Polyphosphoinositide hydrolysis is associated with exocytosis in adrenal medullary cells

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[^{3}H]inositol-labelled products are released from adrenal medullary cells during exocytotic secretion. In 'leaky' cells in which small molecules readily enter and leave the cytoplasm, addition of micromolar calcium ions in the presence of ATP stimulates exocytosis and causes the release of inositol polyphosphates. These data support the idea that hydrolysis of plasma membrane polyphosphoinositides may be an essential step in exocytotic secretion.

Calcium *Exocytosis* *Phosphoinositide* *Membrane fusion* *Phospholipase C* *Adrenal medulla*

1. INTRODUCTION

There is substantial evidence in some types of secretory cells that polyphosphoinositide hydrolysis precedes the increase in cytoplasmic free calcium which triggers exocytosis [1] and that it is a necessary precursor to secretion [2]. The hydrolysis products are second messengers [3] which act by releasing calcium from internal stores and by activating a plasma membrane-associated protein kinase [4]. In some circumstances, the rate of exocytosis is modulated by both calcium and protein kinase activation [5–7]. However, in other secretory cell types, hydrolysis of phosphatidylinositol bisphosphate ($PtdInsP_2$) and phosphatidylinositol phosphate (PtdInsP) seems to be a consequence rather than a cause of an increased cytosolic calcium [8–11]. We have demonstrated that hydrolysis of $PtdInsP_2$ is tightly coupled to exocytosis in sea urchin eggs [12]. I show here that hydrolysis of $PtdInsP_2$ occurs during exocytosis in adrenal medullary cells.

2. MATERIALS AND METHODS

Adrenal medullary cells were obtained by enzymatic dissociation of bovine adrenal glands [14] in a physiological saline solution of 150 mM NaCl, 5 mM KCl, 1.8 mM $MgCl_2$, 1.8 mM $CaCl_2$ and 10 mM Hepes, pH 7.2. The dissociated cells were embedded in agar (Sigma, Poole) and agar slices 0.5 mm thick were made with razor blades to permit efflux measurements to be made. Cells in agar slices were incubated with [*myo*-^{3}H]inositol (30 μCi/ml; Amersham) in saline solution for 2 h at 37°C. [^{3}H]Inositol efflux was measured by incubating slices (10^5 cells) in 2 ml gassed (100% O_2) solution. Each change of solution was assayed for catecholamine content [15] and radioactivity by measured liquid scintillation counting. At the end of each experiment, the catecholamine and [^{3}H]-inositol remaining in the slice was determined by extracting with Triton X-100. Efflux is expressed as a percentage of the slice content at the time at which the sample was taken.

'Leaky' cells were made by exposing the slices to an intense electric field [13] in a solution similar in composition to cytoplasm: 140 mM potassium glutamate, 5 mM $MgCl_2$, 2.5 mM ATP, 1 mM EGTA and 20 mM Pipes, pH 6.7. Free calcium concentration in calcium containing solutions was 5.9 μM (calcium/EGTA ratio 0.838 [12]).

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3. RESULTS

Fig.1a shows that adrenal medullary cells respond to an increase in extracellular potassium concentration by transiently secreting catecholamines. Elevated extracellular potassium concentrations are thought to induce secretion by stimulating an influx of calcium [14]. Fig.1b and c show that increasing extracellular potassium also stimulates the efflux of [^{3}H]inositol from the cells. Other agonists such as carbachol and veratridine stimulate [^{3}H]-inositol efflux (not shown). These results are consistent with the idea that a calcium-stimulated polyphosphoinositide hydrolysis occurs during secretion, but other explanations, for example, calcium-dependent alterations in inositol transport across the plasma membrane, are equally plausible.

I have used leaky adrenal cells to determine whether the inositol efflux which occurs during secretion can be ascribed to hydrolysis of $PtdInsP_2$. Fig.2a shows that subjecting cells to brief, intense electric fields makes them permeable to [^{3}H]inositol. Immediately after the cells are made leaky, there is a rapid loss of four-fifths of the label. In 6 experiments 85 ± 1.9% (mean ± SE) [^{3}H]inositol was rapidly released from leaky cells. Fig.2a also indicates that there is a slower component of inositol efflux in leaky cells. Fig.2b demonstrates that exposing cells to these brief, in-

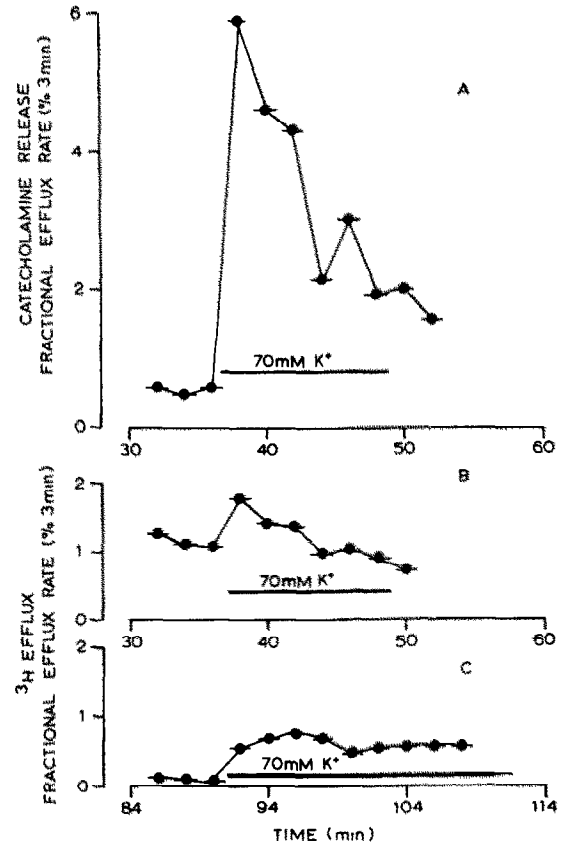

Fig.1. Efflux of catecholamine and [^{3}H]inositol from adrenal cells embedded in agar and stimulated to secrete by elevated extracellular potassium concentration (potassium increased at the expense of sodium). In this and other figures, $t = 0$ is the time at which cells were removed from the labelling solution.

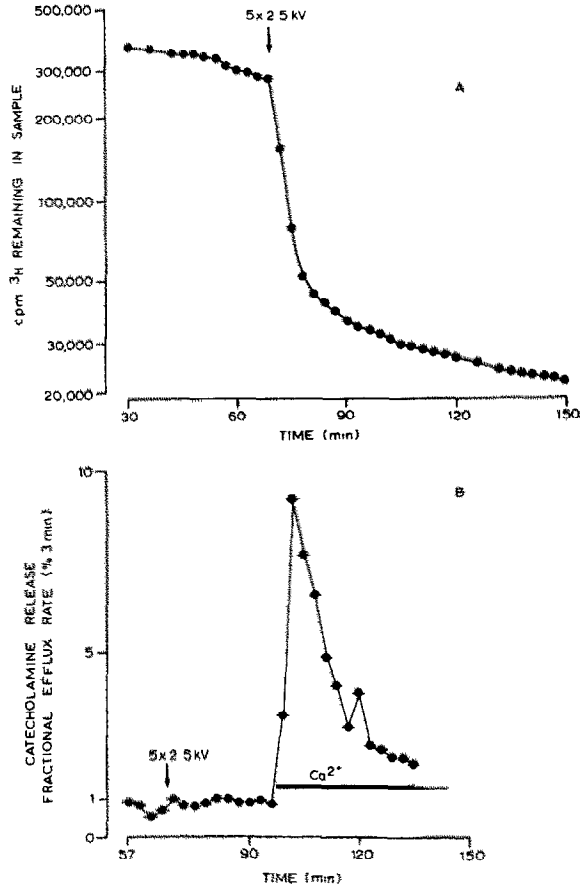

Fig.2. (a) ^{3}H counts associated with agar-embedded cells before and after application of five 2.5 kV pulses at the arrow. (b) Stimulation of catecholamine secretion in 'leaky' agar-embedded cells by addition of 6 μM free calcium.

tense electric fields causes no loss of catecholamines, but that subsequent addition of 6 μM calcium stimulates secretion of catecholamines. The catecholamines are released by exocytosis [13].

The slow component of [^{3}H]inositol efflux in leaky cells might correspond to a compartment within the cell from which [^{3}H]inositol which had not been incorporated into lipid was slowly released. Fig.3 shows that [^{3}H]inositol phosphate (InsP) and [^{3}H]inositol bisphosphate ($InsP_2$) are present in this component of the efflux. Inositol phosphates are produced by hydrolysis of $PtdInsP_2$ and PtdInsP, rather than by phosphorylation of free inositol [2]. The slow component of [^{3}H]inositol efflux in leaky cells appears to correspond to loss of [^{3}H]inositol and [^{3}H]-inositol phosphates from polyphosphoinositide phospholipids. In intact cells, the species leaving the cell is almost entirely [^{3}H]inositol. This is consistent with the finding that inositol phosphates do not readily cross the plasma membrane [16].

Fig.3 also shows that addition of micromolar calcium ions to leaky cells in the presence of ATP, or addition of ATP in the presence of micromolar calcium ions, stimulates the efflux of inositol phosphates. Either of these manoeuvres also results in exocytotic secretion [17]. Stimulation of exocytosis in intact cells by 70 mM K^+ causes an increased efflux of inositol. These data suggest that during exocytosis there is an increased hydrolysis of polyphosphoinositide phospholipids and production of inositol polyphosphates which in intact cells do not cross the plasmalemma.

This idea is supported by the data of fig.4, which indicate that polyphosphoinositide hydrolysis occurs only if calcium and ATP are both present. Addition of calcium in the absence of ATP does

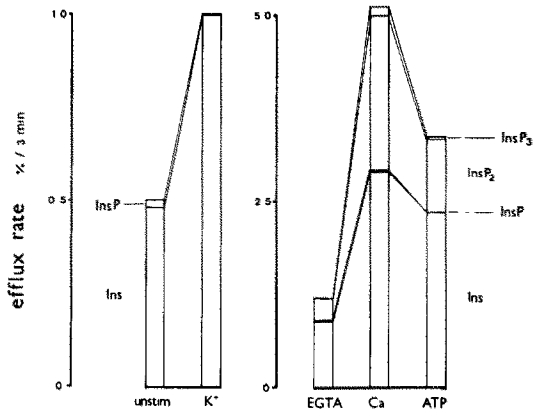

Fig.3. Composition of the ^{3}H efflux in intact and 'leaky' cells. Unstim, cells in physiological saline. K^+, cells stimulated by 70 mM external potassium ions. EGTA, cells in glutamate medium (see section 2). Ca, cells stimulated by addition of 6 μM free calcium in the presence of 2.5 mM MgATP. ATP, cells stimulated by addition of 2.5 mM MgATP in the presence of 6 μM free calcium.

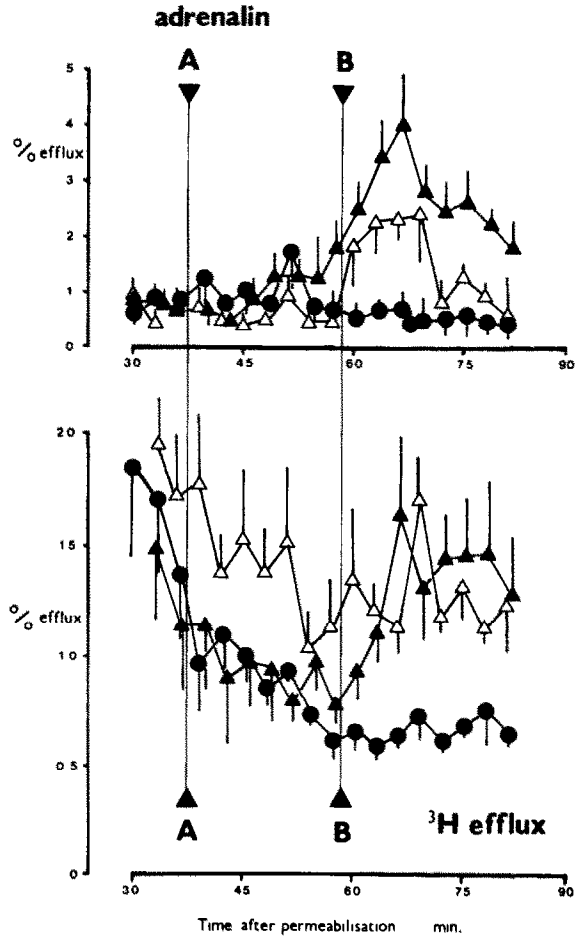

Fig.4. Simultaneous measurements of catecholamine secretion and ^{3}H efflux. Mean and SE of 6 experiments are shown. In each experiment 3 experimental conditions applied. Until A, each set of cells was incubated in glutamate medium (see section 2). (●) No change at A; 6 μM free calcium added at B. (▲) 2.5 mM MgATP added at A; 6 μM free calcium added at B. (△) 6 μM free calcium added at A; 2.5 mM MgATP added at B.

not cause exocytosis; nor is there any increase in [^{3}H]inositol efflux. Addition of ATP in the absence of calcium causes neither exocytosis nor increased [^{3}H]inositol efflux. Addition of ATP in the presence of calcium or calcium in the presence of ATP causes both exocytosis and polyphosphoinositide hydrolysis to occur.

4. DISCUSSION

It appears that exocytosis in adrenal medullary cells is accompanied by polyphosphoinositide hydrolysis, and that adrenal medullary cells possess a calcium-activated phospholipase C. The polyphosphoinositide hydrolysis products may play a part as second messengers. $InsP_3$ may release calcium from the cell's internal calcium stores. There is evidence for a calcium-stimulated contribution to increased intracellular calcium concentration during secretion [18]. The complementary hydrolysis product, DAG, may modulate secretory activity [7], since secretion in leaky cells has been shown to be sensitive to DAG [8]. An alternative possibility is that, as in sea urchin eggs, polyphosphoinositide hydrolysis is more intimately related to the mechanism of exocytosis itself. Neomycin, a drug which inhibits polyphosphoinositide hydrolysis [19], inhibits exocytosis in leaky adrenal cells (P.F. Baker and D.E. Knight, unpublished) and mast cells [20]. It is an attractive possibility that the generation of fusogenic lipids (DAG and its metabolites [21,22]) by hydrolysis of $PtdInsP_2$ and PtdInsP is responsible for the fusion of secretory granules with the adrenal cell plasma membrane.

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