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Electrochemical Modifications Induced by Amino Acids in Rat Pancreas Isolated and Perfused *in situ*

The processes involved in the synthesis and secretion of pancreatic enzymes have been extensively studied (3). However, the mechanisms by which pancreatic secretagogues regulated these secretory processes, have not received much attention until recently.

The biochemical and electrolytic changes induced at the initial stages of secretion by secretagogues are being elucidated using uptake techniques, perfusion techniques or isolated cell techniques. Important information is also being obtained by the adaptation of electrophysiological techniques to diverse types of cell preparations.

In this work which is a continuation of previous work by members of the same group (5-9) the mechanisms which mediate transport of amino acids at the level of the basolateral pole of the exocrine epithelium of the pancreas is further studied. In addition, associated ionic changes, and other metabolic and hormonal factors, which are believed to be responsible for the high rates of protein synthesis displayed by this organ, have been analyzed.

Using isolated and perfused pancreas as previously described (7), from anaesthetized adult male Wistar rats weighing between 200-250 g fed with standard laboratory diet, tap water *ad libitum* and fasted 16-18 h before initiating perfusion, the electrical potential across the pancreas according to the technique originally described for the rat intestine (2) has been measured, as well as the ionic changes in the perfused organ, following a bolus injection of various amino acids diluted in control and in Ca^{+2} free perfusion medium.

The results indicate that injecting a bolus of L-arginine into the perfusing fluid, induces an increase in the potential difference across the pancreas and, at the same time, an increase in calcium concentration of perfusate (table I). This increase in the calcium concentration disappears gradually as the value of the potential difference returns to the values taken as control. The changes in the calcium concentration at the effluent after L-arginine injection in perfusing fluid containing calcium, were not significantly different from those seen after injection in calcium-free perfusion medium. These results suggest that the arginine uptake brings about changes in the intracellular free-calcium levels.

In contrast, the values of the measured concentration of Na^{+} and K^{+} in the effluent did not change significantly after L-arginine injection either in the presence or absence of Ca^{+2} in the perfusing fluid.

Similar experiments with the neutral amino acids L-phenylalanine and L-serine, brought about no changes in the potential difference or in the concentrations of Ca^{+2}, Na^{+} or K^{+} (results not shown). However, after injection of L-aspartate, there was a decrease in the Ca^{+2} concentration of the perfusate fluid (table I).

Clasic secretagogues movilize intracellular calcium via mediators type IP_3 (1, 10). Electric changes are not affected by the extracellular calcium concentration (11).

Taken together the results suggest that L-arginine has a secretagogue effect involving outflux of cellular calcium with significant variations of PD, and that an opposite effect is induced by L-aspartate. These opposite effects probably result from electrogenic phenomena mediated by the charge of the amino acids on the exocrine

Table I. *Ionic concentrations in the perfusate fluid and transpancreatic potential difference (PD) in the isolated rat pancreas.*

The perfusing medium was Krebs-Henseleit buffer supplemented with glucose 3.5 mM and 2 % BSA, equilibrated with 95 % O_2/5 % CO_2 at 38°C and pH 7.3-7.4. Perfusion was carried out for 1 h. A resting period of 10 min was intercalated between amino acid pulses (30 s) to allow the organ to restore its basal state. Concentrations and PD are given in mEq/l and mV units, respectively. The number of experiments was 6. Mean values ± S.E.M. are given. Paired t-test was used (* $p < 0.001$). Ionic concentrations were measured by ion-selective method (Microlyte) and PD was measured by means of two reversible calomel electrodes connected through saturated KCl agar bridges and inserted in both arterial and venous side of preparation by mean of T tubes.

	Ca^{+2}	Na^+	K^+	PD
Control (with Ca^{+2})	2.6 ± 0.3	146 ± 7	4.9 ± 0.5	1.4 ± 0.9
L-Arginine (with Ca^{+2})	11.2 ± 5.4*	150 ± 25	5.2 ± 0.5	6.9 ± 2.1*
L-Aspartate	1.8 ± 0.1*	146 ± 2	4.9 ± 0.9	— 1.2 ± 0.6*
Control (without Ca^{+2})	0.2 ± 0.2	145 ± 18	4.5 ± 0.2	3.5 ± 0.7
L-Arginine (without Ca^{+2})	0.7 ± 0.1*	151 ± 5	4.1 ± 1.6	6.3 ± 0.5*

pancreatic cells, which are not modified by the extracellular calcium concentration (table I). Similar action on intracellular Ca^{+2} outflux was found (2, 12), using different secretagogues as stimulus-secretion coupling as that obtained in this work after the bolus injection of non-neutral amino acids.

Key words: Amino acids, Electrochemical modifications, Pancreas, Rat.

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