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EFFECT OF A SYNTHETIC CHOLECYSTOKININ DERIVATIVE ON HORMONE SECRETION IN HUMAN FETAL PANCREATIC TISSUE CULTURE

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KEY WORDS: pancreatic cell culture; hormones; secretion; cholecystokinin analog; regulation.

The gastrointestinal hormone cholecystokinin (CCK) participates in the regulation of pancreatic endocrine function. Experiments in vivo and in vitro have shown that CCK stimulates basal secretion of insulin and glucagon [4], alters the sensitivity of the β- and α-cells to glucose [5], and modifies the paracrine effects of insular hormones [6]. It has been suggested that CCK derivatives, by selectively stimulating insulin secretion, may be used in the clinical management of diabetes. Indian research workers have synthesized an analog of the C-terminal tetrapeptide CCP (CCK-4), namely pro-Met-Asp-Phe-NH_2 (PMAP). In concentrations of 10^{-10}-10^{-6} M, PMAP stimulated insulin secretion in a culture of rat islets just as effectively as native CCK-4 [2]. Meanwhile PMAP (but not CCK-4) did not stimulate glucagon secretion [3].

In the investigation described below the effect of PMAP on insulin and glucagon production was studied in cultures of microfragments (Mfr) of the human fetal pancreas.

EXPERIMENTAL METHOD

To prepare primary histotypical cultures the pancreas from three 20-week human fetuses was used. The method of culture and details of the morphology of the Mfr were described previously [1]. The Mfr were grown in dishes in medium 199 (glucose concentration 5.5 mM) with 10% fetal calf serum, 5 mM essential amino acids, and 5 mM of a vitamin mixture. The prepared Mfr from each pancreas (on the 5th-6th days in vitro) were divided into four groups and transferred in 24-well planchets. The Mfr of each group were distributed so that for each time of the experiment there were three wells, containing from 8 to 15 Mfr. Nfr of group 1 (control) were incubated in medium 199 with 2.5% serum, amino acids,

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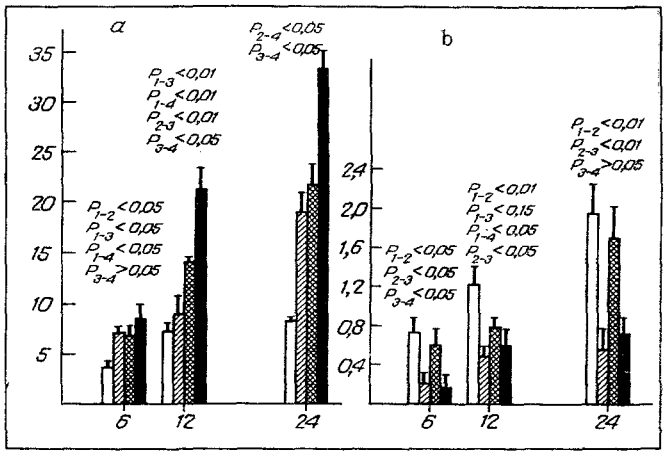

Fig. 1. Effect of glucose and PMAP on insulin (a) and glucagon (b) production in cultures of Mfr of human fetal pancreas. Abscissa, duration of incubation (in h); ordinate, concentration of hormones in medium (in mg/Mfr/2 ml). The results are given in the form $m \pm S_x$ ($n = 9$). Unshaded columns – control group 1; obliquely shaded columns – group 2 Mfr (medium with 17 mM glucose); cross-hatched columns – group 3 Mfr (medium with 1 μM PMAP); black columns – group 4 Mfr (medium with 17 mM glucose and 1 μM PMAP). Significance of differences shown between hormone concentrations in different groups of Mfr.

vitamins, and aprotinin (140 μg/ml) at 37°C in a CO_2-incubator (5% CO_2, 95% air). Mfr of group 2 were incubated under these conditions in medium with the addition of glucose to a final concentration of 17 mM. The Mfr of group 3 were incubated in medium with the addition of PMAP to a final concentration of 1 μM. The Mfr of group 4 were incubated in medium with glucose (17 mM) and PMAP (1 μM). In all cases the volume of medium in the well was 2 ml. Samples of medium were taken from the wells 6, 12, and 24 h after the beginning of incubation. Concentrations of insulin and glucagon in the medium were determined by radioimmunoassay in RIA-Ins-^{125}I-M (Minsk) and Glucagon Biodata (Serono, Switzerland) kits. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

PMAP intensified basal and glucose-stimulated insulin secretion (Fig. 1a). The insulin concentration in Mfr cultures of group 3 exceeded the insulin concentration in the group 1 cultures at all times of the experiment (by 1.8-2.6 times).

The secretogenic activity of PMAP did not differ significantly from that of glucose. The maximal insulinotropic effect of PMAP was observed in the presence of 17 mM glucose: the insulin concentration in the cultures of group 4, 12 and 24 h after the beginning of incubation exceeded the insulin concentration in the group 1 cultures by 2.9 and 4.1 times respectively.

Glucose in a concentration of 17 mM depressed basal secretion of glucagon (Fig. 1b). The glucagon concentration in cultures of group 2 Mfr at all times of the experiment was 2.3-2.6 times lower than in cultures of group 1 Mfr. PMAP had virtually no effect on basal glucagon production and did not intensify the inhibitory action of glucose on release of this hormone. These results are in agreement with those obtained by Indian workers [2, 3]. It can be concluded that PMAP specifically stimulates basal insulin secretion and does not affect glucagon secretion in cultures of human fetal pancreatic Mfr. It was shown for the first time that PMAP in vitro potentiates the stimulating effect of glucose on insulin production and does not modify the action of glucose on glucagon secretion. It was shown previously that CCK and CCK-4 stimulate glucagon production in the islets of adult rats [3, 5]. We obtained the opposite result, indirect evidence that PAMP does not interaction with CCK/CCK-4 receptors on human embryonic α-cells or that embryonic α-cells, unlike the α-cells of adult animals, do not possess CCK/CCK-4 receptors. A detailed study of the biological effects of PMAP in vitro may help

to decipher the mechanisms regulating secretion of insular hormones. To solve the problem of whether PMAP and peptides similar to it may be used to correct diabetes, further investigations in vivo are necessary.

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EFFECT OF 5-HT_{1A} RECEPTOR AGONISTS ON AMINO ACID- AND DOPAMINERGIC RESPONSES OF NEURONS

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The anxiolytic action of buspirone and its structural analogs (gepirone, ipsapirone, campirone, etc.) is due to their activating effect on 5-HT_{1A} receptors of brain nerve cells [10]. The result of activation of 5-HT_{1A} receptors in somatodendritic synapses of neurons of the mesencephalic nuclei raphe and neurons of the limbic system of the brain is depression of their functional activity [6, 7, 11]. Depression of the function of these cells may be the result of changes in the passive properties of the cell membranes arising during hyperpolarization or the result of modulation of the response to the action of nervous influences converging on the neurons. It has been shown that despite the weakness [3] or absence [8] of a hyperpolarizing influence on the hippocampal pyramidal cells, buspirone, if given by the systemic or intrahippocampal routes [9], and also in brain slices [8], suppresses excitatory postsynaptic potentials (EPSP) evoked in pyramidal neurons by stimulation of hippocampal afferent fibers. However, it is not clear whether this effect is the result of the pre- or postsynaptic action of buspirone.

The present investigation showed that agonists of 5-HT_{1A} receptors, acting postsynaptically, potentiate the effects of gamma-aminobutyric acid (GABA), but significantly reduce the inhibitory effects of dopamine and the excitatory effect of aspartate on nerve cells.

EXPERIMENTAL METHOD

The effect of buspirone and campirone (10 μmoles/liter) on responses of motoneurons of the isolated sagitally divided spinal cord of Central Asiatic frogs, evoked by GABA or aspartate, and on GABA- or dopamine (DA)-induced neuronal responses of spinal sensory ganglia of adult rats, were studied. The test objects were superfused with salt solutions

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