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EFFECTS OF AMINO ACIDS, GLUCAGON, INSULIN AND ACETYLCHOLINE ON CYCLIC NUCLEOTIDE METABOLISM AND AMYLASE SECRETION IN ISOLATED MOUSE PANCREATIC FRAGMENTS

JAIPAUL SINGH
The Physiological Laboratory, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

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Abstract—The effects of amino acids, exogenous islet hormones and acetylcholine on cyclic nucleotide metabolism and amylase secretion in the isolated mouse pancreas have been investigated. The changes in levels of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) and guanosine 3′,5′-cyclic monophosphate (cyclic GMP) were measured at different times during exposure of pancreatic fragments to amino acids (L-alanine and L-arginine), islet hormones (insulin and glucagon) or acetylcholine (ACh). L-Alanine (1–20 mM) evoked a transient increase in cyclic AMP concentration accompanied by an initial decrease and subsequent increase in the tissue concentration of cyclic GMP. L-Arginine (1–20 mM) induced a complex triphasic change in cyclic AMP concentrations involving an initial rise and a delayed sustained elevation. The changes in levels of cyclic GMP increased only transiently. The effects of insulin (10^{-6} M) and to some extent glucagon (5×10^{-7} M) resembled those seen with L-arginine. The effects of amino acids and islet hormones were all dose-dependent. ACh (10^{-7} M) elicited a marked reduction in cyclic AMP concentration and this was accompanied by a concomitant increase in the level of cyclic GMP. The amino acids and the islet hormones had no significant effect on amylase secretion whereas ACh, of course, evoked a large increase in amylase output. The results with the amino acids and islet hormones reveal a clear dissociation between cyclic nucleotide changes and amylase secretion and further suggest that the marked reciprocal changes in cyclic AMP and cyclic GMP concentrations may constitute an important physiological role for the cyclic nucleotides to regulate amino acid transport in the pancreas.

The actions of secretin and vasoactive intestinal polypeptide (VIP) on pancreatic acinar cells are associated with changes in cyclic AMP levels [1, 2] whereas the actions of ACh, cholecystokinin-like and bombesin-like peptides are associated with release of intracellular Ca^{2+} and an increase in cyclic GMP concentration [1–3].

A number of amino acids can be taken up into cells by a sodium–amino acid co-transport mechanism [4–6]. Sodium gradient-driven amino acid transport across pancreatic plasma membranes has also been demonstrated [7] and a number of neutral amino acids like L-alanine and glycine, as well as the basic L-arginine, have been shown to depolarise and increase the conductance of the pancreatic acinar plasma membrane [8–11]. Amino acid transport mechanisms have been studied in the greatest detail in the intestine [12] and there are several reports which suggest a role for cyclic AMP in the regulation of amino acid transport into a variety of cells and organs [13–16], possibly mediated through changes in membrane sodium permeability [17–21].

The experiments described in this paper are concerned with the effects of amino acids, both the neutral L-alanine and the basic L-arginine, on cyclic AMP and cyclic GMP metabolism and amylase secretion in isolated mouse pancreatic fragments. There are several reports that arginine is a potent releaser of endogenous islet hormones [22, 23]. The action of exogenous insulin and glucagon on cyclic nucleotide changes and enzyme secretion was therefore also investigated mainly to test whether these hormones could mimic the actions of L-arginine.

MATERIALS AND METHODS

Perfusion procedure. All experiments were performed on small isolated segments (5–10 mg) of adult mice pancreas. Approximately 50–100 mg of tissues were placed into each of eight small Perspex flow chambers (volume = 1.0 ml). Four of the chambers served as 'test' preparations and the remaining four served as control 'partner' preparations. The tissues were superfused with Krebs Henseleit solution [composition (mM): NaCl, 103; KCl, 4.7; $CaCl_2$ 2.56; $MgCl_2$ 1.13; $NaHCO_3$ 25; NaH_2PO_4 1.15; D-glucose, 2.8; Na pyruvate 4.9; Na fumarate 2.7; and Na glutamate 4.9] at the rate of 1 ml/min. The solution was gassed with 95% O_2 and 5% CO_2 maintained at 37° and in all experiments (except those in which acetylcholine was used) atropine (10^{-5} M) was added. In the experiments in which the tissues were stimulated with either L-alanine or L-arginine, appropriate concentrations (range 1–20 mM) of either D-alanine or D-arginine were added to the control perfusing medium in order to maintain the same osmolarity throughout the experiment.

The tissue flow cells were perfused with Krebs solution for approximately 30–40 min prior to stimulation. During stimulation, the fluid flowing through the 'test' chamber was replaced with Krebs solution

containing appropriate concentrations of either L-alanine or L-arginine, insulin or glucagon or ACh. At various times during the onset of stimulation with the different agents, both the 'test' and control 'partner' preparations (i.e. tissue plus chamber) were rapidly frozen in liquid nitrogen and then stored until required for extraction and assay purposes.

Extraction and cyclic nucleotide assay procedures. Details of cyclic nucleotide extraction and assays are given elsewhere [24]. Frozen tissues were pulverised in a stainless-steel mortar and pestle (also cooled in liquid nitrogen) and afterwards extracted with acidic ethanol (1 ml of 1 N HCl in 100 ml of absolute alcohol). The solvent was blown off in a stream of gaseous nitrogen, and the residue taken up in Tris–EDTA buffer (0.05 M Tris, pH 7.5, containing 4 mM EDTA). Cyclic AMP and cyclic GMP levels were then determined using the Radiochemical Centre's assay kits, TRK 432 and TRK 500, respectively. Precise details of the procedures used are to be found in the publications which accompany the assay kits (Radiochemical Centre, Amersham, U.K.). Total protein was estimated using the Biuret method [25] and cyclic nucleotide concentrations obtained are expressed throughout in pmole/mg protein. The values plotted in the figures represent the difference in cyclic nucleotide concentrations between the 'test' and control 'partner' preparations.

Appropriate control experiments have shown that neither the amino acids nor the islet hormones nor ACh interfere with the cyclic nucleotide assays. The recoveries of cyclic AMP and cyclic GMP by the extraction procedure were approximately 90 and 91%, respectively.

Amylase secretion. Pancreata were cut into small segments (5–10 mg) and a total weight of around 100–150 mg placed in a Perspex flow chamber (volume = 1.0 ml) which was perfused with Krebs solution at a rate of 1 ml/min. Amylase in the effluent was assayed by the method of Rinderknecht and Marbach [26] as modified by Matthews *et al.* [27] and α-amylase (Sigma type IIA) was used as standard for calibration. The amino acids, islet hormones and secretagogues were added directly to the superfusing solution.

RESULTS

Mean control levels (±S.E.) of tissue cyclic AMP and cyclic GMP concentrations (pmole/mg protein) were: 2.94 ± 0.09 and 0.57 ± 0.03 (n = 131), respectively. There was some small fluctuation in control levels of cyclic nucleotides during the different experimental conditions, however, their levels remained virtually constant during a particular time course or dose-dependent response. A more detailed discussion of the variability in control levels of cyclic AMP and cyclic GMP is found elsewhere [24, 28]. The cyclic nucleotide concentration in a particular experimental situation was always expressed as the difference between the concentration in the test and the corresponding control preparation. Each data point in Figs. 1–5 therefore represents the difference between two single measurements. The significance of changes in cyclic nucleotide levels can perhaps be assessed by inspection of the dose-dependent response depicted in Figs. 1, 2 and 4.

Effects of amino acids on cyclic nucleotide levels

Figure 1 shows the time course of the changes in tissue cyclic AMP (A) and cyclic GMP (B) levels following superfusion of isolated pancreatic segments with different concentrations (range 1–20 mM) of L-alanine. Each point represents the cyclic nucleotide concentration (test minus control values) at a particular time following the onset of stimulation. L-Alanine evoked a rapid increase in cyclic AMP rising to a maximum within 30 sec of stimulation. This initial transient rise was followed by a slow decline reaching about 0.25 pmole/mg protein after 4 min. The early rapid increase in cyclic AMP was accompanied by a fall in cyclic GMP levels reaching a maximum after 30 sec. Thereafter, cyclic GMP rose, exceeding the control level (horizontal broken line) after 55–65 sec and continued to increase after 3–4 min. These time-dependent changes in endogenous cyclic AMP and cyclic GMP concentrations were dose-related (Fig. 1).

The L-arginine-evoked changes in cyclic nucleotide levels, expressed in pmole/mg protein and plotted as a function of time, are shown in Fig. 2 for different concentrations (range 1–20 mM) of the amino acid. L-Arginine induced a large and very rapid increase in endogenous cyclic AMP levels (A) reaching a maximum within 5–15 sec. This initial quick rise was followed by an abrupt fall to below the control level (horizontal broken line) after 20–40 sec. Cyclic AMP levels then rose again but more slowly to reach

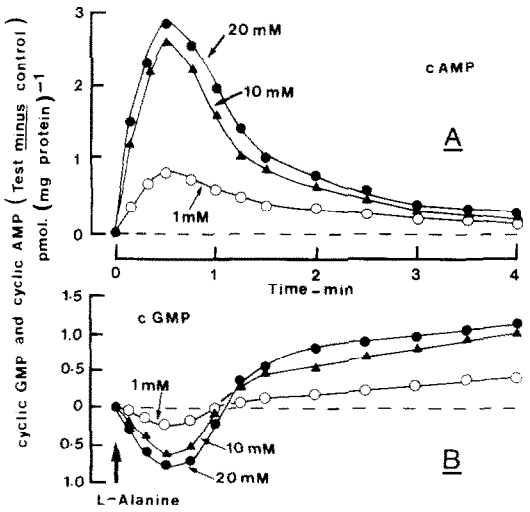

Fig. 1. Time course of changes in the tissue concentration of cyclic AMP (A) and cyclic GMP (B) during superfusion with different concentrations (range 1–20 mM) of L-alanine. Each point represents the difference in cyclic nucleotide concentration (pmole/mg protein) between two similar preparations, one serving as test and the other as a control. Atropine (10^{-5} M) was present throughout. The levels of cyclic AMP and cyclic GMP in control preparations were 2.48 ± 0.08 and 0.84 ± 0.04 pmole/mg protein, respectively (n = 39).