Islet-activating protein

ENHANCED INSULIN SECRETION AND CYCLIC AMP ACCUMULATION IN PANCREATIC ISLETS DUE TO ACTIVATION OF NATIVE CALCIUM IONOPHORES*

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The mechanism whereby "islet-activating protein" (IAP) purified from the culture medium of Bordetella pertussis potentiates insulin secretion was studied by experiments in vitro with islets of rats once injected with IAP (0.5 μg/100 g of body weight, 3 days before killing) or with islets that had been exposed to IAP (0.1 to 100 ng/ml) for 24 h. The IAP treatment markedly enhanced insulin secretory responses and cAMP accumulation in islets, facilitated the efflux of 46Ca through the cell membrane, and abolished the α-adrenergic action of epinephrine (and somatostatin) to inhibit glucose-induced insulin release, cAMP accumulation, and 46Ca uptake. These effects of the IAP treatment were reduced when islets were incubated in a low calcium medium. Based on these results, it was concluded that IAP interacts directly but slowly with the islet B cell in such a manner as to render more calcium available to the stimulus-secretion coupling mechanism as a result of sustained activation of native calcium ionophores on the cell membrane.

The plasma concentration of insulin increased very markedly in response to various insulin secretagogues in rats when they had been injected once with pertussis vaccine 3 to 10 days before (1, 2). Enhanced insulin secretion from their pancreas was markedly inhibited in the normal rat pancreas, but was increased when islets were incubated in a low calcium medium. Based on these results, it was concluded that pertussis vaccine was found on the insulin secretory response of pancreas to epinephrine; during perfusion, the release of insulin was increased from pancreas of these pertussis-sensitized rats more than from pancreas of nonsensitized rats (3). A new protein was released from pancreas of these pertussis-sensitized rats as did pertussis-sensitized rats than from pancreas of nonsensitized rats (3). A new protein responsible for this unique action was then purified from the culture medium of Bordetella pertussis (4). This protein of molecular weight 77,000, termed islet-activating protein (IAP), was found on the islet B cell in such a manner as to render more calcium available to the stimulus-secretion coupling mechanism as a result of sustained activation of native calcium ionophores on the cell membrane.

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1 The abbreviations used are: IBMX, 3-isobutyl-1-methylxanthine; KIU, kallikrein inhibitory units; IAP, islet-activating protein, BtCAMP, dibutyryl cyclic AMP; "IAP islets," the islets isolated from the rat treated with IAP 3 days before; "normal islets," the islets isolated from the nontreated rat.

2 The methods of Lacy and Kostianovsky (9) with minor modifications.

3 The plasma concentration of insulin increased very markedly in response to various insulin secretagogues in rats when they had been injected once with pertussis vaccine 3 to 10 days before (1, 2). Enhanced insulin secretion from their pancreas was substantially by perfusion experiments in vitro; in response to the addition of glucose, arginine, sulfonylureas, and IBMX1 to perfusate, much more insulin was released from pancreas of these pertussis-sensitized rats than from pancreas of nonsensitized rats (3). A new protein responsible for this unique action was then purified from the culture medium of Bordetella pertussis (4). This protein of molecular weight 77,000, termed islet-activating protein, exerted the same enhancing effect on insulin secretory responses as did pertussis vaccine when it was injected intravenously into rats at a dose as low as 1 μg per animal (4, 5).

The most striking effect of the treatment of rats with pertussis vaccine was found on the insulin secretory response of pancreas to epinephrine; during perfusion, the release of insulin was inhibited in the normal rat pancreas, but was stimulated in the pertussis-sensitized rat pancreas, very markedly by the catecholamine (3). The inhibition was abolished by the simultaneous addition of an α-adrenergic antagonist, while the stimulation was antagonized by a β-adrenergic blocking agent (3). Thus, the adrenergic mechanism underlying insulin secretion was profoundly affected by the treatment of rats with pertussis in vivo, thereby leading to attenuation of hyperglycemia otherwise induced by epinephrine (6).

CAMP and calcium are known to play important roles as a link in stimulus-secretion coupling in the pancreatic B cell; most of the insulin secretagogues caused intracellular CAMP accumulation and transmembrane calcium flux in pancreatic islets (7, 8). It is possible, therefore, to expect that changes in CAMP contents and calcium movements in pancreatic islets in response to insulin secretagogues will be influenced by the treatment of pancreas donor rats with IAP. The present paper examines this possibility.

EXPERIMENTAL PROCEDURES

Materials—IAP was purified by Dr. M. Yajima (R & D Project, Kakenuku Kako, Shiga) from the 3-day culture supernatant of B. pertussis cells (Tohama strain, phase I) according to the procedure described elsewhere (4). Aprotinin, rat insulin (Novo Laboratories), glucagon, verapamil, and glibenclamide were kind gifts from Hoocho Japanese Co. Ltd. (Tokyo), Kodama Ltd. (Tokyo), Eli Lilly and Co. (Indiana), Eisai Co. (Tokyo), and Yamanouchi Pharmaceutical Co. (Tokyo), respectively. A23187 was donated by Dr. R. L. Hanil in Lilly Research Laboratories. Reagents for microradioimmunoassay of CAMP, i.e. 125I-labeled CAMP tyrosine methyl ester, anti-CAMP rabbit antiserum, and cAMP*, were also gifts from Yamash Shoyu Co. (Choba, Chiba). Propranolol was provided by Otsuka Pharmaceutical Co. (Tokushima). Somatostatin was a kind gift from Professor N. Yanaihara, Shizuoka College of Pharmacy, Shizuoka.

Commercial sources of other chemicals are: collagenase (type 4, Lot 46S0099P), Worthington Biochemical Co. (Freehold, N. J.); bovine serum albumin (Fraction V) and dextan (clinical grade, average molecular weight, 80700), Sigma Chemical Co. (St. Louis); fetal bovine serum, Flow Laboratories Inc. (Bethesda, Md.); 4CaCl2 and d [1-3H]mannitol, New England Nuclear (Boston); 125I-labeled insulin, Daiabot Radioisotope Laboratories (Tokyo); phenolamine, CIBA-GEIGY-Japan (Tokyo), epinephrine, E. Merck (Darmstadt); IBMX, Aldrich Chemical Co. (Milwaukee); tissue culture medium 199, Nakai Chemicals, Ltd. (Kyoto). Other reagents used are of analytical grade.

Treatment of Rats with IAP—Male rats of the Wistar strain were injected intravenously with IAP at a dose of 0.5 μg/100 g of body weight. These IAP-treated rats, together with the nontreated (normal) rats injected with saline instead of IAP, were used as pancreas donors 3 days later. They had been fasted for 20 h before killing unless otherwise stated.

Preparation and Incubation of Pancreatic Islets to Estimate Insulin Release and CAMP Accumulation—Islets were isolated by the method of Lacy and Kostianovsky (9) with minor modifications. In brief, the pancreas was distended with Hanks' solution containing 1% albumin and 1% dextan, minced, and then digested by collagenase (10 mg/ml) in a mixture additionally containing 400 KIU/ml of the protease inhibitor, aprotinin. Digestion was performed by agitation at 37°C for 10 min.
Following this digestion, the mixture was diluted with the above medium and allowed to settle for 1 min. The supernatant was then removed with a syringe and discarded. This washing procedure was repeated several times. The islets were then transferred to a Petri dish and viewed with microscopic acid (×10). Large islets were siphoned through a micropipette and transferred to incubation flasks containing 200 µl of Krebs-Ringer bicarbonate medium fortified with (0.5% albumin and 500 KIU/ml of aprotinin ("basal medium") further supplemented with 3.3 mM glucose. After the first incubation for 45 min, the islets were further incubated in 200 µl of the fresh "basal medium" containing additions such as insulin secretagogues for 30 or 60 min. Both incubations were carried out with shaking at 120 strokes/min at 37°C under a gas mixture of 95% O₂, 5% CO₂.

**Insulin and cAMP Determination**—After incubation of islets (usually five islets per flask), an aliquot (20 µl) of the incubation medium was taken and assayed for insulin content by the radioimmunochemical method described previously (3) using rat insulin as the standard. For cAMP assay, HCl and EDTA were quickly added to the reaction mixture to make final concentrations of 0.1 mM and 5 mM, respectively. The acidified mixture was then put in a boiling means of a Millipore filter or a micropipette, washed with the fresh medium, and suspended in 0.5 ml of distilled water in a vial which was further added with 5 ml of Triton/toluene scintillation fluid. Islets were gradually disintegrated by virtue of Triton X before being counted in a liquid scintillation spectrometer. Alternatively, the second incubation was terminated by a 60-min incubation in nonradioactive Tris-buffered medium fortified with 2 mM LaCl₃ which was employed to wash out extracellular and superficially bound ⁴⁵Ca without altering the intracellular concentration of the isotope (12). The radioactivity retained in islets after the LaCl₃ washing was referred to as "lanthanum-nondegradable" ⁴⁵Ca.

In studies of ⁴⁵Ca efflux, islets, which had been first incubated for 45 min as described above, were loaded with the isotope in the fresh "basal medium" added with 16.7 mM glucose for 120 min. They were then incubated for 60 min or for various periods in nonradioactive medium containing test substances as required. These experiments, too, were ended by 60 min of washing with 2 mM LaCl₃.

**Long Term Exposure of Islets to IAP**—In some experiments, islets were treated with IAP in vitro as follows. Islets isolated from normal fasted rats were transferred to sterile plastic Petri dishes (35 mm in diameter) which contained a tissue culture medium (TCM199 with Earle's modified salts) supplemented with 10% fetal bovine serum, streptomycin (0.1 mg/ml), penicillin (100 i.u./ml), 16.7 mM glucose, and IAP in various concentrations. The dishes were stored in humidified air containing 5% CO₂ for 24 h at 37°C. Thereafter, the islets were rinsed with the "basal medium" containing 3.3 mM glucose to be freed of IAP and then were preincubated for 1 h in the medium of the same composition. During this preincubation period, the rate of insulin release subsided to the basal level as reported by Frankel et al. (13). Finally, the islets were incubated for 30 min with or without 0.7 µM epinephrine in the presence of 16.7 mM glucose under the same conditions as described above for the fresh islets.

**RESULTS**

**Kinetics of Insulin Release and cAMP Accumulation**—When pancreatic islets isolated from normal rats were incubated with 16.7 mM glucose, insulin was released progressively in response to glucose for the first 30 min (Fig. 1A). The rate of insulin release from islets of fed rats was about twice as high as the rate from islets of fasted rats. The addition of IBMX, a potent inhibitor of cAMP phosphodiesterase, to the incubation medium was also effective in enhancing insulin release from islets of fasted rats; insulin was released with IBMX at a rate 3-fold higher than without IBMX.

Increased secretion of insulin was associated with increased production of cAMP under these conditions, although the time course of the cAMP accumulation (Fig. 1B and C) was markedly different from that of insulin release. cAMP accumulated very rapidly within 5 min. Afterwards, the rate of accumulation was markedly reduced with a trend to level off within 30 min. Thus, the cAMP response of islets to glucose or IBMX was very rapid. Only a fraction of cAMP in the tissue was released into the incubation medium; the release occurred linearly with time as did insulin release. Since any effect exerted on the total cAMP content rapidly up to 5 min was still retained essentially in the same degree or was somewhat enhanced, at 30 min (Fig. 1B), we determined the total (i.e. tissue plus medium) cAMP response of islets in the following experiments in this paper. The results in Fig. 1 confirmed the previous findings (14, 15) that more insulin was released in association with more cAMP generation from islets of fed rats than from islets of starved rats.

**Effect of IAP Treatment on Insulin Contents of Pancreatic Tissues and Islets**—The wet weights of pancreases isolated from rats (body weight, 223 ± 4 g) treated with IAP as described above, "CaCl₂ (4 µM), D-[³H]mannitol (0.1 mM, 4 µCi), and other test substances as indicated in figures and tables were added to the second incubation which was carried out for 120 min. Radioactive mannitol was used as extracellular marker; the islet content of ³H in excess of "H after incubation was determined as intracellular uptake of "H. For measurement of "total" intracellular ³H, islets were rapidly separated from the incubation medium by means of a Millipore filter or a micropipette, washed with the fresh medium, and suspended in 0.5 ml of distilled water in a vial which was further added with 5 ml of Triton/toluene scintillation fluid. Islets were gradually disintegrated by virtue of Triton X before being counted in a liquid scintillation spectrometer.
The slight reduction of the insulin content of pancreas following IAP treatment may have resulted from enhanced secretion of insulin in vivo up to the time of sacrifice. Histological studies did not detect any morphological change in islets and B granules in pancreas of IAP-treated rats.

These results show that the islet mass in pancreas and insulin content of islets were comparable in control and IAP-treated rats. Thus, functions in vitro, such as insulin secretion and cAMP accumulation, of islets isolated from IAP-treated rats were compared with those of islets from control rats in the following experiments.

Insulin Secretory and cAMP Responses of Islets to Various Insulin Secretagogues as Potentiated by the Treatment of the Pancreas Donor Rats with IAP—For brevity, the islets isolated from the IAP-treated rats 3 days after the treatment and the islets isolated from the nontreated rats will be henceforth referred to as "IAP islets" and "normal islets," respectively. The contents of insulin and cAMP in these islets after incubation were plotted as a function of glucose concentration in Fig. 2. Raising the glucose concentration in the incubation medium from 3.3 to 16.7 mM gave rise to increases in both insulin release and cAMP content whether or not IBMX was added. When incubation was carried out in 3.3 mM glucose without IBMX, there was no difference in insulin release and cAMP content between "normal islets" and "IAP islets." At higher glucose concentrations without IBMX, however, more insulin was released from "IAP islets" than from "normal islets," without any enhancement of the cAMP response to glucose (Panels A and B). The addition of IBMX to incubation medium was very effective in increasing not only cAMP content but also insulin release at any concentration of glucose (compare Panels C and D with Panels A and B). Under these conditions, the cAMP content was much higher in "IAP islets" than in "normal islets" (Panel D). The higher content of cAMP was associated with enhanced insulin release (Panel C).

Effect of various insulin secretagogues on insulin release and cAMP accumulation in pancreatic islets was studied next as summarized in Table I. When pancreatic islets were incubated at a low (3.3 mM) concentration of glucose without IBMX, about a 2-fold increase in insulin release was caused by glucagon, arginine, and glibenclamide, although the increase induced by glucagon was not statistically significant due to variability of the data. These increases in insulin release were not associated with increased accumulation of cAMP, except for the case of glibenclamide addition, which, by itself, elevated the cAMP level slightly in "normal islets" and markedly in "IAP islets." In the incubation medium fortified with IBMX, both arginine and glibenclamide caused pronounced increases in cAMP accumulation and insulin release; under these conditions, much more insulin was released and much more cAMP accumulated in "IAP islets" than in "normal islets."

The glucagon effect was further studied in the incubation medium supplemented with 10 mM glucose. In the absence of IBMX, the cAMP content was elevated only slightly, but the insulin release was enhanced markedly by the addition of glucagon; more insulin was released from "IAP islets" than from "normal islets," without a significant change in the cAMP content, in response to glucagon. In the presence of IBMX, glucagon was effective in enhancing both insulin release and cAMP accumulation; much higher values were obtained for both parameters when the pancreas donor rats had been treated with IAP.

Since increases in the cAMP content of islets were invariably accompanied by enhanced insulin release in Figs. 1 and 2 and Table I, the effect of Bt-cAMP on insulin release was studied in Table II. Bt-cAMP enhanced insulin release in agreement with earlier reports (16, 17); the enhancement was more marked in the media supplemented with 10 mM glucose than with 3.3 mM glucose. More insulin was released from "IAP islets" than from "normal islets" when the derivative of cAMP was added to the media containing 10 mM glucose.

Inhibition by Epinephrine of Insulin Release and cAMP Accumulation in Pancreatic Islets via a-Adrenergic Receptors and Lack of the Inhibition in "IAP Islets."—In Fig. 3, "normal islets" were incubated with 16.7 mM glucose in the absence or presence of epinephrine at concentrations from 1.4 nM (0.25 ng/ml) to 11 μM (2 μg/ml). The glucose-induced insulin release and cAMP accumulation were both inhibited effectively by epinephrine at higher doses than 2.7 nM (0.5 ng/ml) in a dose-dependent manner. Epinephrine was similarly inhibitory in this regard in the presence of IBMX (data not shown here, but see Table VII). The concentrations of epinephrine required for the half-maximal and the maximal
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TABLE I

Insulin release and cAMP contents in pancreatic islets after incubation with various insulin secretagogues

Islets from IAP-treated (IAP) or nontreated (normal) rats were incubated as in Fig. 2. The concentrations of additions were: glucagon, 0.35 μM; arginine, 0.13 mM; glibenclamide, 0.1 μM. The mean value ± S.E. is recorded in this and the following tables.

<table>
<thead>
<tr>
<th>Additions</th>
<th>IBMX</th>
<th>No. of observations</th>
<th>Insulin release</th>
<th>cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>IAP</td>
</tr>
<tr>
<td></td>
<td>1 mM</td>
<td></td>
<td>Microunits/islet</td>
<td>fmol/islet</td>
</tr>
</tbody>
</table>

With 3.3 mM glucose

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Normal</th>
<th>IAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>−</td>
<td>8</td>
<td>9 ± 0.9</td>
<td>8 ± 0.4</td>
</tr>
<tr>
<td>Glucagon</td>
<td>−</td>
<td>6</td>
<td>21 ± 5.7</td>
<td>20 ± 5.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>−</td>
<td>8</td>
<td>21 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>−</td>
<td>6</td>
<td>22 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>6</td>
<td>26 ± 1.5</td>
<td>99 ± 3.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arginine</td>
<td>+</td>
<td>4</td>
<td>93 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>217 ± 28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>+</td>
<td>4</td>
<td>138 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>290 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

With 10 mM glucose

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Normal</th>
<th>IAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>−</td>
<td>4</td>
<td>24 ± 1.2</td>
<td>68 ± 5.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucagon</td>
<td>−</td>
<td>4</td>
<td>74 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>131 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>4</td>
<td>195 ± 15</td>
<td>290 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucagon</td>
<td>+</td>
<td>4</td>
<td>247 ± 14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>368 ± 24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Effect of additions is significant (p < 0.01).
<sup>b</sup> Effect of IAP treatment is significant (p < 0.01).
<sup>c</sup> Effect of additions is significant (p < 0.05).
<sup>d</sup> Effect of IAP treatment is significant (p < 0.05).

Inhibitions were around 10 nM and at least 1 μM, respectively, for both parameters. When similar experiments were repeated with “IAP islets,” however, neither insulin release nor cAMP accumulation was inhibited by epinephrine at a dose as high as 11 μM (Table III).

A possible involvement of an α- or β-adrenergic mechanism in the epinephrine-induced inhibition of insulin release and cAMP accumulation as observed in Fig. 3 was studied with the use of an α-adrenergic antagonist, phentolamine, and a β-adrenergic antagonist, propranolol (Table IV). Neither insulin and CAMP in islets nor their responses to epinephrine were affected by propranolol regardless of whether the islets were isolated from normal rats or from IAP-treated rats. In contrast, the epinephrine-induced inhibition of CAMP accumulation in “normal islets” was completely reversed by the addition of phentolamine. This reversal of CAMP accumulation was associated with significant attenuation of the action of epinephrine to inhibit insulin release: epinephrine caused a 90% inhibition in the absence of phentolamine and only 28% inhibition in its presence. Even when epinephrine was lacking or ineffective in “IAP islets,” phentolamine caused slight but significant increases in insulin release and CAMP accumulation. It also enhanced insulin release from “normal islets” in the absence of epinephrine.

Thus, the data in Table IV revealed that the inhibition of insulin release and CAMP accumulation induced by epinephrine in “normal islets” resulted from stimulation of α-adrenergic...
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**Table IV**

Effect of epinephrine on glucose-induced insulin release and cAMP accumulation in the presence of adrenergic antagonists

<table>
<thead>
<tr>
<th>Antagonist added</th>
<th>Epinephrine</th>
<th>Insulin release</th>
<th>cAMP accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM</td>
<td>Normal (µM)</td>
<td>IAP (µM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7 µM</td>
<td>0.7 µM</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>90 ± 3.6</td>
<td>162 ± 6 *</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>9 ± 1.7</td>
<td>144 ± 13 *</td>
</tr>
<tr>
<td>Propranolol (6.5)</td>
<td>-</td>
<td>93 ± 6.1</td>
<td>160 ± 19 *</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>6 ± 0.8</td>
<td>155 ± 11 *</td>
</tr>
<tr>
<td>Phentolamine (8.7)</td>
<td>-</td>
<td>130 ± 9 *</td>
<td>295 ± 17 *</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>94 ± 3.4 b, d</td>
<td>249 ± 25 *</td>
</tr>
</tbody>
</table>

*p < 0.01, effect of IAP treatment is significant.

*p < 0.01, effect of antagonist is significant.

**Table V**

Effect of somatostatin on glucose-induced insulin release and cAMP accumulation

<table>
<thead>
<tr>
<th>Somatostatin</th>
<th>Insulin release</th>
<th>cAMP accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (µg/ml)</td>
<td>IAP (µg/ml)</td>
</tr>
<tr>
<td></td>
<td>microunits/islet</td>
<td>fmol/islet</td>
</tr>
<tr>
<td>Normal</td>
<td>98 ± 5.7</td>
<td>197 ± 8 *</td>
</tr>
<tr>
<td>Normal</td>
<td>66 ± 6.7 *</td>
<td>187 ± 13 *</td>
</tr>
<tr>
<td>1</td>
<td>44 ± 5.0 *</td>
<td>177 ± 7.5 *</td>
</tr>
</tbody>
</table>

*p Effect of IAP treatment is significant (p < 0.01).

*p Effect of somatostatin is significant (p < 0.01).

Failure of Somatostatin to Inhibit Insulin Release and cAMP Accumulation in "IAP islets"—The addition of somatostatin to the incubation medium fortified with 16.7 mM glucose caused inhibition of insulin release and cAMP accumulation in "normal islets" (Table V), although the degree of inhibition was rather smaller than that induced by epinephrine (Fig. 3). No significant inhibition was induced by somatostatin, however, when "IAP islets," instead of "normal islets," were incubated under the same conditions.

In "normal islets" incubated with phentolamine, somatostatin caused only a 16% inhibition for both insulin release and cAMP accumulation. This inhibition was significant only at a 5% level (number of observations, five). Likewise, the increase in the calcium concentration in the incubation medium reduced somatostatin-induced inhibition to 22% (p = 0.01, number of observations, four) for insulin release in agreement with previous reports (18, 19) and to 13% (not significant, number of observations, four) for cAMP accumulation. Thus, the inhibitory action of somatostatin on insulin release and cAMP accumulation was partly antagonized by either the addition of phentolamine or increasing calcium concentration in the incubation medium.

Effect of a Calcium Antagonist and Calcium Insufficiency in the Incubation Medium on Insulin Secretion and cAMP Accumulation—Verapamil, a potent calcium antagonist, is known to inhibit insulin release from isolated pancreatic tissues or islets in vitro (20, 21). In Table VI, the addition of verapamil at concentrations from 1 to 100 µM inhibited glucose-induced insulin release and cAMP accumulation either in the presence or absence of IBMX in a dose-dependent manner. In contrast to the inhibitions induced by epinephrine or somatostatin (Fig. 3 and Table V), verapamil-induced inhibition was observed in "IAP islets" to essentially the same extent as in "normal islets".

Table VII shows the results of experiments in which the extracellular concentration of calcium was reduced by incubating islets in the low calcium or calcium-free medium. The results will be summarized as follows. 

(a) In "normal islets," a reduction in the extracellular calcium concentration caused blunted insulin release with decreased accumulation of cAMP; the decrease in cAMP accumulation was less marked than the decrease in insulin release. 
(b) This unfavorable effect induced by lowering the extracellular calcium concentration was more marked in "IAP islets" than in "normal islets"; consequently, the treatment of islets donor rats with IAP enhanced neither insulin release nor cAMP accumulation in islets when they were incubated in the low calcium or calcium-free medium. 
(c) When incubation was made in the low calcium or calcium-free medium, epinephrine became inhibitory to insulin secretion and CAMP accumulation in "normal islets" or "IAP islets" but was not in "IAP islets." These effects of glucose and IAP on insulin release and CAMP accumulation were partly antagonized by either the addition of phentolamine or increasing calcium concentration in the incubation medium. 

**Table VI**

Effect of somatostatin on glucose-induced insulin release and cAMP accumulation

<table>
<thead>
<tr>
<th>Somatostatin</th>
<th>Insulin release</th>
<th>cAMP accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (µg/ml)</td>
<td>IAP (µg/ml)</td>
</tr>
<tr>
<td></td>
<td>microunits/islet</td>
<td>fmol/islet</td>
</tr>
<tr>
<td>Normal</td>
<td>98 ± 5.7</td>
<td>197 ± 8 *</td>
</tr>
<tr>
<td>Normal</td>
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<td>187 ± 13 *</td>
</tr>
<tr>
<td>1</td>
<td>44 ± 5.0 *</td>
<td>177 ± 7.5 *</td>
</tr>
</tbody>
</table>

*p Effect of IAP treatment is significant (p < 0.01).

*p Effect of somatostatin is significant (p < 0.01).

Calcium Uptake in "Normal Islets" and "IAP Islets"—The increase of glucose concentration in the incubation medium from 3.3 mM to 16.7 mM caused a marked increase in the uptake of calcium by islets (lines 1 and 2 in Table VIII), in confirmation of the previous results observed under similar conditions (12, 22, 23). There was no difference in the islet calcium uptake under these conditions between normal rats and IAP-treated rats. The glucose-induced increase in the calcium uptake was inhibited by epinephrine in "normal islets" but was not in "IAP islets." These effects of glucose and epinephrine were observed whether the calcium uptake was measured in terms of the total or lanthanum-nondisplaceable, i.e., intracellular calcium.

The islet content of the lanthanum-nondisplaceable calcium was also reduced by the addition of somatostatin or verapamil in the glucose-rich medium bathing "normal islets." Somatostatin failed to inhibit the calcium uptake by "IAP islets," whereas verapamil inhibited the calcium uptake to the same extent whether islets had been isolated from normal rats or IAP-treated rats. Thus, these results related to the islet calcium uptake in Table VIII are very similar to changes in the islet content of cAMP in response to these stimuli and the IAP treatment.

Efflux of Calcium from Islets of Normal or IAP-treated Rats—Islets loaded with ⁴⁵Ca were further incubated in the fresh medium to estimate the ⁴⁵Ca efflux in exchange with nonradioactive calcium. A time course of the ⁴⁵Ca efflux was estimated with batches of islets from one rat; the similarly designed experiments were repeated with eight normal rats and the mean ± S.E. for the islet content of ⁴⁵Ca from these...
Mechanism for the Action of Islet-activating Protein

**TABLE VI**

Inhibition by verapamil of glucose-induced insulin release and cAMP accumulation in both normal islets and IAP islets

<table>
<thead>
<tr>
<th>Verapamil (μM)</th>
<th>IBMX</th>
<th>Insulin release</th>
<th>cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 mM</td>
<td>NORMAL</td>
<td>IAP</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>109 ± 4</td>
<td>185 ± 12</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>74 ± 9.2</td>
<td>147 ± 22</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>37 ± 4.1</td>
<td>77 ± 11</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>10 ± 1.1</td>
<td>29 ± 4.7</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>315 ± 9</td>
<td>434 ± 18</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>214 ± 28</td>
<td>284 ± 22</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>126 ± 20</td>
<td>199 ± 14</td>
</tr>
</tbody>
</table>

*p < 0.01, effect of IAP treatment is significant.

*<sup>1</sup> *p < 0.05, effect of verapamil is significant.

*<sup>2</sup> *p < 0.05, effect of verapamil is significant.

*<sup>3</sup> *p < 0.05, effect of IAP treatment is significant.

**TABLE VII**

Effects of epinephrine on glucose-induced insulin release and cAMP accumulation in islets at various calcium concentrations in the incubation medium

"Normal islets" or "IAP islets" were incubated as in Fig. 3 with 16.7 mM glucose. Number of observations: for "normal islets," on the left; for "IAP islets," on the right.

<table>
<thead>
<tr>
<th>Calcium in medium (mM)</th>
<th>Without IBMX</th>
<th>With IBMX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of observations</td>
<td>8; 6</td>
</tr>
<tr>
<td>2.5</td>
<td>-</td>
<td>97 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>9 ± 1.2</td>
</tr>
<tr>
<td>0.25</td>
<td>-</td>
<td>39 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>5 ± 0.8</td>
</tr>
</tbody>
</table>

*p < 0.01, effect of IAP treatment is significant.

*<sup>1</sup> *p < 0.05, effect of epinephrine is significant.

*<sup>2</sup> *p < 0.05, effect of IAP treatment is significant.

*<sup>3</sup> *p < 0.05, effect of epinephrine is significant.

**TABLE VIII**

Uptake of labeled calcium by islets

"Normal islets" or "IAP islets" were incubated for 120 min with 45Ca, and the uptake of 45Ca was determined, as described under "Experimental Procedures." Number of observations is shown in parentheses.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Islet content of 45Ca pmol/islet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>With 3.3 mM glucose</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.5 ± 1.1 (6)</td>
</tr>
<tr>
<td>With 16.7 mM glucose</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2.6 ± 4.5 (6)</td>
</tr>
<tr>
<td>Epinephrine (0.7 μM)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Epinephrine (1 μM)</td>
<td>13.3 ± 2.1 (6)</td>
</tr>
<tr>
<td>Somatostatin (2.5 μg/ml)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Verapamil (0.1 mM)</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*<sup>1</sup> *p < 0.05, effect of increasing glucose concentration from 3.3 to 16.7 mM or effect of addition in the presence of 16.7 mM glucose is significant (*p < 0.01).

*<sup>2</sup> *p < 0.01, effect of IAP treatment is significant.

*<sup>3</sup> *p < 0.01, effect of epinephrine is significant.

Eight experiments are plotted as open circles in Fig. 4. The additional eight experiments were performed in the same way with "IAP islets"; the results being shown in solid circles in Fig. 4. As illustrated in Fig. 4, 45Ca was discharged from "IAP islets" more rapidly than from "normal islets" for the first 30 min. At 30 min, the 45Ca content of islets of IAP-treated rats was the same as that in islets of normal rats at 60 min. Afterwards, the 45Ca efflux from both kinds of islets proceeded at essentially the same rate. As a result, there were significant
differences in 45Ca retained at 30, 60, and 90 min between "normal islets" and "IAP islets". 

Table IX shows 45Ca retained the islets at 60 min of incubation which was carried out as in Fig. 4. The 45Ca efflux was stimulated by A23187, a calcium ionophore. Dimethyl sulfoxide used as a vehicle to dissolve the calcium ionophore was without effect on the 45Ca efflux by itself. In the presence of A23187, there was no significant difference in the 45Ca content between "normal islets" and "IAP islets." Thus, these results in Fig. 4 and Table IX indicate that the transmembrane calcium flux occurs at a higher rate in "IAP islets" than in "normal islets."

**Direct Action of IAP on Islets during Long Term Incubation**—No influence had been exerted by IAP so far when it was added in vitro to the incubation medium of islets or to perfusate of pancreatic tissues (3). A possibility that IAP interacts with islet cells directly but only slowly was then studied by exposure of "normal islets" to the protein for 24 h in a cell culture. After the 24-h exposure, islets were washed and further incubated with or without epinephrine in the ordinary medium supplemented with 16.7 mM glucose (Table X).

The addition of IAP at concentrations above 1 ng/ml caused significant potentiation of glucose-induced insulin release in the absence of epinephrine. In the presence of epinephrine which was markedly inhibitory to insulin release in control islets, IAP above a concentration as low as 0.1 ng/ml increased insulin release in a larger magnitude than in its absence. In other words, the epinephrine-induced inhibition was reduced by IAP until it became insignificant when the concentration of the protein was increased beyond 10 ng/ml. Close resemblance of these actions in vitro of IAP to in vivo observations makes it very likely that properties unique to "IAP islets" as shown by the foregoing experiments in this communication have resulted from a direct effect of IAP on islet cells in vivo.

**DISCUSSION**

In the present study, a new protein, IAP, purified from the culture medium of R. pertussis cells was injected intravenously into rats at a dose as low as 0.5 μg/100 g of body weight, and pancreatic islets isolated therefrom 3 days later ("IAP islets"), together with islets from nontreated rats ("normal islets"), were incubated to study insulin release and cAMP accumulation during incubation. More insulin was released and more cAMP accumulated in "IAP islets" than in "normal islets" under certain conditions. This reflects enhanced responses of "IAP islets" in vitro, since there was no significant difference in the contents of insulin and cAMP before incubation between these two kinds of islets. The features of IAP-induced changes in response of pancreatic islets in vitro will be summarized as follows.

**Potentiation of Insulin Secretory Responses of Islets by IAP Treatment**—The IAP-induced potentiation of insulin release was observed only upon the incubation of isolated islets with insulin secretagogues; i.e. more insulin was released from "IAP islets" than from "normal islets" only when insulin secretion was stimulated in vitro by the addition of glucose (at concentrations higher than 10 mm), arginine, or glibenclamide. When insulin secretion from islets remained at the "basal" rate in the incubation medium containing a low (3.3 mM) concentration of glucose, or when insulin release was not significantly increased by glucagon alone, there was no significant difference in insulin release between "IAP islets" and "normal islets." Thus, IAP does not provoke insulin release by itself but only potentiates insulin secretory responses to secretagogues, in accord with earlier conclusions reached by Sumi and Uji (1) based on the experiments in vivo with pertussis vaccine.

**Enhanced Generation and Degradation of cAMP in "IAP Islets"**—When more insulin was released from "IAP islets" than from "normal islets" as observed upon the incubation with insulin secretagogues, there was no difference in cAMP...
contents between “IAP islets” and “normal islets,” unless the incubation medium was fortified with IBMX. In the presence of IBMX, more cAMP accumulated in “IAP islets” than in “normal islets.” Rather exceptionally, the cAMP content was higher in “IAP islets” than in “normal islets” even in the absence of IBMX if glibenclamide, which by itself increased both cAMP and insulin release, was added to the incubation medium (Table I). This increase in cAMP contents was associated with enhanced insulin release. Such a similarity of glibenclamide to IBMX, however, is not surprising since sulfonamide (24–27) are inhibitors of cAMP phosphodiesterase just as methylxanthines. Thus, the cellular level of cAMP was higher in “IAP islets” than in “normal islets” only when the breakdown of cAMP was prevented by the addition of a phosphodiesterase inhibitor. The increase in the cAMP content upon inhibition of its breakdown may result from enhanced production, whereas failure to increase the cAMP content despite enhanced production may arise from enhanced breakdown. It is concluded, therefore, that not only the generation but also the degradation of cAMP in islet was activated by the IAP treatment.

Activation by the IAP Treatment of the Stimulus-Secretion Coupling Process at the Site(s) Distal to CAMP Generation—Enhanced insulin release without increased cAMP contents as observed in “IAP islets” incubated in the absence of IBMX suggested that the action of cAMP to provoke insulin release in cell was potentiated under these conditions. In order to gain insights into this problem, the correlation of insulin release to the cAMP content in islets is depicted in Fig. 5, in which the values obtained in Fig. 2 and Table I are plotted. In “normal islets,” increases in insulin release caused by raising glucose concentration from 3.3 to 16.7 mM were proportional to increases in the cAMP content, as visualized by a linear regression in Fig. 5. When glucagon was combined with 10 mM glucose, the relationship of insulin release to cAMP accumulation obeyed the same regression. This would suggest that the enhancement of insulin release induced by glucose and glucagon might be accounted for solely in terms of increased cAMP generation under these conditions.

In “IAP islets” as well, a linear relationship was found to exist between insulin release and cAMP contents at different glucose concentrations with or without glucagon in the absence of IBMX; but the slope of the linear regression obtained was more steep than that for “normal islets” (Fig. 5). Thus, the manner in which more insulin was released from “IAP islets” than from “normal islets” is clearly distinct from the mechanism underlying glucose- or glucagon-induced insulin release. The intracellular process mediated by cAMP (or distal to cAMP generation) eventually leading to insulin release appears to be the additional site that is activated by the IAP treatment. In support of this conclusion, more insulin was released from “IAP islets” than from “normal islets” in response to the addition of Bt_3cAMP in the presence of 10 mM glucose (Table II).

Saturation Kinetics of cAMP-dependent Insulin Release Unaffected by IAP Treatment—In the case of IBMX addition, the increase in islet CAMP induced by treatment of the donor rat with IAP tended to be more marked than the accompanying increase in insulin release, especially when insulin release was stimulated by the addition of glucose (10 mM or more) or glucose plus glucagon (Fig. 2 and Table I). It would appear that, under these conditions, cAMP generated in “IAP islets” was less effective than the nucleotide generated in “normal islets.” The relationship between the insulin release and cAMP accumulation in the presence of IBMX is illustrated in Fig. 6, in which amounts of the insulin that were released from islets in response to glucose and glucagon are plotted as a function of cAMP contents of the same islets. All points in Fig. 6 can be connected to form a hyperbolic pattern, regardless of whether they have been derived from “normal islets” or “IAP islets.” Thus, cAMP-dependent insulin secretion exhibited a saturation kinetics when cAMP accumulated in the presence of IBMX. It is then concluded that the IAP treatment was incapable of increasing the maximal insulin secretory response to the saturating concentration of cAMP.

Insulin and cAMP data obtained with 3.3 mM glucose were not included in a plot in Fig. 6; these values were located far below the hyperbola drawn based on those obtained at higher glucose concentrations. This is not surprising in view of the fact that most of the insulin secretagogues failed to elicit marked insulin release at this low glucose concentration de-

Fig. 5. Correlation of insulin release to cAMP accumulation during incubation of islets with various concentrations of glucose in the presence (triangles) or absence (circles) of glucagon. The mean values obtained without IBMX in Fig. 2 and Table I are plotted with the concentration of glucose being shown in the panel. Open symbols, islets from normal rats; solid symbols, islets from IAP-treated rats. Vertical and horizontal bars represent S.E.

Fig. 6. Saturation kinetics of insulin release as a function of cAMP accumulation. The mean values obtained with IBMX in Fig. 2 and Table I are plotted as in Fig. 5. See legends to Fig. 5 for explanation of symbols and figures. The values obtained with normal islets incubated with 10 mM and 16.7 mM glucose in the absence of IBMX and glucagon (which were shown as open circles in Fig. 5) are reproduced by small open circles in the panel for comparison.
spite their ability to stimulate insulin release at higher glucose concentrations. Probably, glucose above its critical threshold concentration would be required for a sufficient amount of insulin to be discharged from islets in response to CAMP-generated within the cell as well as to insulin secretagogues added.

Reversal by IAP Treatment of α-Adrenergic Actions to Inhibit Insulin Release and cAMP Accumulation in Islets and Its Role in Enhanced Insulin Secretory Responses of "IAP Islets"—"IAP islets" were characterized by their failure to respond to epinephrine, which in "normal islets" inhibited insulin release and reduced cAMP contents very markedly dependent on the stimulation of α-adrenergic receptors. Numerous publications have shown that epinephrine and noradrenaline inhibit insulin secretion in vivo and in vitro via a-adrenergic receptors (see Ref. 28 for review). Islet contents of cAMP were also reported to be lowered by epinephrine in vitro (29, 30), probably due to inhibition of adenylate cyclase (31, 32). Based on the results obtained in vivo (1, 6) and in vitro with perfused rat pancreas (3), it has been proposed that β-adrenergic stimulation of insulin secretion is predominant over the α-adrenergic inhibition of the secretion in rats treated with IAP or pertussis vaccine. The present results obtained with isolated pancreatic islets are compatible with this earlier proposal.

The lowest dose of epinephrine required for significant inhibition of the glucose-induced insulin release and cAMP accumulation in "normal islets" was 0.5 ng/ml (Fig. 3), which is essentially equal to the minimum dose used for the inhibition of insulin release from mouse pancreatic islets (33). Likewise, insulin release from perfused canine pancreas was reported to be inhibited by epinephrine or norepinephrine at 2 ng/ml (34). These concentrations of catecholamines are in the same order as concentrations in human plasma (35, 36) and in perfusate of canine pancreas during glucose deprivation (37) or are lower than those in rat plasma (38) and in homogenates of whole mouse pancreas (39). Actually, insulin secretion was inhibited by direct neural input to the pancreas in the dog (40) or by norepinephrine released from adrenergic nerve terminals in isolated pancreatic islets perfused in vitro (41); these inhibitions were mediated by α-adrenergic receptors.

Therefore, insulin secretory responses in vivo to various stimuli are likely to be blunted under physiological conditions as a result of the stimulation of α-adrenergic receptors by endogenous catecholamines. Thus, the reversal by the IAP treatment of α-adrenergic receptor-mediated functions could be largely, if not totally, responsible for enhanced insulin secretory responses of IAP-treated rats to various stimuli.

The inhibitory actions of somatostatin on glucose-induced insulin release and cAMP accumulation were also abolished by the treatment of the islet donor rats with IAP. Eistendal et al. (42) were the first to report that somatostatin-induced inhibition of insulin release was associated with a reduction of cAMP contents in isolated pancreatic islets. Phentolamine was effective in attenuating the inhibitory actions of somatostatin in "normal islets" in accordance with recent reports that it failed to inhibit insulin release (43, 44) and glucagon release (45) from canine pancreas in vivo when phentolamine was infused simultaneously. Conceivably, "IAP islets" may provide a condition that is unfavorable for cAMP-dependent insulin secretion to be inhibited effectively via α-adrenergic receptors.

Enhanced Calcium Influx in "IAP Islets"—The capability of "IAP islets" to secrete large amounts of insulin in association with accumulation of intracellular cAMP under the conditions as discussed above showed a tendency to be attenuated when the extracellular concentration of calcium was lowered. Even in the presence of 16.7 mM glucose plus IBMX, the amount of insulin released from, and of cAMP produced in, "IAP islets" did not differ significantly from those in "normal islets" when these islets were incubated in the calcium-deficient medium (Table VII). Nor was the reversal of epinephrine-induced inhibitions, which was otherwise characteristic of "IAP islets," observable in the calcium-deficient medium.

Significance of calcium in IAP-induced modification of insulin secretory responses was further indicated by the 45Ca experiments. The glucose-induced islet uptake of 45Ca was inhibited by epinephrine (46, 47), somatostatin (47), and calcium antagonist such as verapamil (48) in agreement with previous reports. The inhibition of the 45Ca uptake caused by these agents was modified by the prior treatment of the pancreas donor rat with IAP in just the same manner as the inhibitions of insulin secretion and cAMP accumulation: the inhibition by epinephrine and somatostatin was abolished, but the inhibition by verapamil was not, by the IAP treatment. It is conceivable that the calcium flux through the B cell membrane is closely related to the cAMP-dependent insulin secretion. In fact, the initial rate of the 45Ca efflux was significantly higher in "IAP islets" than in "normal islets." Calcium ionophores, A23187 (Table IX) and X-537A (49), were also effective in this regard. Thus, it is very likely that in "IAP islets," more calcium became available to the insulin secretory machinery within the B cell as a result of the activation of the channel for calcium influx through the cell membrane.

A Direct, Slow in Onset, and Durable Interaction of IAP with Islet Cells—For the purpose of studying influences of IAP on islet activities, islets isolated from rats treated in vivo with IAP (or "IAP islets") were employed in most experiments in this communication, because the addition of IAP to pancreatic preparations in vitro had been thus far without effect. In a series of preliminary experiments shown in Table X, however, islets that had been exposed to IAP in vitro over a period as long as 24 h exhibited enhanced insulin secretory responses to glucose and blunted susceptibility to the epinephrine inhibition just as observed with islets isolated from rats treated with the protein in vivo. The action of IAP in vivo was characterized by its long duration; more insulin was secreted in response to a glucose load even at 60 days after IAP injection than before the injection (5). Thus, it can be concluded that IAP interacts directly and slowly with islet cells eventually to cause sustained changes in secretory activities of the B cell in such a fashion as will be discussed below.

A Plausible Mechanism for IAP Action: Activation of Native Calcium Ionophores in B Cells—Based on the present results and the foregoing discussion, we would like to propose that a single injection of IAP into rats exerted sustained influences on the native divalent cation ionophore located at the islet B cell membrane (50), in such a manner as to provoke the inward movement of extracellular calcium into the intracellular pool that triggers the insulin secretory process. As has been discussed above, the generation and degradation of intracellular cAMP as well as its permissive effect on glucose-induced insulin secretion were all more marked in "IAP islets" than in "normal islets." These alterations characteristic of "IAP islets" would be accounted for in terms of the increased calcium availability to the intracellular pool because calcium is known to stimulate adenylate cyclase (or to increase cAMP content in the presence of methyloxanthines) in pancreatic islets (51, 52), to activate phosphodiesterase in various tissues (53), and to enhance cAMP-induced insulin release (54) or to exhibit a permissive effect at the site(s) distal to the cAMP-sensitive step of the secretory mechanism (52).
The α-adrenergic action of epinephrine to inhibit the release of insulin from cultured pancreatic cells was reversed by enhancing the calcium influx (54). It is conceivable, therefore, that the increased influx of calcium in "IAP islets" would be responsible for the unique abolition of epinephrine-induced inhibitions of insulin release and cAMP accumulation. Probably, the stimulation of α-adrenergic receptors on the B cell membrane by catecholamines would enhance the outward flux of intracellular calcium which otherwise functions not only as an activator of adenylate cyclase but also as a link in the stimulus-secretion coupling. The activation of the native calcium ionophore on the B cell membrane by the IAP treatment would prevent the efflux of this functional calcium because of the much higher extracellular than intracellular concentration of calcium. This would be the reason why the effect of the IAP treatment was no longer observable upon incubation of islets in the calcium-deficient medium. In contrast, the verapamil-induced inhibitions of 45Ca uptake, insulin release, and cAMP accumulation were observed in "IAP islets" to the same extent as in "normal islets." In other words, the actions of verapamil and IAP were mutually independent or "additive." Conceivably, the site of action of the calcium antagonist would not be the native ionophore per se; the antagonist would reduce the calcium influx regardless of whether the ionophore has been activated or not.

A vast amount of cAMP accumulated in "IAP islets" incubated with IBMX. As has been illustrated in Fig. 6, insulin release dependent on such saturating concentrations of cAMP did not appear to be particularly activated in "IAP islets" in comparison with "normal islets." Conceivably, such high concentrations of cAMP would cause a marked translocation of calcium within the B cell from an organelle-bound pool to a functional cytoplasmic pool (55), thereby minimizing the stimulative effect of enhanced calcium influx on insulin secretion. It might be possible to postulate that the treatment with IAP did not affect the intracellular translocation of calcium in the B cell.

The results of experiments in vivo showed previously that α-adrenergic actions including those on the pancreatic insulin secretion and on carbohydrate metabolism in other tissues were favored at the expense of β-adrenergic actions during acidosis in rats, and that the reverse was the case during alkalosis or after the treatment of rats with hydrocortisone (56, 57). It was suggested in these reports that one of the important factors determining such relative functions of α- to β-adrenergic receptors might be calcium availability; more calcium would be available in cells during acidosis or after hydrocortisone therapy, whereas the tissue concentration of calcium would be lowered during alkalosis. Thus, the present results add an example, presumably a more convincing one, to the proposed role of calcium in determining the shift of adrenergic receptor-mediated functions between α and β types.

Although much more work will be required for the mechanism of the IAP actions to be fully elucidated, the present results clearly show that the treatment of the pancreatic donor animal with IAP (or a long term incubation of islets with IAP) will provide a promising tool for studying roles of calcium and cAMP in the stimulus-secretion coupling process in the pancreatic B cell. IAP was recently reported to be dissociated into three kinds of subunits, one of which was, although inactive by itself, afforded a new biologically active protein upon association with any other subunit (58, 59). Whether the treatment of rats with IAP activates glucose metabolism and proinsulin synthesis in the B cell remains subjects for further investigation.

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Mechanism for the Action of Islet-activating Protein

Islet-activating protein. Enhanced insulin secretion and cyclic AMP accumulation in pancreatic islets due to activation of native calcium ionophores.

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