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TWO DIFFERENT TYPES OF ELECTROGENIC AMINO ACID ACTION ON PANCREATIC ACINAR CELLS

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Summary

The electrogenic action of the basic amino acid, L-arginine, has been compared with the action of the neutral amino acids, L-alanine and glycine, in mouse pancreatic acinar cells. All three amino acids cause membrane depolarization, but while the reversal potential for the action of the neutral amino acids is close to the calculated value of the Na equilibrium potential (+30 mV) the reversal potential for the L-arginine effects is +7 mV. The neutral amino acids exhibit mutual inhibition, but L-arginine did not inhibit the L-alanine- or glycine-evoked depolarization nor did the neutral amino acids inhibit the action of L-arginine. While L-alanine markedly depressed acetylcholine-evoked depolarization, L-arginine had no such effect. It is concluded that there are at least two quite different types of electrogenic amino acid action in pancreatic acinar cells.

Introduction

Several neutral amino acids, L-alanine, L-valine and L-proline, have been shown to evoke depolarization of the pancreatic acinar cell membrane [1]. The depolarization is mainly due to an increase in membrane Na conductance [1] and it is likely that the previously demonstrated Na-amino acid cotransport system [2—5] provides the Na conductance pathway [1,6]. We have now carried out a close comparison of the actions of two neutral amino acids, L-alanine and glycine, and a basic amino acid, L-arginine. The L-arginine-evoked depolarization is of a nature totally different from that of the neutral amino acids,

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since the null (equilibrium) potential is less positive, the depolarization is independent of Na and the action of L-arginine does not, in contrast to the effect of L-alanine, inhibit acetylcholine-induced depolarization. While L-alanine and glycine exhibit mutual inhibition, the action of L-arginine is not reduced in the presence of L-alanine or glycine nor does L-arginine inhibit L-alanine- or glycine-evoked depolarizations. The pancreatic acinar plasma membrane therefore possesses at least two different types of electrogenic amino acid transport mechanism.

Methods

The experiments were carried out on isolated superfused segments of mouse pancreas, using intracellular microelectrode recording (two separate microelectrodes) and extracellular ionophoretic micropipette applications of the neutral amino acids, glycine and L-alanine, and the basic L-arginine [6]. In some experiments L-arginine was added directly to the tissue bath. The superfusion solution had the following composition (in mmol/*l*): NaCl, 126; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13: D-glucose, 2.8; Tris base, 3: and was gassed with 100% O_2. The pH was adjusted to 7.4 with HCl. The solution was prewarmed to 37° C.

Results

The effects of both L-arginine and glycine had a very short latency (less than 50 ms) as previously shown for L-alanine [1]. As seen in Fig. 1, the depolarization evoked by L-alanine was markedly decreased in the presence of glycine and vice versa even though the experiment was carried out in such a way that effects were always compared at exactly the same membrane potential. In contrast, glycine or L-alanine did not interfere with the L-arginine-evoked depolarization and L-arginine did not inhibit L-alanine- or glycine-evoked depolarization. Five complete experiments in five different tissues of the kind shown in Fig. 1 were carried out, all with identical results. In a few experiments the effect of adding L-arginine directly to the tissue bath to achieve concentrations between 0.5 and 10 mM was tested. This evoked membrane depolarizations with amplitudes between 2 and 6 mV.

The mechanism of action of L-arginine was investigated by assessing the null or equilibrium potential for the membrane effect. It has previously been shown that the null potential for the actions of L-alanine ($E_{\text{L-ala}}$), L-valine and L-proline was about +20 to +40 mV [1]; this has been confirmed (Fig. 2). E_{gly} has been found to have the same value (Fig. 2) (five experiments: range +20 to +32 mV). In contrast, $E_{\text{L-arg}}$ was consistently less positive than $E_{\text{L-ala}}$ when determined in the same experiments (Fig. 2). $E_{\text{L-arg}}$ and $E_{\text{L-ala}}$ were determined in the same cells in 14 separate experiments. $E_{\text{L-ala}}$ had a mean value of +29.5 ± 1.7 mV (S.E.) while $E_{\text{L-arg}}$ was +6.8 ± 1.4 mV (S.E.). This difference is statistically significant ($P < 0.01$). While $E_{\text{L-ala}}$ and E_{gly} correspond reasonably well to the Na equilibrium potential (E_{Na}) [1] this is clearly not the case for $E_{\text{L-arg}}$. While the depolarizing action of L-alanine is immediately and reversibly reduced by removal of Na from the superfusion solution [1,6] this was not so

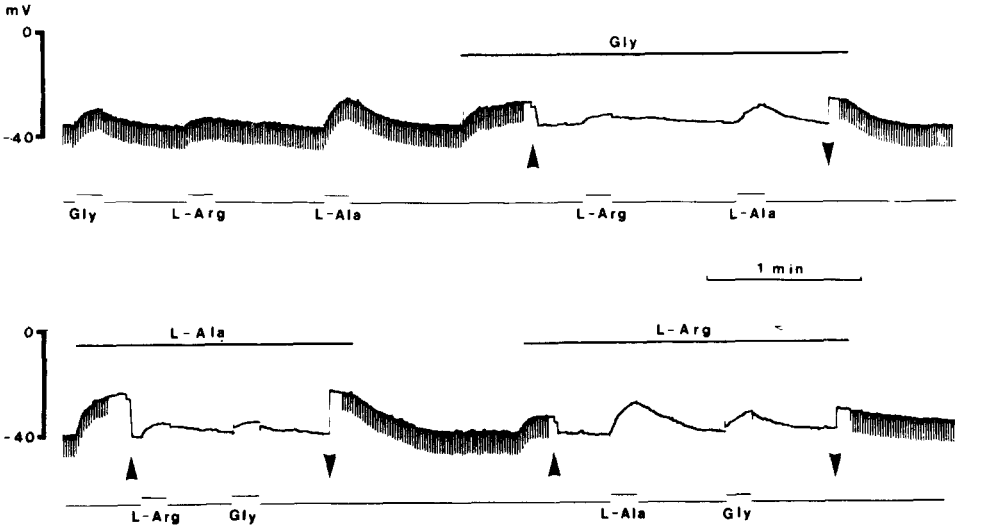

Fig. 1. Effects of glycine, L-arginine and L-alanine on membrane potential and resistance in the same mouse pancreatic acinus. Two intracellular microelectrodes inserted into two closely coupled neighbouring cells were employed. One was used as a recording electrode, the other was used for injecting current, either as short-lasting hyperpolarizing pulses (100 ms, 1 nA) or as direct current. The amino acids were applied directly to the outside of the acinus under visual control (phase contrast × 400) from three separate micropipettes filled with 1 M concentrations of the respective amino acids. Ejection of amino acids occurred by positive currents of 600 nA for glycine and L-alanine and 1000 nA for L-arginine. Duration of amino acid applications is indicated by the labelled bars or marker signals. The two traces shown are excerpts from one continuous recording from one acinus. First, the effects of short pulses of the three amino acids are shown. Thereafter, sustained glycine application is initiated. After a stable depolarization has been attained the repetitive intracellular current pulse applications are discontinued and thereafter hyperpolarizing direct current is adjusted to such a level as to bring back the membrane potential to the value observed prior to the glycine stimulation (▲); short pulses of L-arginine and L-alanine are then applied. The direct hyperpolarizing current is now switched off (▼) (causing a sharp depolarization) and repetitive current injection resumed (in order to permit continuous assessment of membrane resistance). Glycine stimulation is stopped and the membrane potential returns to the control level. The lower trace shows exactly the same type of experiments with two other ways of combining the three amino acids. Atropine ($2 \cdot 10^{-6}$ M) was present throughout.

for L-arginine. In nine separate experiments in which the effects of L-alanine and L-arginine were investigated in the same cells, replacement of superfusion fluid Na by Tris, choline or sucrose reduced the responses to L-alanine to about one third of the control within 3 min, whereas the effects of L-arginine were unchanged.

Pancreatic secretagogues such as acetylcholine and peptides belonging to the cholecystokinin-gastrin and the bombesin families evoke acinar cell membrane depolarization [1,7,8]. The mechanism of the secretagogue-evoked depolarization is very different from that of the amino acid-induced depolarization [1], since the null potential for the membrane actions of all the secretagogues is about —15 mV [8] as compared to the +30 or +7 mV for the different amino acids. Nevertheless, the secretagogue-evoked depolarization involves, amongst other effects, an increase in Na conductance [7]. Interactions between the effects of, e.g., acetylcholine and neutral amino acids might therefore be expected. Fig. 3 shows the effect of acetylcholine in the absence or presence of L-alanine at the same membrane potential. The action of acetylcholine is

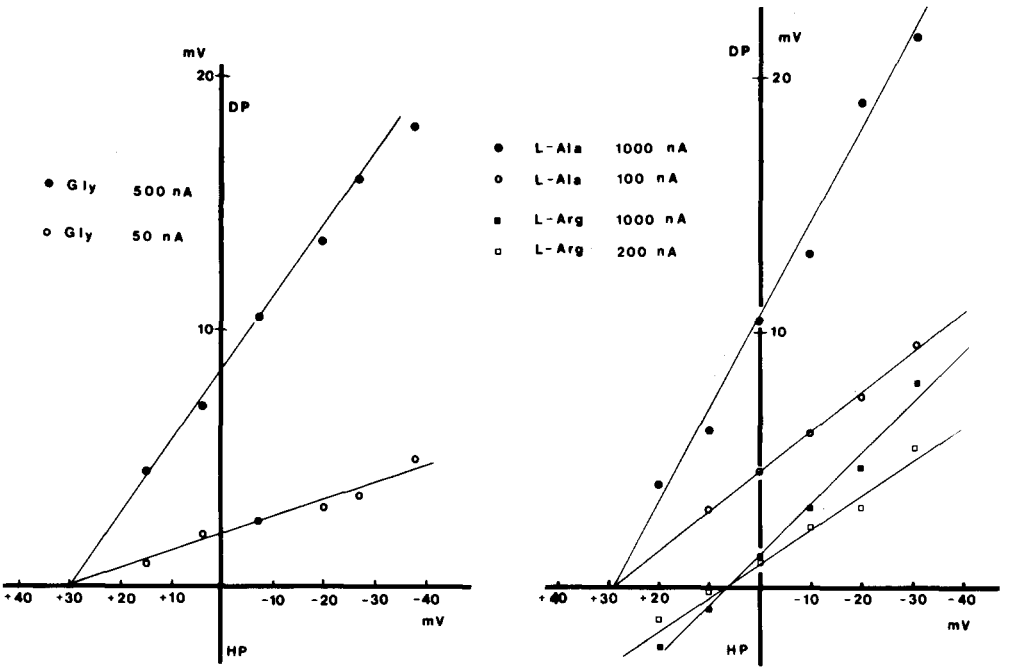

Fig. 2. The change in membrane potential evoked by L-alanine, L-arginine and glycine (ordinate) as a function of the level of the pre-set membrane potential (abscissa). The level of the membrane potential was adjusted by direct current injection into the acinus. The amino acids were applied for 10 s by iontophoresis as indicated. All the results for L-alanine and L-arginine were obtained on the same acinus. The glycine results are from a different experiment. Atropine ($2 \cdot 10^{-6}$ M) present throughout.

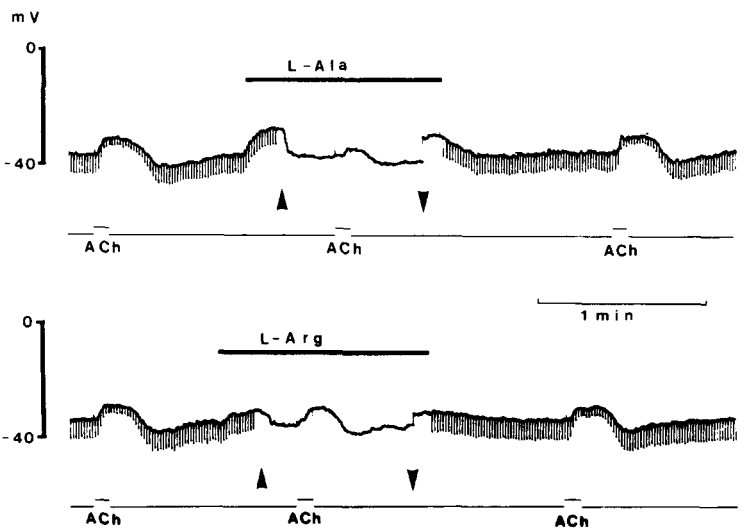

Fig. 3. The effect of acetylcholine (ACh) in the presence and absence of L-alanine or L-arginine. Repetitive intracellular current injection (100 ms, 1 nA) reveals membrane resistance and it is seen that acetylcholine (100 nA ejecting current, between acetylcholine applications retaining current of 30 nA) causes more marked resistance reduction than L-alanine. During the continuous L-alanine application (700 nA) hyperpolarizing direct current is injected intracellularly to adjust the membrane potential to the pre-stimulation level (▲) (resistance measurement discontinued in this period) and the effect of acetylcholine at the same membrane potential as previously tested is now markedly reduced. The lower trace shows the same type of experiment with L-arginine (1000 nA ejecting current) instead of L-alanine.

markedly and reversibly reduced by L-alanine. In contrast, L-arginine did not inhibit the acetylcholine action (Fig. 3.) Four separate experiments of the type shown in Fig. 3 were carried out, all giving the same result. It seems likely that L-alanine-evoked Na uptake reducing the transmembrane Na gradient caused the inhibition of the acetylcholine response. L-Arginine presumably does not increase Na permeability and probably therefore failed to inhibit the acetylcholine effect. Acetylcholine also dramatically reduced (virtually abolished) the membrane depolarization evoked by L-alanine in four experiments.

Discussion

The results presented here demonstrate two entirely different electrogenic amino acid actions on the pancreatic acinar cells, probably reflecting two types of transmembrane amino acid transport. This conclusion is mainly based on two lines of evidence. (1) Interaction experiments of the type shown in Fig. 1 indicate that there is virtually no inhibition of L-arginine-evoked depolarization by glycine or L-alanine nor indeed any inhibition of L-alanine or glycine-evoked depolarization by L-arginine. On the other hand, as previously described [6], neutral amino acids exhibit strong mutual inhibition as also confirmed in the present experiments in regard to L-alanine and glycine. We have also demonstrated here that L-alanine but not L-arginine markedly inhibits acetylcholine-evoked depolarization (Fig. 3). (2) The reversal potential for the action of L-arginine is consistently and markedly different from that of the neutral amino acids (Fig. 2), indicating a different ionic mechanism underlying its effect. As yet, we have no positive evidence to offer in regard to the mechanism by which L-arginine exerts its effects, but it is interesting that the L-arginine-evoked depolarization is less Na dependent than the action of the neutral amino acids previously described [6].

Arginine is a very potent releaser of pancreatic islet cell hormones [9]. However, it is unlikely that the electrogenic arginine effects described here are indirect via release of islet peptides, since the latency was very short (much shorter than that of the pancreatic secretagogue hormones [8]). If an electrogenic arginine action as described here for the acinar cells also exists in the islet cells, then this could explain why arginine is a powerful secretagogue for the endocrine pancreas. None of the amino acids tested here cause pancreatic enzyme secretion (unpublished observations). However, the acinar cells are electrically inexcitable whereas the islet B-cells fire action potentials when depolarized [7].

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