THE EFFECT OF A PURIFIED PREPARATION OF LECITHINASE C ON IODIDE TRANSPORT

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Treatment of porcine thyroid slices with highly purified lecithinase C leads to a marked reduction in the ability to accumulate iodide ion. Although this response is less sensitive as a function of lecithinase concentration than is the ability to respond to thyrotropin with an increase in glucose oxidation, phospholipid synthesis or adenyl cyclase activity, it occurs when the baseline values of these parameters are not substantially altered.

1. Introduction

It has been shown that iodide accumulation by thyroid slices is sensitive to a variety of agents that are presumed to react at the plasma membrane [1, 2]. Since phospholipids are important constituents of the membrane and have also been implicated in the recognition mechanism for iodide and related anions [3, 4], one approach to an understanding of the possible relationship between phospholipids and transport is to study iodide transport after treatment of thyroid slices with phospholipases that hydrolyze specific types of phospholipids in membranes. Like many transport systems, iodide accumulation by thyroid slices has been shown to be inhibited by *crude* commercial phospholipase C preparations [1]. It is, however, clear from previous studies [5, 6] that these preparations are very impure and contain many enzymatic activities that can influence the behavior of cell membranes. A purified preparation of lecithinase C obtained from the growth medium of *Clostridium perfrigens* can abolish, in thyroid slices, the effect of TSH on $^{32}P_i$ incorporation into phospholipids and on 1-^{14}C-glucose oxidation without changing the basal rates of these processes [6]. We therefore tested the effect of this purified enzyme on iodide accumulation by hog thyroid slices.

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2. Materials and methods

The sources of all materials used have been previously described [6]. Enzyme purification, glucose oxidation, phospholipid synthesis analysis of adenyl cyclase activity and iodide accumulation were measured in fresh hog thyroid slices as previously described [2–10]. Thyroid glands, obtained at the local abattoir, were sliced with a Stadie-Riggs microtome, washed in Krebs-Ringer bicarbonate buffer and preincubated with or without the purified enzymes for 60 min at 37° in Krebs-Ringer bicarbonate buffer containing 0.1% bovine serum albumin, at pH 7.4. The slices were then washed briefly in fresh buffer and incubated under the experimental conditions indicated in the tables. Iodide accumulation is expressed as T/M [I^-]: the concentration ratio of $^{131}I^-$ in the tissue to that of the medium in the steady state.

3. Results and discussion

Lecithinase C decreased both the TSH stimulation and the accumulation of iodide in thyroid slices. Concentrations of lecithinase that entirely abolished the effects of TSH on $^{14}CO_2$ production, on ^{32}P-incorporation into phospholipids and on cyclic-AMP formation, caused a small but significant reduction in the T/M [I^-] (table 1). This suggests that the TSH-site is

Table 1
Inhibition of TSH effects and of iodide accumulation in hog thyroid slices by lecithinase C.

Lecithinase C (μg/ml)	$^{14}CO_2$ produced (cpm/mg/hr)		^{32}P-Incorporation into phospholipid (cpm/mg/hr)		cAMP-^{32}P formed (pmoles/100 mg tissue/5 min)			T/M [I^-]
	Control	TSH (100 mU/ml)	Control	TSH (100 mU/ml)	Control	TSH (50 mU/ml)	NaF (40 mM)	
None	34 ± 2	60 ± 3	106 ± 3	190 ± 3	76	120	240	50 ± 4
1.0	35 ± 3	36 ± 2	104 ± 2	103 ± 4	88	87	270	42 ± 3
5.0	30 ± 1	31 ± 3	95 ± 3	94 ± 2	86	88	260	23 ± 2
10.0	25 ± 2	24 ± 1	86 ± 4	85 ± 1	89	86	275	8 ± 1

Slices were preincubated with lecithinase C for 1 hr, briefly washed and then incubated for 1 hr in 1 ml KRB-buffer containing 1 mg of albumin, 1 mg of glucose and 0.3 μCi of 1-^{14}C-glucose for glucose oxidation studies. For the phospholipid studies each slice was incubated in 1 ml of KRB-buffer containing 1 mg albumin, 1 mg glucose and 0.8 μCi of ^{32}P-phosphate. For the assay of the adenyl cyclase activity, the washed, preincubated slices *after enzyme treatment*, were minced with a razor blade and about 200 mg of tissue was homogenized in 1 ml of ice-cold buffer tris-HCl 25 mM containing sucrose 0.25 M, and the conversion of α-^{32}P-ATP to ^{32}P-3′,5′-AMP was measured with this material as previously described [9, 10]. For the iodide accumulation studies, the enzyme-treated slices were incubated individually in 3 ml of KRB-buffer pH 7.4 containing 3 mg of albumin, 1 mM methyl mercaptoimidazole and 1 μM iodide labeled with $^{131}I^-$. The specific activity of lecithinase C was 3200 U/mg. The results are the means of four slices ± S.E.

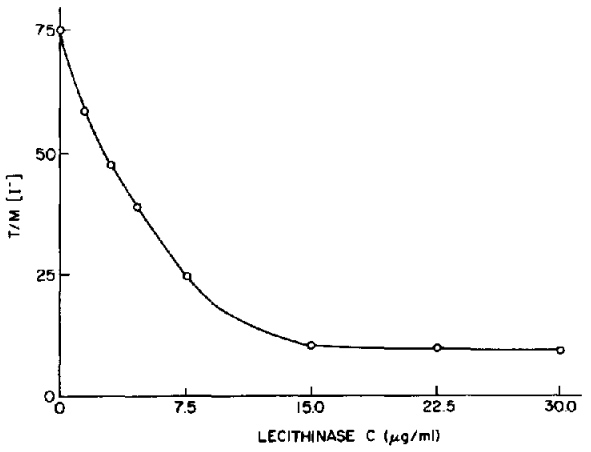

Fig. 1. The effect of lecithinase C concentration on the iodide accumulation in hog thyroid slices. The experimental conditions were identical with those of table 1. The results are the means of four slices ± S.E. at each point.

more sensitive to lecithinase C than the iodide-accumulating system. When thyroid slices were preincubated with increasing concentrations of lecithinase C there was a progressive decrease in the accumulation of iodide (fig. 1). The T/M [I^-] reached the plateau at 15–20% of the control values over a range of enzyme concentrations 1–10 μg/ml (fig. 1). Even enzyme concentrations higher than 10 μg/ml, that significantly reduced the basal ^{32}P incorporation into phospholipids or $^{14}CO_2$ production from glucose-1-^{14}C, never reduced the T/M [I^-] to one or near one. This suggests that there may be two components to the iodide accumulating system and lecithinase inhibits only one of these.

It is of interest that the larger concentrations of lecithinase C, while inhibiting the TSH response, had a slight stimulatory effect on basal and F^--activated adenyl cyclase activity. This increase may be akin to that seen in the presence of the phenothiazines [11].

The lecithinase C used was highly specific for lecitin; analysis, by thin-layer chromatography, of the phospholipid content of thyroid slices exposed to 4 μg/ml of lecithinase C (when the response to TSH was completely abolished and the T/M [I^-] was reduced to 50%

of the control values) showed that 50% of total thyroid lecithin was hydrolyzed while the other phospholipids were unaffected.

In contrast to the findings with lecithinase C, we have observed in preliminary experiments that another lipase, sphingomyelinase [7], *stimulates* iodide accumulation as well as glucose oxidation and phospholipid synthesis in thyroid slices. Although this stimulation was never greater than 50% above the controls, it was statistically significant ($p < 0.01$) in six of eight experiments. Under these conditions sphingomyelinase did not inhibit the TSH effect on thyroid slices [8] and hydrolyzed only sphingomyelin.

Although the iodide accumulating system of the thyroid gland is less sensitive to purified lecithinase than is the TSH "receptor", it seems likely that membrane-bound lecithin plays a crucial role in iodide transport or accumulation.

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