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Photodynamic action of rose bengal on isolated rat pancreatic acini: stimulation of amylase release

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The halogenated fluorescein derivative, rose bengal, upon photon activation, elicits amylase secretion from isolated, perifused pancreatic acini. This effect is due to production of highly reactive singlet delta oxygen which can permeabilize the cell membrane and may also react chemically with secretagogue receptors, or other functional components of the membrane such as the G-proteins. The profile of photodynamically induced amylase secretion is anion-dependent: it becomes biphasic when the chloride ion is substituted by the glutamate ion, an effect attributed to the action of glutamate on the ionic transport systems of the zymogen granule membrane.

Photodynamic action; Rose bengal; Pancreatic acini; Secretion; Amylase release; (Rat)

1. INTRODUCTION

Halogenated fluorescein derivatives have been reported to block sodium channels in squid giant axon [1], cause inhibition of ouabain binding to *Digitalis* receptors in rat brain and guinea-pig heart [2], cause an increase in membrane potassium permeability of isolated molluscan ganglia [3], and cause membrane permeabilization of murine thymocytes [4] and smooth muscle cells of guinea-pig *Taenia coli* [5].

The most recent studies [4,5] suggest that the halogenated fluorescein compounds produce their effects via a photodynamic action which requires the simultaneous presence of a photodynamic agent, light, and ground state molecular oxygen, thereby producing highly reactive singlet delta molecular oxygen [6]. Anionic fluorescein derivatives, preferentially taken up and retained by the cell membrane because of their negative charge [7,8] can be activated by light of appropriate wavelengths. Singlet oxygen will then be produced locally at the plasma membrane, where it can interact with various membrane components, and so permeabilize the cell membrane [5]. In an attempt to permeabilize selectively the plasma membrane of secretory cells, and to directly manipulate the intracellular environment for investigation of the molecular events of secretion, we report here the photodynamic effects of the halogenated fluorescein molecule, rose bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodo-fluorescein), on the secretion of amylase from freshly isolated, perifused rat pancreatic acini. Rose bengal was chosen for this initial study because of its high quantum yield, θ, of singlet oxygen ($\theta = 0.76$) [9,10], the reactive intermediate of photodynamic action.

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2. MATERIALS AND METHODS

Isolated pancreatic acini were prepared according to methods previously reported [11], i.e., by sequential collagenase digestion of the rat pancreas. The cells so prepared were found to be 95% viable by the eosin exclusion test. Routinely, 1 ml of acini suspension was mixed with 250 mg Biogel beads (P2) and loaded into a tissue column for perifusion. The tissue column, constructed from a 2 ml plastic hypodermic syringe, was mounted in a brass support and immersed in a water bath (37°C).

Amylase released from the perifused pancreatic acini was assayed fluorometrically by the automated method of Matthews

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et al. [12] with minor modification. The substrate employed was amylopectin anthranilate (Sigma); this, upon hydrolysis by amylase, releases the dialyzable molecule anthranilate which is fluorogenic and can be measured by reference to standard concentrations of amylase.

When required the pancreatic acini were exposed to light from a Schott KL 1500 quartz-halogen light source equipped with a heat filter. The light emission was >400 nm, and colour temperature 3200 K. The light output was evenly divided and conveyed to the preparation by three fibre-optic light guides (diameter 4.5 mm) arranged symmetrically around, and orthogonal to, the tissue column. The output of illuminance to the central axis of the tissue column at a set distance from the end of each light guide was measured by a Minolta T-1H illuminance meter.

The isolated acini were perifused with either a normal extracellular solution, or a cytosolic medium, of the following composition. Extracellular solution (in mM): 118 NaCl; 4.7 KCl; 1.16 $MgCl_2$; 2.0 $CaCl_2$; 1.16 NaH_2PO_4; 2 $mg \cdot ml^{-1}$ glucose; 25 Hepes; pH adjusted to 7.3 with 1 N NaOH. Cytosolic solution (in mM): 4.70 NaCl; 118 KCl or K glutamate; 1.16 $MgCl_2$; 1.16 NaH_2PO_4; 14 glucose; 25 Hepes; 2.0 EGTA; 5.0 ATP; pH adjusted to 6.8 with 1 N KOH.

All solutions were bubbled with 100% O_2 for at least 25 min before use and then continuously gassed with O_2 throughout the experiment. Ionized calcium concentrations present in the cytosolic medium were determined with a computer programme based on the calculations of Fabiato and Fabiato [13].

All chemicals were purchased from Sigma (England) with the exception of bethanechol which was from Koch-Light and Biogel beads (P2) from Biorad (England).

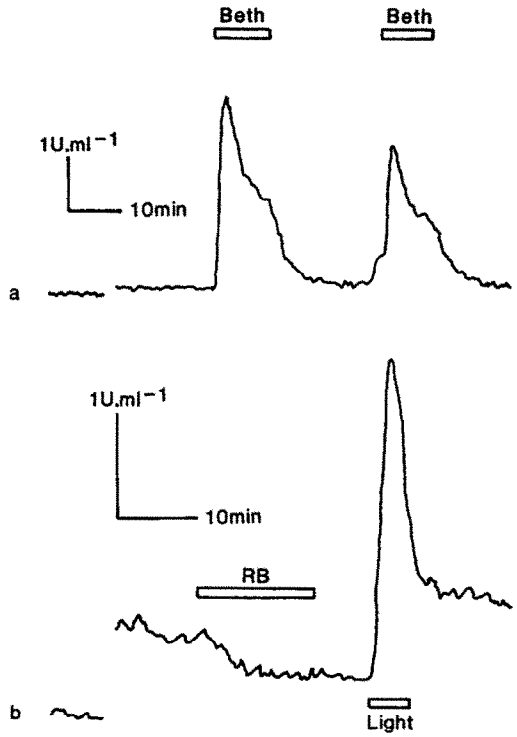

Fig.1. Amylase release from perifused acini elicited (a) by exposure to 100 μM bethanechol (Beth); (b) by the photodynamic action of 1 μM rose bengal (RB) on irradiation with light at 39910 lux.

3. RESULTS

The functional responsiveness of freshly prepared acini was tested by exposure to the muscarinic agonist, 100 μM bethanechol (fig.1a). The rate of amylase release from the perifused acini increased very rapidly upon exposure to bethanechol, followed by an initial rapid decline from the maximum and a subsequent slow decrease in output. Amylase release returned to the resting level upon withdrawal of bethanechol from the perifusion medium. A second exposure to bethanechol gave a smaller response which may indicate a progressive decrease in the releasable store of amylase, desensitization of muscarinic receptors, or of subsequent reactions coupling receptor activation to cellular secretion.

A typical record of amylase release induced photodynamically by rose bengal is shown in fig.1b. Isolated acini were perifused with a normal extracellular solution containing 1 μM rose bengal for 20 min during which the acini took up and retained rose bengal. Free rose bengal in the extracellular space was then washed out by continued perifusion with medium containing no rose bengal. Subsequent photon activation of the bound rose bengal led to a rapid increase in the rate of amylase release. The rate of amylase output decreased from the initial peak before termination of irradiation, and the rate was then maintained at a level higher than that of basal release until the end of the experiment. Light alone in the absence of rose bengal pretreatment had no effect on secretion.

For plasma membrane permeabilization studies of the photodynamic action of rose bengal, a cytosolic-like medium is necessary. In these experiments, acini were first perifused with an extracellular medium containing 2 $mg \cdot ml^{-1}$ bovine serum albumin (BSA) for 30 min before perifusion with a cytosolic medium containing 1 μM rose bengal but no BSA. A small but rapid increase in amylase release was consistently observed upon switching from the extracellular to the cytosolic medium (fig.2). This could be due to concomitant removal of BSA from, and addition of a high con-

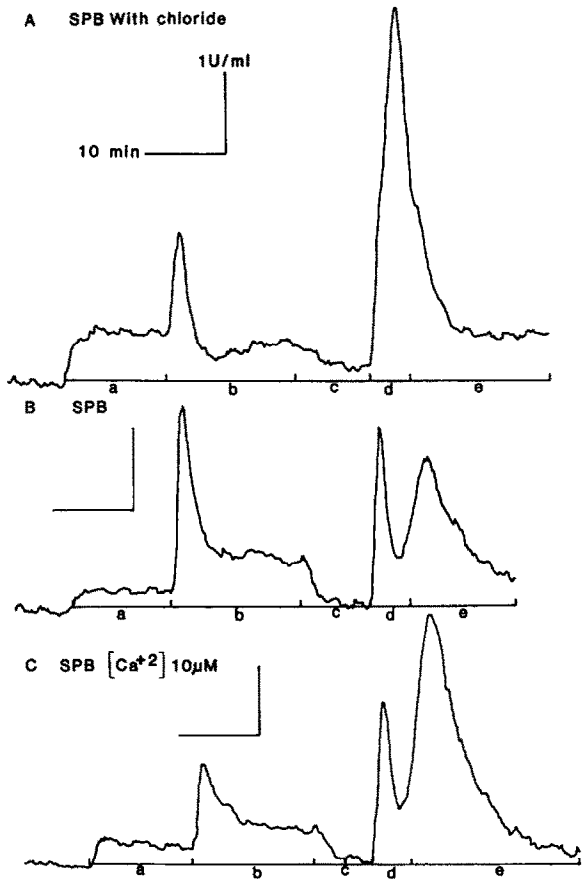

Fig.2. Photodynamic action of rose bengal on amylase release in the presence (A) or absence (B and C) of chloride. Acini were perifused with extracellular solution containing chloride, before and during period a, and subsequently, for periods b, c, d and e, with cytosolic medium containing chloride rather than glutamate in (A), and 10 μM ionized calcium in C only. Acini were exposed to 1 μM rose bengal during period b and to light (39910 lux) during period d. The total amylase content of the acini was 2175, 1750 and 1713 units for A, B and C, respectively.

centration of potassium to, the medium since neither BSA removal nor high potassium alone caused significant stimulation of amylase secretion (data not shown).

The photodynamic action of 1 μM rose bengal on amylase release from acini perifused with a cytosolic medium is shown in figs 2 and 3. It is obvious that in the absence of ionized calcium, photodynamic action (1 μM rose bengal; light, 39910 lux) still caused a prominent increase in amylase release (fig.2B). However, 10 μM ionized calcium did enhance the effect (e.g., cf. 2B and C)

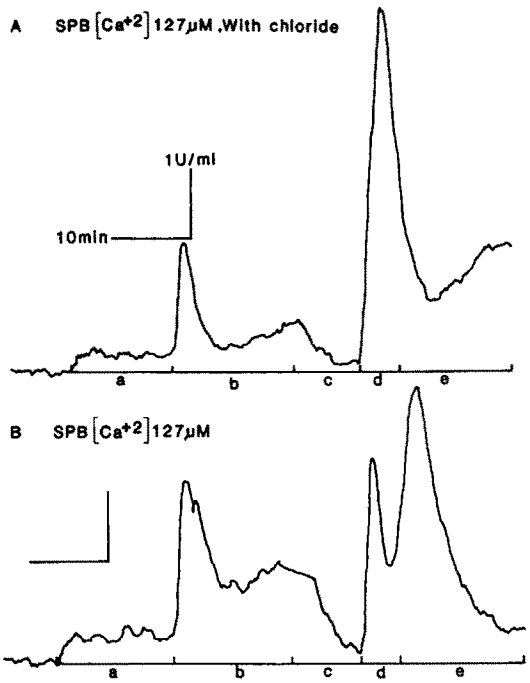

Fig.3. Photodynamic action of rose bengal on amylase release in the presence of 127 μM ionized calcium and the presence (A) or absence (B) of chloride. Other details as fig.2. The total amylase content of the acini was 1625 and 1650 units for A and B, respectively.

because the percentage of the total amylase content released in the first 15 min of photodynamic action was increased significantly ($P < 0.02$) from 1.8 ± 0.3% ($n = 6$) to 3.1 ± 0.2% ($n = 5$). Increasing the ionized calcium to 127 μM did not further enhance amylase release. In other experiments an increase in ionized magnesium from 1 to 6 mM had no effect on the amount of amylase released in the absence of ionized calcium, nor did the omission of ATP.

Interestingly, the major anion (chloride or glutamate) in the cytosolic medium exerts a profound influence on the kinetic pattern of amylase output. When the major anion is chloride (see fig.2A) amylase release is monophasic, and addition of 127 μM ionized calcium did not change the monophasic nature of the response (fig.3A) but, when the major anion in the cytosolic medium was glutamate, the amylase release became biphasic (fig.2B), consisting of an initial rapid peak followed by a second, broader output peak. This pattern was seen in all experiments with glutamate as the major anion ($n = 31$). Addition of ionized calcium,

10 μM or 127 μM did not affect the biphasic nature of the response (figs 2C and 3B). However, although the profile of amylase release was anion-dependent, the total amount of amylase released during the response period was not affected by the change of anion.

4. DISCUSSION

These results establish that cellularly bound rose bengal upon activation by light can stimulate amylase secretion from physiologically functioning rat pancreatic acini, both in a balanced extracellular solution and in a cytosol-like medium. Although micromolar calcium can enhance photodynamically induced amylase secretion, calcium is not absolutely essential for the secretion initiated by photodynamic action. It is therefore likely that membrane permeabilization and subsequent Ca^{2+} influx contributed only partly to the photodynamically induced secretion in normal physiological saline. In the cytosolic medium containing no calcium, secretion must obviously be due to factors other than Ca^{2+} influx through the plasma membrane permeabilized during photodynamic action [5]. The present observations and those of previous experiments [4,5] are consistent with a photodynamic action restricted to the plasma membrane but these latest results suggest that other membrane-located effects are produced by photodynamic action: effects which may actually precede membrane permeabilization [14]. Thus when singlet oxygen is generated by membrane-bound rose bengal after light activation [9,10], it may react with and activate the membrane proteins of secretagogue receptors or their coupled G-proteins or effectors (adenylate cyclase, phospholipase C) which are known to exist in isolated pancreatic acini [15]. Activation of these functional proteins leads to the production of second messengers and ultimately secretion.

The anion dependence of the kinetics of photodynamically induced amylase secretion is striking and may be related to the properties of the zymogen granule membrane which possesses at least three transport systems: a potassium conductance, a chloride conductance, and a chloride/bicarbonate antiporter [16]. When acinar cells are stimulated with secretagogues, these transport systems are believed to be activated via a phosphorylation process; K^+ and Cl^- then enter the granules, leading to exocytosis by osmotic swelling. In our experiments, when acinar cells are subjected to photodynamic action by rose bengal, the plasma membrane is permeabilized. Since the permeabilization occurs in a solution containing a high concentration of glutamate rather than Cl^-, glutamate will be the major anion to gain access to the granules. The glutamate ion is much larger than the chloride ion and may well cause a rapid blockage of chloride conductance or of the chloride/bicarbonate antiporter, leading to changes in granule membrane permeability [17] and a delayed or diminished exocytosis. The first phase of amylase secretion may therefore reflect receptor activation before plasma membrane permeabilization; glutamate entry into the cytosol after membrane permeabilization then results in a decreased or delayed secretion.

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