INVOLVEMENT OF THE α-ADRENERGIC RECEPTOR IN THE PHOSPHOLIPID EFFECT IN RAT PAROTID

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1. Introduction

It has been shown, mainly by the Hokins, that in a variety of tissues, particularly in exocrine glands, hormonal stimulation is accompanied by an increased incorporation of $^{32}P_i$ into phospholipids [1–7]. Although the phospholipid effect has been amply demonstrated, its relationship to enzyme secretion [3, 7] and its role in hormonal stimulation of other target tissues [4–6] are little understood.

We have recently found that epinephrine activates two independent processes in the rat parotid gland: enzyme secretion mediated by a β-adrenergic receptor via cAMP, and K^+ release mediated by an α-adrenergic receptor [8–10]. The present communication demonstrates that the phospholipid effect is associated with activation of the α-adrenergic receptor. Induction of massive enzyme secretion does not lead to an increased incorporation of $^{32}P_i$ into phospholipids, as long as the α-adrenergic receptor is blocked or remains dormant.

2. Methods

Parotid gland slices were prepared and incubated at 37°C, as described previously [9]. Krebs–Ringer bicarbonate medium, from which phosphate was omitted, was used throughout for incubation and washings. The specific radioactivity of the nucleotide phosphate fraction was determined on TCA supernatant of slices equivalent to one gland [11]. Lipids were extracted from tissue homogenates [12], separated by two dimensional chromatography [13] and determination of specific radioactivity was carried out on phospholipid fractions [14, 15].

3. Results and discussion

Rat parotid slices incorporated $^{32}P_i$ into phospholipids. The phosphatidyl inositol phosphatidyl serine fraction contained in the largest amount of $^{32}P_i$ The specific radioactivity of phosphatidyl choline was an order of magnitude lower than that of the phosphatidyl inositol phosphatidyl serine fraction. The labelling of other phospholipids was very low.

Addition of epinephrine resulted in an increased incorporation only in the phosphatidyl inositol phosphatidyl serine fraction (table 1). This effect of epinephrine could not be attributed to an increase in the specific radioactivity of ATP, which did not change upon hormonal stimulation (fig. 1).

Determination of the phospholipid composition failed to reveal any significant change in the relative amounts of the phospholipid classes assayed. Taking this fact into consideration, it seems that changes in specific radioactivity represent turnover increase rather than net synthesis. The possibility of rapid synthesis of a very small fraction of one phospholipid class cannot, of course, be excluded.

These findings are in agreement with previous reports on the phospholipid effect in pancreas [3, 7], in liver [5], brain [16, 17, 19] and isolated fat cells [6]. However, in contrast to previous work on the phospholipid effect, the fraction of phosphatidic acid plus cardiolipin was very weakly labelled and was not affected by epinephrine. This does not necessarily ex-

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Table 1
$^{32}P_i$ incorporation into phospholipids of rat parotid slices.

Exp.	Additions		Sphingomyelin*	Phosphatidyl choline	Phosphatidyl inositol phosphatidyl serine	Phosphatidyl ethanolamine	Phosphatidic acid cardiolipin *
			(cpm/nmole)	(cpm/nmole)	(cpm/nmole)	(cpm/nmole)	(cpm/nmole)
1	None		43 (500)	79 (5800)	538 (21200)	8 (320)	10 (80)
	Epinephrine	0.10 mM	15 (230)	58 (6800)	1076 (39900)	7 (320)	7 (70)
	Isoproterenol	0.10 mM	39 (690)	52 (6800)	543 (24100)	6 (310)	6 (80)
2	Epinephrine	0.10 mM	18 (350)	59 (8000)	1431 (50500)	4 (410)	4 (50)
	Epinephrine	0.10 mM					
	+ phentolamine	0.02 mM	3 (40)	79 (6800)	748 (18400)	6 (220)	6 (40)
	Epinephrine	0.016 mM					
	+ propranolol	0.02 mM	19 (310)	80 (8600)	1366 (42900)	7 (470)	3 (40)
	Isoproterenol	0.10 mM	39 (680)	89 (11600)	729 (34000)	7 (480)	8 (100)
3	None		4 (30)	44 (3400)	440 (10400)	5 (190)	5 (30)
	Phentolamine	0.02 mM	4 (40)	35 (3500)	405 (12900)	4 (280)	5 (50)
	Propranolol	0.02 mM	4 (60)	39 (3400)	407 (11300)	4 (240)	5 (40)

* The specific radioactivities of minor components were determined on the basis of a calculated phosphorus content, derived from a separate determination of phospholipid composition.

Rat parotid slices were incubated for 30 min in the presence of $^{32}P_i$ (0.2 mCi/ml). The slices were then washed three times with non-radioactive medium (8 ml per gland). Slices, equivalent to approx. 4 glands, were transferred to vessels containing 8 ml of medium with the indicated catecholamines and inhibitors. After a further 30 min incubation, the tissue was taken for lipid extraction, followed by determination of specific radioactivities of the phospholipid fractions.

Phosphatidyl inositol and phosphatidyl serine were not completely resolved by the chromatographic procedure and were assayed together as a single fraction. Phosphatidic acid and cardiolipin were also assayed as a single fraction.

The figures represent the ratio of the mean of four independent determinations of both radioactivity and organic phosphorus, and are expressed as specific radioactivities (cpm/nmole P). Total radioactivities incorporated into each spot (cpm) are shown in parentheses.

clude phosphatidic acid as a precursor of phosphatidyl inositol. A very small, rapidly labelled fraction of phosphatidic acid would not be detected against the background of a relatively large amount of inert phosphatidic acid and cardiolipin.

The epinephrine effects on the rat parotid slice system have been recently resolved into α- and β-adrenergic responses [8–10]. The following experiments show (table 1) that the phospholipid effect is associated with activation of the α-adrenergic receptor, and not with the β-adrenergic receptor.

1) Isoproterenol, which specifically activates the β-adrenergic receptor, and induces massive enzyme secretion, had no effect on the incorporation of $^{32}P_i$ into phospholipids;
2) Epinephrine in the presence of the β-blocker, propranolol, was as effective in induction of the phospholipid effect as epinephrine alone;
3) Phentolamine, which selectively inhibits the α-adrenergic response, completely abolished the effect of epinephrine on the incorporation of $^{32}P_i$ into the phosphatidyl inositol phosphatidyl serine fraction.

The characterization of the phospholipid effect as part of the α-adrenergic response seems of great interest. Unlike the β-adrenergic response, of which the molecular mechanism has been elucidated [18], the biochemistry of the α-adrenergic response is still an enigma.

Employing the phospholipid effect as a test system, some insight into the mechanism of the α-adrenergic response in a cell-free system, might be attainable.

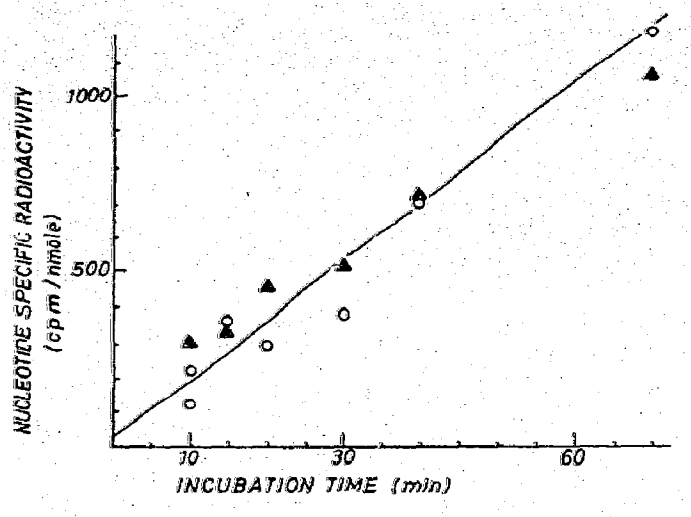

Fig. 1. $^{32}P_i$ incorporation into the nucleotide phosphate fraction of rat parotid slices in the presence and absence of epinephrine. Portions of slices (equivalent to 8 glands) were preincubated for 20 min and transferred into vessels containing $^{32}P_i$ orthophosphate (2 μCi/ml) in 16 ml medium. After 10 min of labelling, epinephrine was added to one vessel to the concentration of 10^{-4} M. At various time intervals, tissue samples were removed and the nucleotide phosphate specific radioactivity was assayed. Slices incubated in the presence of epinephrine (o—o—o); slices incubated in the absence of epinephrine (Δ—Δ—Δ).

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