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EICOSANOIDS EVOKE THE RELEASE OF AMYLASE AND INCREASE CYTOPLASMIC CALCIUM IN RAT PAROTID CELLS

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The effects of various eicosanoids on cytoplasmic calcium and the release of amylase were examined in isolated rat parotid cells. Arachidonate and several of its metabolites increased amylase release and elevated cytoplasmic calcium. Melittin, a stimulator of arachidonate mobilization, and lyso-phosphatidylcholine also released amylase and elevated calcium. These results suggest that the metabolites of arachidonate may have an important role in amylase secretion.

Arachidonic Acid (AA) is stored within the membrane attached at the sn-2 position of the phospholipids. During secretion, AA is hydrolyzed from the phospholipids by phospholipase A2 (PLA2) [1]. Unesterified AA is metabolized into a number of other molecules including prostaglandins, thromboxanes and leukotrienes. Some of these directly evoke secretion of peptides or modulate the second messenger activity of cytoplasmic calcium (Ca_i) or cyclic AMP [2-5]. Since the release of amylase from parotid cells is known to be controlled by cyclic AMP and Ca_i [6], it is possible that AA or some of its metabolites might initiate or modulate the release of amylase.

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Abbreviations: AA, arachidonic acid; PLA2, phospholipase A2; lyso-PC, lysophosphatidylcholine; 5-HETE, 5-hydroxy-6,8,11,14-eicosatetraenoic acid; 12-HETE, 12-hydroxy-5,8,10,14-eicosatetraenoic acid; 15-HETE, 15-hydroxy-5,8,11,13-eicosatetraenoic acid; 5,6-EET, 5,6-epoxy-8,11, 14-eicosatrienoic acid; 5-HPETE, 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid; 12-HPETE, 12-hydroperoxy-5,8,11,14-eicosatetraenoic acid; Ca_i, cytoplasmic free calcium; PGE_2, prostaglandin E2; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid; KHB, Krebs Henseleit Bicarbonate solution; CLO, cyclooxygenase; LP, lipoxygenase; Cyt-P450, cytochrome P450.

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To test the hypothesis that the AA cascade is important in the control of the release of amylase, we exposed aequorin-loaded parotid cells to AA and some of its metabolites. We found that melittin (an activator of AA mobilization), AA, lysophosphatidylcholine (lyso-PC), 5,6-EET, 5-HETE, PGE_2 and 15-HETE increased Ca_i and amylase release. These results are consistent with the hypothesis that the eicosanoids may have an important role in amylase release.

METHODS

Isolated Parotid Cells: The method used to isolate parotid cells was an adaptation of that of Watson et al. [7]. In our studies, 300 gm rats (Outbred Sprague-Dawley, Simonsen Co., Gilroy, CA) were anesthetized with ether and exsanguinated by an aortic incision. The parotid glands were removed and minced in a petri dish containing oxygenated Hank's Solution (Gibco, Freehold, NJ) fortified with 20 mM HEPES buffer (pH = 7.4). The mincate was then transferred to a 25 ml Erlenmeyer Flask containing collagenase (Worthington Scientific, Freehold, NJ) and hyaluronidase (Sigma Chemical Co., St. Louis, MO). The pH of the digestion was maintained at 7.4 with a pH titrator (McKee-Pedersen Inst. Danville, CA). After 1 hr, the suspension was filtered through nylon mesh and the cells were washed with Krebs Henseleit Bicarbonate (KHB) plus 4% BSA to remove the enzymes and assayed for viability with trypan blue (Sigma Chemical Co., St. Louis, MO). KHB contained 120 mM NaCl, 5 mM KCl, 24 mM $NaHCO_3$, 1 mM $MgSO_4$ 1 mM KH_2PO_4, 1 mM $CaCl_2$ and 5 mM dextrose. We used populations of cells 90% viable.

Measurements of Cytoplasmic free calcium with aequorin: The procedures for loading cells with aequorin, measuring cytoplasmic calcium with aequorin and calibration procedures have been described in detail [8].

Amylase Release in static incubation: To compare the effectiveness of AA metabolites to release amylase, parotid acini were evenly distributed among 5-10 Erlenmeyer Flasks containing 10 mls of KHB and gassed with 95% O_2 5% CO_2 (pH 7.4) and incubated at 37°C in the Orbital Shaker Bath set at 130 rev/min. A one ml aliquot was transferred to a centrifuge tube and then centrifuged at 200 g for 5 minutes. One ml of the supernatant was removed and assayed for amylase content as described by Rinderknecht et al [9]. A second 1 ml aliquot was removed from the cell suspension 0.5 hr after the addition of either carbachol, isoproterenol, PGE_2, 5-HETE, 5,6-EET, AA or an appropriate volume of ethanol (vehicle control). This aliquot was centrifuged and 0.5 ml of the supernatant was analyzed for amylase content. The net change in amylase content of the supernatant was used as an index of the rate of amylase secretion. The data was expressed as a percentage of the amylase released by 40 mM isoproterenol and reported as the mean ± standard error. Student's t-test was used to test for statistically significant differences between drug and control groups.

Materials: Melittin, PGE_2 and lyso-PC were purchased from Sigma Chemical Co. (St. Louis, MO), AA, 5-HETE, 15-HETE and 5,6-EET were synthesized and purified as previously described [17,19] and stored in benzene at -70° C. Before each addition, the desired aliquot of eicosanoid was transferred to a micro-centrifuge tube, blown to dryness with argon and then resuspended in ethanol. Argon was used to displace the oxygen in the gas phase of all storage containers to avoid oxidation prior to the experiment. Aequorin was purchased from Dr. J. Blinks (Mayo Foundation, Rochester, MN).

RESULTS

Melittin (0.35 nM - 35 uM), a well known stimulant of PLA2, released amylase from rat parotid acini in a dose dependent manner (figure 1). Only high doses of melittin (3.5 - 35 uM) increased the aequorin signal (figure 2). Both AA (40 uM) and lyso-PC (40 uM), the products of PLA2 action, increased Ca_i and the release of amylase albeit with different time courses (figure 3). Therefore, the activation of PLA2 and its products, AA and lyso-PC, activate the release of amylase and elevate Ca_i.

Unesterified AA is metabolized by 3 enzymatic pathways: cyclooxygenase (CLO), lipoxygenase (LP) and cytochrome P450 (Cyt-P450). We tested products of each of these pathways for their ability to release amylase. In order to avoid the influences of cell number, and variability in responsiveness between different populations of cells, the data was expressed as a percentage of the effect of isoproterenol (40 uM). As shown in Table I, AA (40 uM) was the most

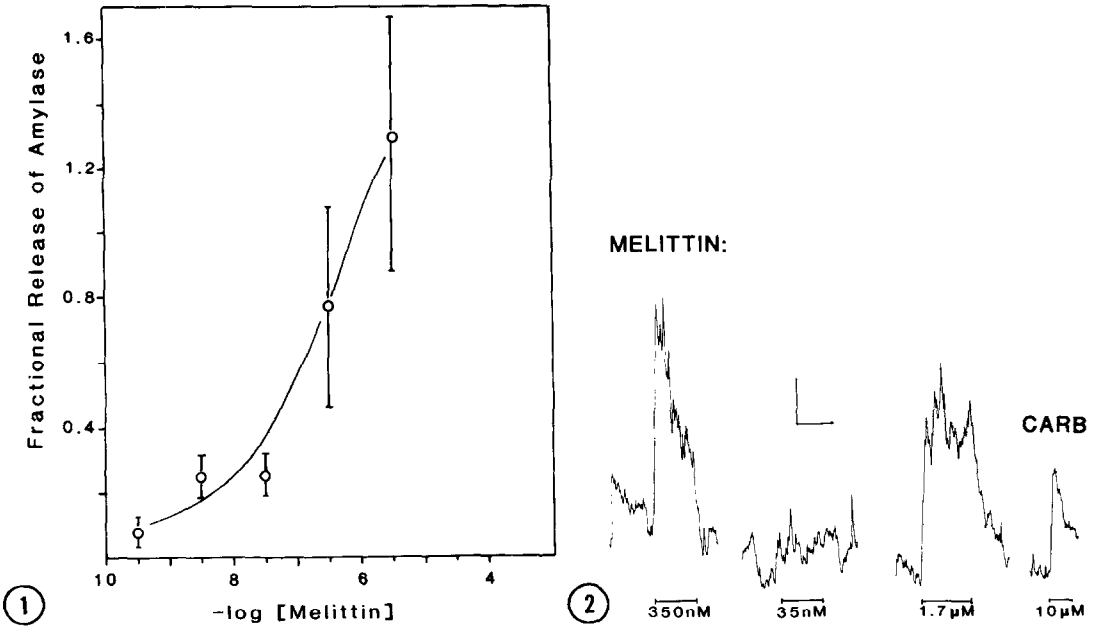

Figure 1: The dose dependent release of amylase from rat parotid cells evoked by melittin. Released amylase is expressed as a fraction of that remaining in the cells. Each point represents the mean of 5-7 observations ± standard error. *P< 0.05 by Student's t-test.

Figure 2: The effect of 35 nM, 350 nM and 1.7 uM melittin and 10 uM carbachol on the aequorin signal. Calibration bars: horizontal (10 min), vertical (5 nA). Traces from 1 of 4 similar experiments.

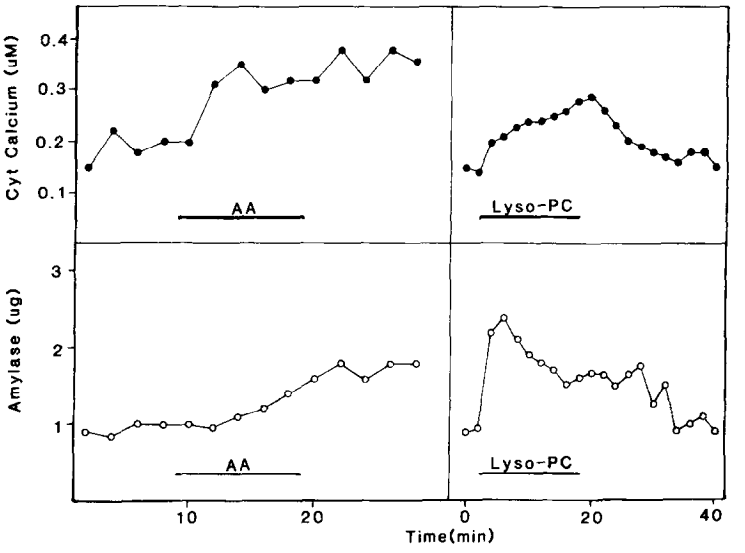

Figure 3: Effects of AA (left) and lyso-PC (right) on Ca_i (top traces) and amylase content in effluent (bottom traces). Amylase content expressed in terms of ug/ml compared to the effect of an alpha-amylase standard (14 units/mg at pH 6.9) from Sigma Chem. (St. Louis, MO).

potent at releasing amylase being nearly equal to 40 uM carbachol. PGE_2 was the next most potent with 15-HETE, 5,6-EET and 5-HETE being less potent. These observations support the notion that products of LP, CLO and Cyt-P450 may function as amylase secretagogues.

DISCUSSION

The role of eicosanoids in the secretion of insulin has been more thoroughly studied than that of any other secretory system [1]. In insulin

TABLE I: EFFECTS OF VARIOUS EICOSANOIDS ON AMYLASE RELEASE

Condition	percent amylase release
Carbachol	107.8 ± 23.9 (5)*
AA	101.4 ± 16.4 (5)*
PGE2	62.2 ± 16.5 (5)*
15-HETE	66.0 ± 17.0 (5)*
5,6-EET	73.4 ± 22.7 (5)*
5-HETE	75.4 ± 10.3 (5)*
Control	28.5 ± 4.8 (5)

[a] The concentration of all agonists was 40 uM. Data is expressed as a % of that evoked by 40 uM Isoproterenol (= 100%). Control refers to the effect with the same volume of (ethanol) (< 0.1%).

* $P < 0.05$ by Student's t-test.

secretion, the elevation of glucose leads to the activation of PLA2 promoting the rapid breakdown of phosphatidylcholine into AA and lyso-PC [1]. AA has been shown to: 1) release calcium ions from intracellular storage sites [2], 2) stimulate soluble guanyl cyclase [3], 3) promote the release of insulin [4] and 4) activate its own mobilization by activating phospholipase C [5]. Lyso-PC also appears to release insulin [10]. Unesterified AA is metabolized by CLO to the prostaglandins in islet cells as indicated by the observations that glucose increases the islet content of the prostaglandins (PGE_2, PGF2alpha, 6-keto-PGF 1 alpha) and thromboxane B2 [11,12]. AA is also oxidized by 5- and 12-lipoxygenase to form 5-HPETE and 12-HPETE, respectively [13,14]. 5-HPETE and its metabolites, the leukotrienes, are insulin secretagogues [15,16] whereas 12-HPETE is the major eicosanoid formed during insulin release [12,14]. Finally, unesterified AA is metabolized by Cyt-P450 which is an ubiquitous hemoprotein that has been shown to hydroxylate endogenous materials such as steroids and fatty acids [17]. Cyt-P450 metabolizes AA to regioisomeric epoxyeicosatrienoic acids (EET's) as well as other hydroxylated metabolites some of which have been shown to release hormones from cells [17-19].

In our study, we have shown that the activation of PLA2 by melittin and its products, AA or lyso-PC, cause the release of amylase. We have also shown that the activation of PLA2 or the administration of AA or lyso-PC increases the concentration of Ca_i. This suggests that there might be some interrelationships between Ca_i and AA metabolism with regards to amylase release. The importance of these findings concerning the metabolites of AA in the secretion of amylase is further underscored by the fact that products of all 3 major pathways of AA metabolism increase the release of amylase. Therefore, we conclude that the AA cascade is involved in the secretion of amylase.

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