7 mM glucose. No significant effect of histamine was observed on insulin secretion in the presence of 16.7 mM glucose, though it caused a

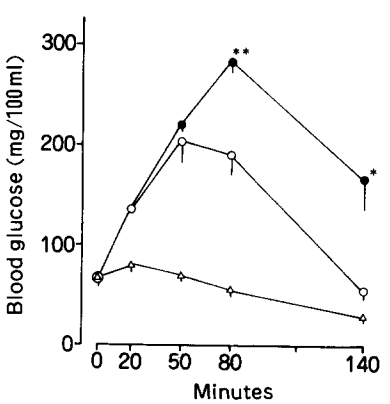

Fig. 6. Effect of anti-insulin serum on histamine-induced hypoglycemia in IAP-treated rats. Rats that had been injected i.v. with IAP (5 μg/kg) 3 days before were injected i.v. with anti-insulin serum (○, ●) or normal serum (△) at time 0 and then injected s.c. with histamine (5 mg/kg, △, ●) 20 min later. The number of observations was four to five. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

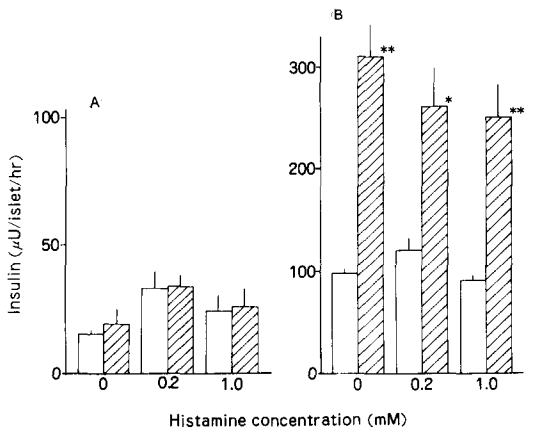

Fig. 7. Effect of histamine on insulin secretion from isolated islets of normal (open column) and IAP-treated rats (hatched column). Islets isolated from IAP-treated (5 μg/kg, i.v., 3 days before) and from nontreated rats were incubated with histamine in the presence of 3.3 mM (A) or 16.7 mM (B) glucose. The number of observations was five. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

slight increase at 0.2 mM in the presence of 3.3 mM glucose. In any case, the pretreatment of pancreas donor rats with IAP failed to change the insulin secretory response of their islets to *in vitro* addition of histamine.

DISCUSSION

The injection of histamine into normal rats caused hyperglycemia accompanied by a slight increase in the concentration of plasma insulin, whereas the injection into IAP-treated rats caused marked hyperinsulinemia followed by severe hypoglycemia (Figs. 1 and 2). This peculiar reversal of histamine action by IAP treatment may be due to altered insulin secretory responses of IAP-treated rats to epinephrine which was secreted from the adrenal medullae following histamine challenge. Epinephrine suppresses insulin secretion from the pancreas via stimulation of α-adrenergic receptors in normal animals [3, 4, 17, 18]; this is one of the reasons why epinephrine gives rise to marked hyperglycemia. In IAP-treated rats, however, epinephrine causes enhancement, rather than suppression of insulin release (Fig. 3, Refs. 2–6); this is because epinephrine stimulates β- rather than α-adrenergic receptors [3, 4] under these conditions. Hyperinsulinemia thus provoked would lead to severe hypoglycemia instead of hyperglycemia. The experimental basis of this conclusion is as follows.

Involvement of endogenous epinephrine in the action of histamine. Histamine is known to stimulate the release of catecholamines, especially epinephrine from the adrenal medullae [19]. The effects of histamine on circulating concentrations of insulin and glucose observed in the present study appear to be mediated indirectly by the secreted epinephrine for the following reasons. First, after adrenodemedullation the injection of histamine did not exert any influence on the concentrations of glucose and insulin. It is known that the amount of circulating epinephrine is very small after adrenodemedullation [20]. Second, the changes in the concentration of blood glucose and plasma insulin following histamine injection were just like those following epinephrine injection (Fig. 3). Both drugs caused significant hyperglycemia with a slight increase in the plasma insulin concentration in normal rats, and marked hyperinsulinemia followed by hypoglycemia in IAP-treated rats. Third, the glycemic and insulinemic actions of ether anesthesia, which provoke the release of epinephrine from the adrenal medullae, were very similar to the actions of histamine (Fig. 4). Fourth, hyperinsulinemia in IAP-treated rats following the injection of histamine was completely abolished by the pretreatment of the rats with a β-adrenergic antagonist (Fig. 5), suggesting that the action of histamine was mediated through β-adrenergic receptors. Finally, histamine was without effect on insulin secretion when it was added directly to the incubation medium of isolated islets (Fig. 7). Thus, the effects of histamine on the circulating level of glucose and insulin are a reflection of the action of epinephrine secreted in response to histamine injection regardless of whether the rats had been treated with IAP or not. Although there was no evidence as to whether or not the release of epinephrine from the adrenal medullae was also modified by IAP treatment, a possibility that the altered secretion of epinephrine was involved in the differential action of histamine would be unlikely because epinephrine injected, just like histamine injected, exhibited extraordinary actions in IAP-treated rats.

Hyperinsulinemia and hypoglycemia in IAP-treated rats. Epinephrine is known to suppress insulin secretion via the stimulation of α-adrenergic receptors. Epinephrine-induced hyperglycemia may be partly explained by the suppression of insulin secretion. Recent papers have shown that experimental animals which had been injected with pertussis vaccine [21, 22] or IAP [2, 5, 6] exhibited potentiated insulin secretory responses to various kinds of stimuli *in vivo*. Enhanced insulin secretion was substantiated in perfusion experiments of the pancreases [3, 18] or incubation of islets isolated [4] from pertussis- or IAP-treated rats. These findings are reproduced in Fig. 7, in which islets isolated from IAP-treated rats secreted much more insulin in response to 16.7 mM glucose than those from normal rats. One of the striking characteristics of IAP was that epinephrine caused enhancement, rather than suppression, of insulin secretion in these animals. Such enhanced secretion of insulin following the injection of epinephrine would result from reversal of the α-adrenergic inhibition of pancreatic insulin secretion in IAP-treated rats. Katada and Ui [4] have suggested that the reversal is closely related to an IAP-induced activation of native calcium ionophores postulated to be located on the islet cell membrane. Enhanced availability of calcium in the IAP-treated cells might be responsible for the abolition of the α-adrenergic action, considering the report [23] that α-adrenergic action to inhibit insulin secretion could be reversed in the cultured pancreatic islets by enhancing the calcium influx. It appears, therefore, that under the condition of an α-adrenergic blockade

in IAP-treated rats epinephrine exclusively stimulates the β-adrenergic receptors and thus results in marked hyperinsulinemia *in vivo*. On the other hand, the α-action to inhibit insulin secretion in normal rats is predominant over the β-action to enhance the secretion, and the injection of epinephrine results in a decrease or no change or only a slight increase (probably resulting from hyperglycemia) in the plasma concentration of insulin. The attenuation of epinephrine-induced hyperglycemia in IAP-treated rats resulted from the hypoglycemic action of insulin markedly secreted following epinephrine challenge [2, 22]. Epinephrine released from the adrenal medullae following histamine administration into IAP-treated rats may enhance insulin secretion via β-adrenergic receptors, because propranolol, a β-adrenergic antagonist, was very effective in preventing histamine-induced hyperinsulinemia and hypoglycemia (Fig. 5). Hypoglycemia did not develop following histamine injection into IAP-treated rats unless the adrenal medullae was functioning; there was no hyperinsulinemia either in adrenodemedullated rats. When the circulating insulin was neutralized by anti-insulin serum, histamine provoked hyperglycemia, rather than hypoglycemia, even in IAP-treated rats (Fig. 6). Thus, histamine-induced hypoglycemia is undoubtedly mediated by increased secretion of insulin in IAP-treated rats.

The severe hypoglycemia following histamine challenge may be partly responsible for the so-called histamine-sensitizing activity of pertussis vaccine [24] or IAP [2]. This problem is currently under study in our laboratory.

Acknowledgements—We are very grateful to Dr. Michio Ui, Professor of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, for valuable advice and discussion during the performance of these experiments and preparation of this manuscript. Thanks are also due to Mrs. H. Kidowaki for her excellent assistance.

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