ACETYLCHOLINE PROMOTES THE SYNTHESIS OF PROSTAGLANDIN E IN MOUSE PANCREAS

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1. Introduction

The major prostaglandins (E_2,$F_{2\alpha}$) are synthesized from arachidonic acid, which can be derived by deacylation of phospholipids or triacylglycerols [1]. In considerations of the precise source of arachidonic acid used for prostaglandin synthesis, much attention has been drawn to the fact that mammalian phosphatidylinositol contains an exceptionally high proportion of arachidonic acid, as compared with other phospholipids. In mouse pancreas, ~80% of the total phosphatidylinositol is the 1-stearoyl, 2-arachidonoyl-*sn*-glycerophosphorylinositol species [2]. Acetylcholine (ACh) promotes a net breakdown of this phosphatidylinositol [2,3] and it seemed possible, therefore, that there might be some arachidonic acid release associated with this breakdown which could act as a substrate for prostaglandin synthesis. An effect of ACh on prostaglandin synthesis has not been reported, however. Here, the effect of ACh on the synthesis of prostaglandin E (PGE) in mouse pancreas was examined under conditions which are known to promote the breakdown of phosphatidylinositol in this tissue. The PGE fraction was assayed since PGE_2 is the major prostaglandin synthesized from arachidonic acid in most tissues [1].

2. Materials and methods

Solvents (reagent grade) were obtained from Fisher Sci. Co., Fair Lawn, NJ and twice glass-distilled prior to use. Florisil® (60–100 mesh, regular grade) was obtained from Sigma Chemical Co., St Louis, MO, and washed with acid prior to use [4]. [5,6,8,11,12, 14, 15-^{3}H]PGE_2 (100–200 Ci/mmol) was obtained from New England Nuclear, Boston, MA and purified prior to use using mini-columns packed with acid-washed Florisil [4]. PGE_2 was generously supplied by Dr John Pike of The Upjohn Co., Kalamazoo, MI. Rabbit anti-prostaglandin E-BSA serum (lot no. PRE-4) was obtained from Miles Res. Products, Elkhart, IN. The Miles PGE anti-sera crossreacts with PGE_2 (100%), PGE_1 (60%), $PGF_{1\alpha}$ (14%) and $PGF_{2\alpha}$ (2%).

Male mice from an inbred Swiss-Webster strain were used. The pancreas tissue was removed and incubated as in [3]. Immediately after incubation, the tissue was homogenized in the total incubation media (1 ml) using a Brinkman Polytron with 50 μl 10 mg/ml solution of indomethacin in ethanol added prior to homogenization. Indomethacin has been shown to be a potent (I_{50}, 0.17 μM) rapid-acting inhibitor of prostaglandin synthesis [5]. It was added to prevent prostaglandin synthesis during homogenization. The homogenate and a 0.3 ml incubation buffer rinse were combined and frozen in crushed dry-ice and then shipped in crushed dry-ice from the University of Wisconsin in Madison to the Louisiana State University Medical Center in Shreveport.

The homogenates were thawed and treated with 0.4 ml 0.1 M HCl followed by 2.6 ml isopropanol–ethyl acetate solution (1:1, v/v). The mixture was vortexed for 30 s. Two phases were formed by centrifugation and the upper, ethyl acetate phase, was removed and saved. Another extraction with 2 ml ethyl acetate was conducted and the two extracts were combined. Solvent was removed under nitrogen, with benzene added to remove traces of water. The E series prostaglandins were isolated as in [4]. For the unincubated pancreases which were homogenized in 1 ml incubation buffer (plus indomethacin) immediately after removal from the animal, 1/10th of the

ethyl acetate extract was used for PGE isolation. For all of the incubated pancreases, only 1/20th of the ethyl acetate extract was used for PGE isolation. PGE was quantified in duplicate using a radioimmune assay, as described, supplied with the Miles PGE anti-sera. [^{3}H]PGE_2 was used to determine PGE loss during extraction and column chromatography. Average recovery for [^{3}H]PGE_2 was 51% with a range of 46–57%. Corrections were made for PGE loss. Two separate aliquots of each ethyl acetate extract were analyzed for PGE and the duplicate values obtained were averaged. The intraassay coefficient of variation was 10.6% and the interassay coefficient of variation was 18.7%.

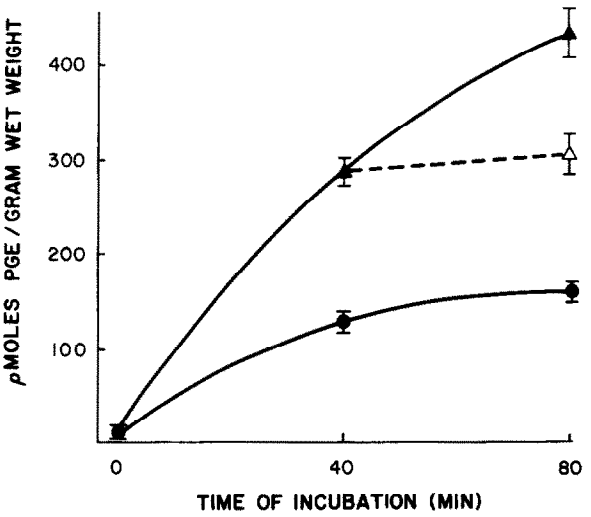

Fig.1. Acetylcholine-dependent prostaglandin synthesis in mouse pancreas. (●) The time-dependent synthesis of prostaglandin E during the incubation of mouse pancreases in the absence of exogenous acetylcholine. (▲) The time-dependent synthesis of prostaglandin E in the presence of 100 μM acetylcholine (plus 100 μM eserine). (△) Prostaglandin E synthesis after the addition of 10 μM atropine to acetylcholine-stimulated (100 μM) mouse pancreases at 40 min incubation. Data shown represent the mean ± SE for 4 different mouse pancreases.

3. Results

The mean value (± SE) for the level of PGE in mouse pancreas in situ (i.e., before incubation) was 12 ± 2 pmol/g fresh tissue. This level is similar to that reported for other secretory tissues where prostaglandins are believed to play an important role in the secretory process. For testes and ovaries, PGE levels in situ have been reported to be ~13 pmol/g tissue [6].

The total level of PGE in the tissue, plus the incubation medium, rose markedly during incubation, indicating that PGE was synthesized during the incubation period (fig.1). Tissue was homogenized in the incubation medium so that total PGE synthesis could be examined. Synthesis of PGE was much increased in the presence of 100 μM ACh (+100 μM eserine) (fig.1). There was little or no net synthesis during the second 40 min incubation in the unstimulated tissue, but PGE synthesis continued during this period in the ACh-stimulated tissue. Increased synthesis due to ACh was 157 pmol/g tissue during the first 40 min incubation and 120 pmol/g tissue during the second 40 min incubation. Increased synthesis of PGE due to ACh was abolished by the addition of atropine (10 μM), a muscarinic ACh antagonist, at the 40 min time interval (fig.1). Atropine alone had no effect on the basal rate of PGE synthesis (table 1). In the ACh-stimulated tissue, the rate of synthesis of PGE averaged 5.31 pmol $min^{-1} . g^{-1}$ over the total 80 min incubation period.

When the effects of different concentrations of ACh were examined (table 1), the mean value for PGE synthesis during an 80 min incubation period in the presence of 1 μM ACh was somewhat higher than the control, but the difference did not reach statistical significance. With 10 μM ACh, synthesis increased ~2.5-fold, and with 100 μM ACh, it increased ~3-fold. The results indicate that there is a net synthesis

Table 1
Effect of acetylcholine on prostaglandin E synthesis in mouse pancreas in vitro

Treatment	PGE synthesis (pmol/g wet wt)	*P*
None	148 ± 12	–
Atropine (10 μM)	151 ± 13	n. s. d.
ACh (1 μM)	208 ± 40	n. s. d.
ACh (10 μM)	358 ± 44	<0.02
ACh (100 μM)	425 ± 60	<0.02

Incubation time was 80 min. Eserine (100 μM) was present in all vessels to which ACh was added. Data represent the mean ± SE of values from 4 mouse pancreases/treatment. Statistical analysis was by the 2-tailed, unpaired *t*-test. n. s. d., not significantly different ($P > 0.05$) from the control (no treatment). PGE levels in situ were 12 ± 2 pmol/g tissue ($N = 4$). This mean in situ value has been subtracted from PGE levels observed after incubation to determine net PGE synthesis

of PGE in mouse pancreas during incubation in vitro, and that this increased in the presence of 10 μM or 100 μM ACh.

4. Discussion

Prostaglandins have been implicated in secretory cell function in a variety of tissues but their role in pancreatic exocrine secretion is, at present, somewhat controversial. Prostaglandins have been reported to have no effect, to be inhibitory, and to enhance both basal release of pancreatic enzymes and release stimulated by ACh and other secretagogues [7]. In dispersed rat acinar cells, no evidence was found for prostaglandin synthesis, as assayed using incorporation of [^{14}C]arachidonate [7].

Since PGE synthesis is stimulated under conditions which produce a net breakdown of phosphatidylinositol in mouse pancreas, it is possible that a release of some arachidonic acid from this phosphatidylinositol pool could provide the essential precursor for PGE synthesis. However, there is a difference of 3 orders of magnitude in the extent of phosphatidylinositol breakdown and the amount of PGE synthesized. Under conditions in which an increased synthesis of 277 pmol PGE/g tissue was found to take place in response to 100 μM ACh, there is a loss of 174 nmol/g tissue of arachidonate from the phosphatidylinositol pool [2]. It may therefore be difficult to establish experimentally whether a part of the net loss of phosphatidylinositol during ACh stimulation of mouse pancreas is directly linked to prostaglandin synthesis. It seems unlikely, however, from this relationship, that the chief function of stimulated phosphatidylinositol breakdown in the pancreas is to provide arachidonic acid for prostaglandin synthesis.

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