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INHIBITION BY MEPACRINE OF AMYLASE SECRETION FROM INTACT AND PERMEABILIZED RAT PANCREATIC ACINI

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In intact rat pancreatic acini, the phospholipase A_2 inhibitor mepacrine did not affect basal amylase release but dose-dependently inhibited the carbachol (IC_{50} 65μM) and CCK-8 (IC_{50} 210 μM)-stimulated amylase release. In permeabilized acini, mepacrine shifted the dose-response curve for calcium to the right by a factor 2 and inhibited the release of amylase stimulated by GTPγS. From these results we conclude that carbachol, CCK-8 and GTPγS probably activate a phospholipase A_2 closely coupled to exocytosis.

The stimulus-secretion coupling of the rat exocrine pancreas is mostly under the control of agonists which stimulate the hydrolysis of polyphosphoinositides. Muscarinic agents, cholecystokinin (CCK) and bombesin activate a G protein which is coupled to the activation of a phospholipase C, the first step in a cascade leading to exocytosis (1). A second phospholipase, phospholipase A_2, has been involved in the response to carbachol or CCK (2). This enzyme might be responsible for the generation of arachidonic acid, a precursor of prostaglandins. The mode of activation of this phospholipase A_2 is still controversial: the plasma membrane receptors might activate a guanylnucleotide binding site coupled to this enzyme (3); or the increase in intracellular calcium could secondarily activate the phospholipase A_2, an enzyme which is calcium-dependent (4) or the activation of calcium-dependent protein kinases could phosphorylate the phospholipase A_2 or a regulator, leading to the activation of the enzyme (5).

We originally proposed that a phospholipase A_2 might be involved in the last steps of the stimulus-secretion coupling (6). This hypothesis, to be testable, required a way to get access to the cytosolic side of the apical plasma membrane without disrupting the integrity of the secretory granules. The use of streptolysin O as a lytic agent has been reported in several tissues (7) and in the exocrine pancreas (8). When pancreatic acini are exposed to the toxin, they exhibit a calcium-dependent amylase-secretion; guanylnucleotides can also activate the release of amylase. In this paper, we test the effect of mepacrine, an inhibitor of phospholipase A_2 on the amylase secretion from intact and permeabilized acini. We show

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that in intact acini, the drug inhibits the amylase secretion stimulated by carbachol and by CCK. In permeabilized acini, the drug inhibits the basal release of amylase and the amylase release induced by calcium; it abolishes the response to GTPγS. From these results we conclude that the activation of phospholipase A_2 by guanylnucleotide is intimately coupled to exocytosis.

MATERIAL AND METHODS

Male Wistar rats (150-300g) were used for these experiments. The animals were fed ad libitum and had free access to water. The collagenase (CLSPA) was from Worthington (Freehold NJ), the streptolysin O (reduced form, lyophilized) was from Wellcome (Erembodegem, Belgium). Carbamylcholine, pyruvate, NAD and the CCK-8 were from Sigma Chemicals (St Louis, MO). The mepacrine was from the Pharmacie Centrale de Belgique (Brussels, Belgium). All the other reagents were of analytical grades.

Preparation and incubation of the acini:

The rats were killed with ether and their pancreas removed. One rat was used for each experiment. After dissection to remove connective and adipose tissue, the acini were prepared and incubated as described previously (9). The amylase was assayed with the method of Noelting and Bernfeld (10).

To permeabilize the acini, the acinar preparation was washed with a "permeabilization medium" containing Kglutamate 140 mM, HEPES buffer 24 mM pH 7.4 (with KOH), $MgCl_2$ 7 mM, ATP 1 mM, glucose 5 mM and 0.2% bovine serum albumin. They were incubated for 10 minutes at 37°C in the same medium in the presence of EGTA 1 mM, the required calcium concentration, streptolysin O 0.1 U/ml and the tested agents. The amylase present in the medium was assayed as described previously. In our incubation conditions, the LDH present in the incubation medium averaged 87 ± 4% and this amount was independent of the calcium concentration in the incubation medium.

RESULTS

Effect of mepacrine on intact acini

Concentrations of mepacrine from 1 μM to 1 mM did not affect the basal release of amylase from intact pancreatic acini. Maximal concentrations of the muscarinic agonist carbachol (10 μM), and of CCK-8 (100 pM) stimulated the amylase release 6-, 5-fold respectively (Figure 1). Mepacrine dose-dependently inhibited the response to the muscarinic agonist. A half-maximal and a maximal inhibition were observed at 65 μM and 1 mM respectively. Mepacrine also inhibited the response to CCK-8 but the shape of the inhibition curve was different from the curve observed with carbachol: the Hill coefficient was lower than 1 (0.8) while it was higher than 2 with carbachol (2.7) and at a maximal 1 mM concentration, mepacrine inhibited the effect of CCK-8 by 80% (instead of 100 % for carbachol). The half-maximal inhibitory concentration of mepacrine was 210 μM.

Effect of mepacrine on permeabilized acini

Calcium by itself was able to promote amylase release from permeabilized acini (Figure 2). The stimulatory effect was already apparent at a 110 nM calcium concentration

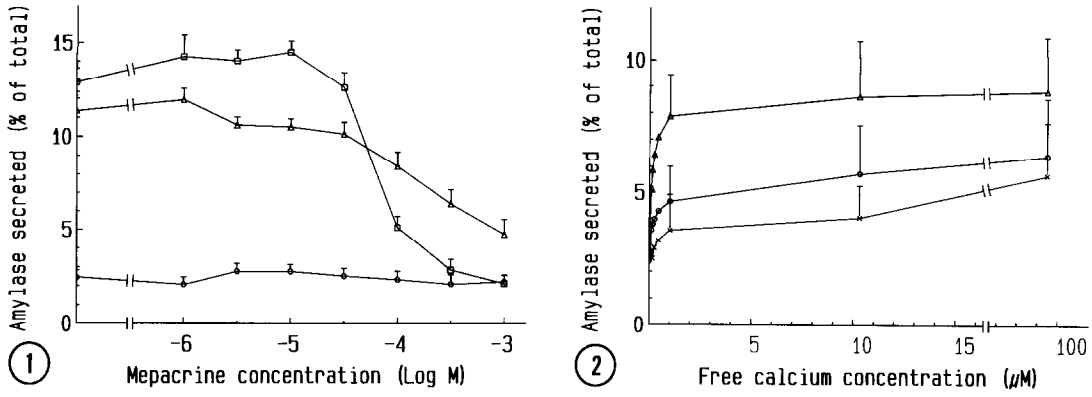

FIGURE 1 . Effect of increasing concentrations of mepacrine on amylase secretion in intact acini.
Rat pancreatic acini were incubated for 20 min at 37°C in the presence of increasing concentrations of mepacrine, in control conditions (o) or in the presence of 10 μM carbachol (□)or of 0.1 nM CCK-8 (Δ). The amylase released in the medium was assayed as described in Methods. Results are expressed as percent total amylase released in the medium during the experiment and are the means ± s.e.m. of 4 experiments.

FIGURE 2 . Effect of increasing concentrations of calcium on the amylase release from permeabilized acini.
The acini were incubated for 10 min at 37°C in the presence of 0.1 U/ml SLO, in the presence of 1 mM EGTA and increasing concentrations of calcium. Free calcium concentrations were calculated with the program of Fabiato and Fabiato (25). The acini were incubated in control conditions (o) or in the presence of 10 μM GTPγS (Δ) or 1 mM mepacrine (x). Results are the means ± s.e.m. of 3 experiments.

and reached a maximum at 10 μM. The half-maximal effect was observed at 0.92 μM. The guanylnucleotide analog GTPγS potentiated the stimulatory effect of calcium: it shifted the half-maximal concentration to the left (to 0.17 μM) and increased the maximal response by 50%. Mepacrine at a 1 mM concentration had the opposite effect at least on the half-maximal concentration: it shifted the dose-response curve for calcium to the right. Assuming that the inhibitory effect of mepacrine is competitive, the half-maximal concentration of calcium would be in the presence of mepacrine around 2 μM.

In the next series of experiments, the acini were incubated in the presence of a 0.25 μM free calcium concentration, a concentration specially suitable to test the effects of stimulants. As can be seen in Figure 3, mepacrine slightly inhibited the basal release of calcium (a 15 % inhibition in these experiments). GTPγS at a 10 and 100 μM concentration increased the basal release of the enzyme by 115 % and 125 % respectively. Concentrations of mepacrine higher than 10 μM inhibited that stimulation. At a 1 mM mepacrine concentration, the inhibition was total. The half-maximal inhibitory concentrations were not significantly different for the two concentrations of GTPγS: 94 μM and 105 μM at 10 and 100 μM GTPγS respectively.

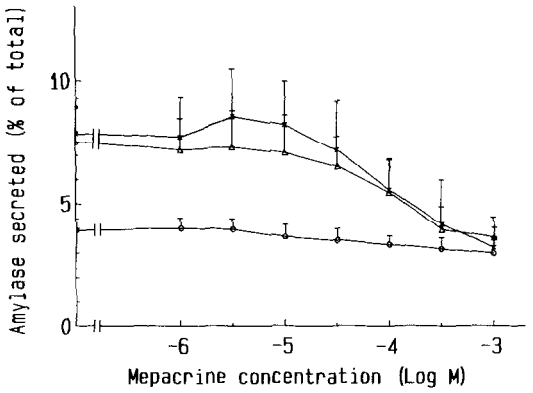

FIGURE 3 . Effect of increasing concentrations of mepacrine on the amylase secretion stimulated by GTPγS in permeabilized acini.
The acini were incubated for 10 min at 37°C in the permeabilization buffer, in the presence of 0.25 μM free calcium and of 0.1 U/ml SLO. The acini were exposed to increasing concentrations of mepacrine in control conditions (o) or in the presence of GTPγS 10 μM (Δ) or 100 μM (x). The results are the means ± s.e.m. of 4 experiments.

DISCUSSION

We have shown that in intact acini, mepacrine can inhibit the release of amylase induced by carbachol and CCK-8. Heisler (11) previously reported that mepacrine did not affect the secretory response to acini while Farese et al. observed that mepacrine could inhibit the secretory response to carbachol but concluded that this inhibition was nonspecific (12). Since mepacrine inhibits the response to either carbachol or CCK-8, two agonists stimulating different classes of plasma membrane receptors, mepacrine probably acts at a step distal to the receptor. This compound is a well-known inhibitor of phospholipase A_2 at least in the 10 μM-1 mM concentration range (13). Considering that the activation of secretion is coupled to the release of arachidonic acid from rat pancreatic acini (14) and considering that activation of endogenous phospholipase A_2 with mellitin (11) or addition of exogenous phospholipase A_2 (15) triggers amylase secretion, the inhibitory effect of mepacrine on secretion could be secondary to its inhibition of endogenous phospholipase A_2. In permeabilized acini, mepacrine shifted to the left the dose-response curve of calcium and inhibited the secretory effect of GTPγS. GTPγS has been reported by several groups to stimulate exocytosis from permeabilized acini (8,16). This stimulatory effect has been dissociated from the activation of a regulatory site coupled to phospholipase C (16). Rather, it has been suggested that GTPγS activates a regulatory site located on the plasma membrane of secretory granules. This hypothesis is consistent with the fact that several low-molecular GTP-binding proteins have been described in the membrane of zymogen granules (17,18). But a phospholipase A_2 is also located in this membrane (19) and GTPγS can

activate this enzyme (20). Considering the inhibition by mepacrine on the secretory effect of GTPγS, we suggest that GTPγS binds on the regulatory sites of the membrane of the zymogen granules, activates via this receptor a phospholipase A_2 which is intimately coupled with exocytosis. Phospholipase A_2 could generate in this membrane lysophospholipids which are fusogens (21), or could release arachidonic acid, which in turn could favor the interaction of proteins like synexin with the plasma membrane and the zymogen granule membrane, triggering exocytosis (22). The arachidonic acid generated by the activation of this distal phospholipase A_2 could also be the precursor of prostaglandins which can modulate both the secretory process in the acinar cells (23) and the activity of ductal cells (24) ensuring a concerted activity of the two components of the secretory process in the exocrine pancreas.

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