Islet-Activating Protein (IAP) Reduces Bethanechol-Stimulated Release of Pancreatic Polypeptide in the Dog

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Abstract

The effect of islet-activating protein (IAP) purified from culture medium of Bordetella pertussis was examined in dogs. This was assessed by the levels of pancreatic polypeptide (PP) as well as the responses of plasma insulin and glucagon to a parasympathomimetic agent, bethanechol. Plasma responses of these pancreatic hormones were measured before and 5 days after IAP injection. Although IAP had no significant effect on the bethanechol-stimulated increase in plasma glucose, insulin and glucagon, the PP response to bethanechol was significantly reduced after IAP treatment compared with that before IAP (p<0.05). In conclusion, IAP significantly and selectively reduced bethanechol-stimulated PP release in the dog although the mechanism remained to be elucidated.

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Materials and Methods

Eight healthy male and female mongrel dogs, weighing between 9.2 and 11.0 kg, were given a daily meal of commercial dog food before experiments but were fasted for 24 hours prior to treatment. They were anesthetized with sodium pentobarbital (25 mg/kg). Two blood samples were taken from a catheterized femoral vein in the basal state before bethanechol injection. Just after the second basal blood sample was taken, bethanechol chloride (donated by Eizai Co., Tokyo) dissolved in saline (0.5 mg/ml) at a dose of 0.1 mg/kg was given. Further blood samples were obtained at 5, 10, 15, 20 and 30 min after bethanechol injection. Each blood specimen was immediately transferred to a chilled tube containing 1 mg of EDTA-2Na and 500 KIU of aprotinin per ml of blood, which was kept on ice. The sample was centrifuged as soon as possible and the plasma was stored at −20°C until assay. Just after the first experiment, 5 µg/kg body weight of IAP dissolved in saline was administered intravenously to each dog. IAP was kindly donated by Dr. Ui (Hokkaido University, Japan). On the fifth day after IAP injection, the entire experimental procedure was repeated on each animal.

Plasma PP was measured by a modification of the radioimmunoassay as described elsewhere (Hanasfusa et al., 1981) using a double-antibody method. The materials for PP radioimmunoassay were generously donated by Dr. R. E. Chance (Lilly Research Laboratories, Indianapolis). Bovine PP (BPP) was labeled by a modification of the chloramine T method (Greenwood et al., 1963), and the tracer was purified on a Sephadex G-50 fine column. Incubation was carried out in 0.05 M phosphate buffer, pH 7.5, containing 0.2% bovine serum albumin. Each assay tube contained a total volume of 1.0 ml consisting of the following components: (a) 0.1 ml standard HPP or plasma sample plus 0.7 ml of the assay buffer; (b) 0.1 ml diluted antiserum (1:200,000); and (c) 0.1 ml 125I-BPP. Incubation of the test sample and antibody for two days at 4°C was followed by the addition of the labeled antigen for an extra day at 4°C. The antibody-bound hormone was precipitated by addition of 0.1 ml diluted normal rabbit serum and 0.1 ml goat anti-rabbit Ig-G serum, and the incubation was allowed to proceed for a further 24 hours at 4°C. Bound PP was separated from free hormone by centrifugation at 3,000 rpm for 30 min. The intraassay and interassay coefficients of variations were 10.0% and 13.7%, respectively. Canine PP (CPP) can be measured without difficulty by this heterologous radioimmunoassay (Chance et al., 1979).

The concentration of plasma immunoreactive glucagon (IRG) was measured by radioimmunoassay using the antiserum (30K) specific for pancreatic glucagon as previously described (Nonaka and Foa, 1968). Plasma immunoreactive insulin (IRI) was measured with “Phadebas Kits” (Pharmacia). Plasma glucose (PG) was measured by a glucose oxidase method using Beckman’s glucose autoanalyzer.

All results are expressed as mean±SEM. Differences in mean values between the results were determined by paired Student’s t-test. P values less than 0.05 were considered to be significant.

Results

Figure 1 represents the responses of

![Graph showing the response of plasma glucose (PG), IRI, IRG, and PP before and after IAP injection.](image-url)
plasma glucose, IRI, IRG and PP to bethanechol before and five days after the injection of IAP. The mean plasma glucose level after IAP treatment tended to become lower than that before IAP injection, although the difference between them was not statistically significant. Plasma IRI and IRG levels after IAP treatment were almost the same as those before IAP injection. On the other hand, bethanechol-stimulated release of PP after IAP treatment was lower than that before IAP injection; a statistically significant difference was observed at 20 min after bethanechol injection (p<0.05).

To estimate the effect of IAP on the responses of IRI, IRG and PP to bethanechol, incremental areas of IRI, IRG and PP responses were calculated. An incremental area was defined as the area between the curve of IRI, IRG or PP from 0 time to 30 min and the mean value of the two basal samples. Results obtained from this calculation are shown in Figure 2.

IAP had no effect on the incremental area of IRI (Figure 2, left panel). Although the incremental area of IRG after IAP injection tended to be smaller than that before IAP injection, the difference between them was not statistically significant (Figure 2, middle panel). On the other hand, incremental area of PP after IAP injection was significantly smaller than that before IAP injection (p<0.05, Figure 2, right panel).

Discussion

The present investigation demonstrated an inhibitory effect of IAP on bethanechol-stimulated PP release. This is the first report that revealed the effect of IAP on PP secretion.

The mechanism by which IAP reduces PP response to bethanechol is not clear. Cholinergic agents such as bethanechol are related to the increase in the tissue content and plasma concentration of cyclic guanosine 3', 5'-monophosphate (cyclic GMP) (Honma and Ui, 1978). Although the influence of IAP on cyclic GMP has not yet been clearly known, a preliminary study showed that the inhibitory effect of IAP on acetylcholine-induced increases in the plasma concentration of cyclic GMP (personal communication from Dr. Ui, Hokkaido University). Taking these data into consideration, it may be hypothesized that the suppressive effect of IAP on bethanechol stimulated PP secretion is mediated through the inhibition of cyclic GMP level.
But the precise mechanism for the influence of IAP on PP secretion remains to be elucidated.

Although IAP was reported to stimulate insulin response to various secretagogues (Yajima et al., 1978 a; b), IAP did not augment insulin release in our experiment. We consider that the discrepancy between our results and the previous reports about the influence of IAP on insulin response might be due to the difference in stimulants. Although several reports (Katada and Ui, 1979 a; b) revealed that the stimulatory effect of IAP on insulin secretion was mediated through the activation of Ca-channel and the resultant increase in cyclic adenosine 3' 5'-monophosphate (cyclic AMP), the mechanism for the bethanechol-stimulated insulin release has not been shown to be related to the increase in cyclic AMP. Since insulin secretion is not mediated through cyclic GMP, which may be related to the reduction of PP response to bethanechol after IAP treatment, as mentioned before, it is possible that insulin response to bethanechol is not affected by IAP treatment.

With regard to the effect of IAP on glucagon secretion, some disagreement has been noted. Toyota et al. (1978) reported that glucagon secretion in response to norepinephrine was reduced after IAP treatment in both the diabetic and the normal rats. On the other hand, Yoshida et al., (1981) showed that the suppressive effect of IAP on glucagon response to arginine or glucose was seen only in alloxan-treated dogs but not in normal dogs. They proposed that improvement in insulin deficiency might have reduced the glucagon response to arginine or glucose in alloxan treated dogs. In the present study glucagon response to bethanechol tended to decrease after IAP treatment, although the difference was not statistically significant. This result does not fully accord either with reports by Toyota et al., or by Yoshida et al. Since glucagon secretion is affected by the insulin concentration, our result concerning the effect of IAP on glucagon secretion might be due to the unchanged insulin level after IAP treatment.

In conclusion, IAP reduced bethanechol-stimulated PP release in the dog. The significance of this effect has not been assessed but its study presents an intriguing field for research.

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