Relationship between Phosphatidylinositol Synthesis and Recovery of 5-Hydroxytryptamine-Responsive Ca^{2+} Flux in Blowfly Salivary Glands

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Each salivary gland contains about 135 pmol of phosphatidylinositol. In glands pre-labelled by incubation for 1 h with [^{32}P]P_i or [^3H]inositol there was a subsequent breakdown of 80% of the labelled phosphatidylinositol over a 2 h incubation period with 10 μM -5-hydroxytryptamine. However, there was no detectable decrease either in total phosphatidylinositol based on phosphorus analysis by chemical estimation or in the radioactivity of [^{32}P]phosphatidylinositol in salivary glands of flies raised from the larval stage on diets containing [^{32}P]P_i and whose phospholipids were uniformly labelled. These results suggest that the pool of phosphatidylinositol involved with Ca^{2+} gating is a small fraction of the total phosphatidylinositol content. Furthermore it is this small compartment that is preferentially radioactively labelled during short-term incubations with radioactively labelled precursors. In salivary glands incubated for 2 h with 10 μM -5-hydroxytryptamine there was a marked decrease in the flux of $^{45}\text{Ca}^{2+}$ across the gland. After removal of the hormone, incubation of salivary glands for 1 h in the presence of 2 mM-inositol, but not choline or ethanolamine, resulted in a recovery of hormone-responsive $^{45}\text{Ca}^{2+}$ flux. Quantitative studies revealed that less than 9 pmol of phosphatidylinositol must be formed to fully restore the 5-hydroxytryptamine-responsive $^{45}\text{Ca}^{2+}$ flux.

The addition of 5-hydroxytryptamine to the isolated blowfly salivary gland stimulates fluid secretion, transepithelial Ca^{2+} transport and the breakdown of labelled phosphatidylinositol. In salivary glands labelled by brief incubation with [^{32}P]P_i or *myo*-[2- ^3H]inositol the addition of 1 μM -5-hydroxytryptamine resulted in 80% breakdown of radioactively labelled phosphatidylinositol over a 2 h incubation period (Fain & Berridge, 1979). The breakdown of labelled phosphatidylinositol was not increased by cyclic AMP or ionophore A23187 and the increase due to 5-hydroxytryptamine was unaffected by the absence of Ca^{2+} from the bathing medium (Fain & Berridge, 1979). Our results support the hypothesis of Michell *et al.* (1977) that the breakdown of phosphatidylinositol is somehow related to the agonist-dependent entry of Ca^{2+} into cells. Berridge & Fain (1979) found that prolonged stimulation of salivary glands with a high concentration of 5-hydroxytryptamine resulted in a marked decline in the rate of Ca^{2+} entry, which seemed to result from a fall in the concentration of phosphatidylinositol.

There was no restoration of 5-hydroxytryptamine-responsive Ca^{2+} flux if glands were allowed to recover for 1 h in control medium, but full recovery was observed when glands were incubated in the presence of 2 mM-inositol (Berridge & Fain, 1979).

The present experiments were designed to investigate how much phosphatidylinositol must be hydrolysed to inactivate Ca^{2+} entry. We have also studied the relationship between the amount of phosphatidylinositol formed during the recovery period and the restoration of 5-hydroxytryptamine-responsive Ca^{2+} flux.

Methods

The experimental procedures used in the present study were the same as those described previously (Fain & Berridge, 1979). The salivary glands were incubated in 50 μl of medium kept under liquid paraffin. In studies where 40 or more glands were used, a volume of 250 μl of medium per 40 glands was utilized and the glands were incubated in small plastic Petri dishes set on edge.

Phospholipids were separated by two-dimensional t.l.c. on glass plates (20 cm $\times$ 20 cm) coated with silica gel H (0.25 mm thick) containing magnesium acetate (1 g/40 g of silica gel). The plates were run in the first dimension for 1.5 h in chloroform/methanol/water/15 M-NH₃ (26:14:1:1, by vol.) and in the second dimension in chloroform/methanol/acetic acid/water (75:25:7:3, by vol.).

The phosphorus content of phospholipids was determined by the ultramicro-procedure of Bartlett

(1959). The absorbance was read at 830 nm in microcuvettes with a 2 cm light path in a Beckmann spectrophotometer. The biggest problem with this sensitive procedure is the presence of trace amounts of phosphorus as a contaminant in silica-gel scrapings. All values are corrected based on analysis of areas on the silica gel plates of the same size as those taken for each phospholipid. The blank areas were taken from the region just above the location of phosphatidylinositol. Phosphatidylinositol accounted for approx. 8% of the total phospholipid content (Fain & Berridge, 1979). To obtain sufficient amounts of phosphorus for analysis and detection of the phosphatidylinositol spot by I_2 vapour staining it was necessary to analyse approx. 40 salivary glands.

Radioactivity present in individual phospholipids was determined by counting a portion of the acid hydrolysate taken for phosphorus analysis (Table 1). In the studies shown in Table 2 the individual phospholipid spots were scraped from the thin-layer plates and the entire sample added to plastic miniscintillation vials containing 0.2 ml of 50% (v/v) methanol followed by 4 ml of Biofluor (New England Nuclear Corp., Boston, MA, U.S.A.). In the other studies utilizing $[2-^3H]$ inositol, $[^{14}C]$ ethanolamine or $[^{14}C]$ choline the synthesis of the respective phospholipids was determined by counting for radioactivity the entire chloroform layer obtained after extraction of the glands with chloroform/methanol (2:1, v/v) and separation of the phases (Fain & Berridge, 1979). Calculation of synthesis in pmoles was based on the incorporation of radioactivity and the specific radioactivity of the added precursor. No correction was made for possible dilution of these precursors with unlabelled inositol, choline or ethanolamine. Radioactivity was determined in

dual-channel liquid-scintillation counters with a counting efficiency for 3H in Biofluor of about 33%. In the dual-labelled experiments the counter was adjusted so that less than 0.1% of the 3H label appeared in the channel used for counting of ^{32}P radioactivity. About 6% of the ^{32}P label appeared in the channel used for counting 3H and this was subtracted from the values for 3H . The background was subtracted from all the values given in c.p.m.

For the Ca^{2+} -transport studies the salivary glands were placed in 50 μ l portions of medium under liquid paraffin and the cut ends of the isolated glands were ligatured with silk threads that pulled these ends out into liquid paraffin (Fain & Berridge, 1979). The medium surrounding the closed end of the glands contained $^{45}CaCl_2$ and the transepithelial flux of Ca^{2+} was determined by measuring the rate at which $^{45}Ca^{2+}$ appeared in the saliva (Berridge & Lipke, 1979).

Results

The breakdown of total and labelled phosphatidylinositol

5-Hydroxytryptamine had no significant effect on the total phosphatidylinositol content of salivary glands, whereas it caused a marked decrease in the amount of radioactively labelled phosphatidylinositol (Table 1). The phosphorus content of the phosphatidylinositol was determined by chemical analysis with 40 salivary glands for each experiment. The salivary glands were incubated with $[^{32}P]P_i$ before being exposed to 5-hydroxytryptamine to study the changes in both total and labelled phosphatidylinositol. Each salivary gland contains about 135 pmol of phosphatidylinositol, which does not change even

Table 1. *Effect of 5-hydroxytryptamine on breakdown of total and labelled phosphatidylinositol*

Salivary glands (80) were incubated for 2 h in 0.5 ml of buffer containing unlabelled 2 mM-inositol and a trace amount of $[^{32}P]P_i$. At the end of the first incubation the glands were washed five to six times in buffer without $[^{32}P]P_i$ over a period of 30 min. The glands were divided into half and then incubated for a further 2 h without or with 0.1 or 1.0 μ M-5-hydroxytryptamine. At the end of this incubation the glands were homogenized in 0.2 ml of ice-cold 7% $HClO_4$. In the two experiments with 1 μ M-5-hydroxytryptamine the amount of ^{32}P present as acid-soluble material in the supernatant obtained after centrifugation of the homogenates was approx. 12000 c.p.m./gland and was unaffected by 5-hydroxytryptamine. The precipitate from the homogenate was extracted with chloroform/methanol and the phosphatidylinositol separated by two-dimensional t.l.c. The amount of radioactive label found in phosphatidylinositol varied considerably between the individual experiments. Values in parentheses indicate the percentage decrease in the value for 5-hydroxytryptamine treatment compared with the corresponding control value.

Expt. no.	Phosphatidylinositol content (pmol/gland)			$[^{32}P]$ Phosphatidylinositol (c.p.m./gland)		
	Control	0.1 μ M-5-Hydroxytryptamine	1.0 μ M-5-Hydroxytryptamine	Control	0.1 μ M-5-Hydroxytryptamine	1.0 μ M-5-Hydroxytryptamine
1	180	195	—	322	230(29)	—
2	105	120	—	292	175(40)	—
3	185	—	161	7295	—	953(87)
4	48	—	94	3592	—	945(74)

after incubation with 5-hydroxytryptamine for 2 h. However, 5-hydroxytryptamine produced a marked decrease in the amount of labelled phosphatidylinositol (Table 1). These observations suggest that the newly made phosphatidylinositol equilibrated with a pool of radioactive label considerably smaller than the total pool.

In another series of studies we labelled the phosphatidylinositol of salivary glands totally by allowing larvae to grow in meat containing [³²P]P_i. After emergence from the pupae, the adults were maintained on a diet of sucrose and water. Salivary glands were removed and incubated with 20 μM-*myo*-[³H]-inositol for 15 min, which resulted in doubly labelled phosphatidylinositol. The glands were then incubated for 2 h with or without 10 μM-5-hydroxytryptamine. There was no significant drop in the [³²P]phosphatidylinositol under conditions in which there was an 80% decrease in the content of the pulse-labelled [³H]phosphatidylinositol (Table 2). There was no effect of 5-hydroxytryptamine on the breakdown of [³²P]phosphatidylethanolamine which was used as a control.

Phosphatidylinositol synthesis and the recovery of 5-hydroxytryptamine-responsive Ca²⁺ flux

After incubation of salivary glands for 2 h with 10 μM-5-hydroxytryptamine, the high rate of ⁴⁵Ca²⁺ transport initially seen after the addition of hormone returned to near basal values (Berridge & Fain, 1979). If glands were washed free of hormone and then incubated for 1 h in the presence of 2 mM-inositol, there was a recovery of 5-hydroxytryptamine-responsive ⁴⁵Ca²⁺ flux. There was no recovery in the absence of added inositol. The effect of varying the concentration of inositol in the medium during the 1 h recovery period after prior exposure for 2 h to 10 μM-5-hydroxytryptamine is shown in Table 3. The 2 h exposure to 10 μM-5-hydroxytryptamine impaired both fluid secretion and ⁴⁵Ca²⁺ flux, which did not recover after treatment with 2 μM-inositol. Increasing the inositol content from 2 to 20 μM gave a near maximal recovery of fluid secretion and approximately half-maximal recovery of ⁴⁵Ca²⁺ flux (Table 3). Full recovery of ⁴⁵Ca²⁺ flux was seen in the presence of 200 μM-inositol and was associated with

Table 2. Preferential breakdown of newly synthesized phosphatidylinositol by 5-hydroxytryptamine

Larvae were grown in the presence of [³²P]P_i, which was added to their diet. Adults were maintained after emergence from the pupal stage on sugar cubes (sucrose) and water. Approx. 1 week after emergence the two salivary glands from each fly were divided between the control and experimental groups and 13 glands in each group were incubated with 20 μM-*myo*-[2-³H]inositol for 15 min. The glands were then washed for 15 min and incubated for 2 h without or with 10 μM-5-hydroxytryptamine. Values in parentheses indicate the percentage change with 5-hydroxytryptamine treatment compared with the corresponding control value.

Expt. no.	[³ H]Phosphatidylinositol (c.p.m./gland)		[³² P]Phosphatidylinositol (c.p.m./gland)		[³² P]Phosphatidylethanolamine (c.p.m./gland)	
	Control	+10 μM-5-Hydroxytryptamine	Control	+10 μM-5-Hydroxytryptamine	Control	+10 μM-Hydroxytryptamine
1	6205	2130 (34)	151	170 (112)	180	170 (94)
2	13810	2070 (15)	180	238 (132)	276	238 (86)
3	3550	856 (24)	110	166 (150)	174	290 (166)
4	3230	338 (10)	138	105 (76)	200	200 (100)
Mean ± S.E.M.	6700	1348 (20 ± 6)	145	170 (117 ± 18)	207	224 (109 ± 20)

Table 3. Recovery of 5-hydroxytryptamine-responsive Ca²⁺ flux in the presence of inositol

Groups of three salivary glands were incubated for 2 h with 10 μM-5-hydroxytryptamine. The glands were washed free of 5-hydroxytryptamine and incubated for 1 h in medium containing 2–2000 μM-[2-³H]inositol. At the end of the 1 h incubation period, glands were removed and analysed for the incorporation of inositol into phosphatidylinositol. These data are means ± S.E.M. for six paired replications. Other sets of glands were incubated under the same conditions with unlabelled 2–2000 μM-inositol for 1 h. Then ⁴⁵Ca²⁺ flux and salivary secretion were measured over the next 20 min at 5 min intervals. The results shown for ⁴⁵Ca²⁺ flux and salivary secretion are means ± S.E.M. for three replicates over the 15–20 min period after restimulation with 10 μM-5-hydroxytryptamine.

Inositol concn. during 1 h recovery period (μM)	[³ H]Phosphatidylinositol synthesis (pmol/gland)	Salivary secretion in response to 10 μM-5-hydroxytryptamine (nl/gland per min)	⁴⁵ Ca ²⁺ flux in response to 10 μM-5-hydroxytryptamine (c.p.m./gland per min)
2	0.3 ± 0.1	7.5 ± 1.5	9 ± 1
20	1.7 ± 0.2	24.3 ± 3.7	53 ± 9
200	8.8 ± 1.5	31.0 ± 7.0	108 ± 28
2000	24.3 ± 7.7	24.7 ± 2.3	88 ± 8

the resynthesis of 8.8 pmol of [^3H]phosphatidylinositol. Increasing the concentration of inositol to 2000 μM resulted in the synthesis of 24.3 pmol of [^3H]phosphatidylinositol, but no greater increase in $^{45}\text{Ca}^{2+}$ flux was seen (Table 3). Since the total phosphatidylinositol content of a salivary gland is approx. 135 pmol (Table 1), the resynthesis of only a small amount of phosphatidylinositol (less than 6.5% of the total amount initially present in the glands) is sufficient to restore full sensitivity.

The time course of phosphatidylinositol synthesis in the presence of 200 μM -*myo*-[^3H]inositol is shown in Fig. 1. The glands were pre-exposed to 10 μM -5-hydroxytryptamine for 2 h and after washing out the 5-hydroxytryptamine the glands were incubated with 200 μM -*myo*-[2- ^3H]inositol for various times. There was virtually no lag before [^3H]inositol from the medium was incorporated into phosphatidylinositol and 6.8 pmol was formed during the 1 h incubation period (Fig. 1). As noted previously (Berridge & Fain 1979), the synthesis of labelled phosphatidylinositol was considerably less in control glands not pre-exposed to 5-hydroxytryptamine. In a parallel experiment we studied the time course for the recovery of 5-hydroxytryptamine-responsive $^{45}\text{Ca}^{2+}$ flux. The results of a typical experiment are shown in Fig. 2. The glands were incubated with 10 μM -5-hydroxytryptamine for 2 h and the rate of $^{45}\text{Ca}^{2+}$ flux was measured over the last 10 min to establish the degree of inactivation. After removing 5-hydroxytryptamine, the glands were incubated in

the absence or presence of 200 μM -inositol for 15 min before re-adding 10 μM -5-hydroxytryptamine (Fig. 2). The glands that had been incubated with 200 μM -inositol showed a large recovery of $^{45}\text{Ca}^{2+}$ flux. However, the recovery was transient and after a brief plateau the rate of $^{45}\text{Ca}^{2+}$ transport began to fall. Presumably the amount of phosphatidylinositol synthesized during the 15 min recovery period was able to sustain transport for a short time only. When glands recovered in 200 μM -inositol for 60 min, the rate of $^{45}\text{Ca}^{2+}$ transport remained at a high value (see Fig. 4).

The time course for the recovery of 5-hydroxytryptamine-responsive Ca^{2+} transport is shown in Fig. 3. The peak rate of $^{45}\text{Ca}^{2+}$ flux during the second 5-hydroxytryptamine treatment was subtracted from the rate before the recovery period to provide a comparative measure of the recovery of 5-hydroxytryptamine-responsive $^{45}\text{Ca}^{2+}$ flux. As noted previously (Berridge & Fain 1979), there was little recovery when glands were incubated in the absence of inositol (Fig. 3). In the presence of 200 μM -inositol, there was little recovery after 5 min, but by 15 min there was half-maximal recovery. Comparison of these results with those in Table 3 and Fig. 1 suggest that there may be a short lag period between the synthesis of phosphatidylinositol and the recovery of $^{45}\text{Ca}^{2+}$ flux. The synthesis of 1.7 pmol of phos-

Fig. 1. Time course of phosphatidylinositol synthesis during recovery from 5-hydroxytryptamine stimulation

Groups of three salivary glands were incubated for 2 h without (control) or with 10 μM -5-hydroxytryptamine. The glands were washed and incubated for 5, 15, 30 and 60 min with 200 μM -*myo*-[2- ^3H]inositol and analysed for incorporation of inositol into phosphatidylinositol ($\circ$). The values are means $\pm$ S.E.M. for four to six replicates. Synthesis of phosphatidylinositol was measured in control glands not previously exposed to 5-hydroxytryptamine only at 60 min ($\bullet$).

Fig. 2. Recovery of 5-hydroxytryptamine-responsive $^{45}\text{Ca}^{2+}$ flux

Groups of 3 salivary glands were incubated for 2 h with 10 μM -5-hydroxytryptamine (first open bar) and the rate of Ca^{2+} transport was measured over the last 10 min. The 5-hydroxytryptamine was washed away and the glands were incubated for 15 min in the presence ($\circ$) or absence ($\bullet$) of 200 μM -inositol (solid bar). After this 15 min recovery period the glands were again stimulated with 10 μM -5-hydroxytryptamine (second open bar) and the rate of $^{45}\text{Ca}^{2+}$ transport was measured at 3 min intervals for 15 min.

Fig. 3. Time course for recovery of 5-hydroxytryptamine-responsive $^{45}\text{Ca}^{2+}$ flux

The protocol for this experiment was identical with that described in the legend to Fig. 2, except that the glands were allowed to recover in the presence (○) or absence (●) of $200\text{ }\mu\text{M}$ -inositol for various times after a 2 h incubation with $10\text{ }\mu\text{M}$ -5-hydroxytryptamine. The recovery of $^{45}\text{Ca}^{2+}$ flux was determined by subtracting the average for the last two measurements for flux during the 2 h exposure to 5-hydroxytryptamine from the average for the flux measurements taken at 6 and 9 min after readdition of hormone. The values are means $\pm$ S.E.M. for three to five replicates.

phatidylinositol over 1 h (in the presence of $20\text{ }\mu\text{M}$ -inositol) gave a half-maximal recovery of $^{45}\text{Ca}^{2+}$ flux, whereas the synthesis of the same amount of phosphatidylinositol over 5 min in the presence of $200\text{ }\mu\text{M}$ -inositol (Fig. 1) did not have much effect on the recovery of $^{45}\text{Ca}^{2+}$ flux (Fig. 3). Comparison of Figs. 1 and 3 again reveals that the recovery of 5-hydroxytryptamine-responsive $^{45}\text{Ca}^{2+}$ flux requires the resynthesis of relatively small amounts of phosphatidylinositol.

The effects of other phospholipid precursors

Unlike inositol, neither choline nor ethanolamine was able to restore the 5-hydroxytryptamine-responsive $^{45}\text{Ca}^{2+}$ flux in glands that had been inactivated after prolonged stimulation with $10\text{ }\mu\text{M}$ -5-hydroxytryptamine. The results for choline are shown in Fig. 4 where the experimental protocol was similar to that used for inositol (Fig. 2). At the end of the 2 h incubation period in $10\text{ }\mu\text{M}$ -5-hydroxytryptamine, the rate of $^{45}\text{Ca}^{2+}$ transport had fallen

Fig. 4. Comparison of the effects of inositol and choline on the recovery of 5-hydroxytryptamine-responsive $^{45}\text{Ca}^{2+}$ flux

The protocol for this experiment was similar to that for the experiment shown in Fig. 2. At the end of the 2 h stimulation with $10\text{ }\mu\text{M}$ -5-hydroxytryptamine (first open bar) the rate of $^{45}\text{Ca}^{2+}$ transport was measured to establish the degree of inactivation. After this initial period of stimulation, the glands were washed free of hormone before being incubated in control medium alone (○), or in the presence of $685\text{ }\mu\text{M}$ -choline (●) or $200\text{ }\mu\text{M}$ -inositol (□). After this recovery period the glands were again stimulated with $10\text{ }\mu\text{M}$ -5-hydroxytryptamine (second open bar) and the rate of $^{45}\text{Ca}^{2+}$ transport was measured.

below $20\text{ c.p.m./gland per min}$. The 5-hydroxytryptamine was washed away and the glands were allowed to recover for 1 h in control medium (no phospholipid precursor) or in medium containing either $685\text{ }\mu\text{M}$ -choline or $200\text{ }\mu\text{M}$ -inositol. When the glands were retested at 180 min, there was almost no recovery of $^{45}\text{Ca}^{2+}$ transport in the control glands or in the glands treated with choline, whereas the glands treated with inositol showed a full recovery (Fig. 4). It is noteworthy that the glands that had recovered in inositol for 60 min did not show the fall off in Ca^{2+} transport seen with the glands that were incubated for only 15 min (Fig. 2). When glands that had been prestimulated with $10\text{ }\mu\text{M}$ -5-hydroxytryptamine were allowed to recover in the presence of $676\text{ }\mu\text{M}$ -[2- ^3H]-inositol, they synthesized much more phosphatidylinositol than the control glands that had not been stimulated (Table 4). Such prestimulation had no effect on the incorporation of labelled choline or labelled ethanolamine into their respective phospholipids (Table 4).

Table 4. *Phospholipid synthesis during recovery after prolonged stimulation with 5-hydroxytryptamine*

Groups of three salivary glands were incubated for 2h either in the presence or absence of $10\mu\text{M}$ -5-hydroxytryptamine. The glands were washed free of 5-hydroxytryptamine and incubated for 1h in medium containing $676\mu\text{M}$ -[2- ^3H]inositol, $685\mu\text{M}$ -[Me- ^{14}C]choline or $664\mu\text{M}$ -[2- ^{14}C]ethanolamine. At the end of the 1h incubation period the glands were washed and the incorporation of radioactive label into phospholipid was measured. The results are means $\pm$ S.E.M. for six paired replicates.

	Phospholipid synthesis (pmol/gland)	
	Control	Glands prestimulated for 2h in $10\mu\text{M}$ -5-hydroxytryptamine
Inositol	3.5 ± 0.4	11.3 ± 2.2
Choline	27.5 ± 2.7	22.0 ± 3.1
Ethanolamine	28.0 ± 2.4	31.5 ± 3.5

Discussion

The present results show that the phosphatidylinositol pool of salivary glands may not be uniform. Newly synthesized phosphatidylinositol seems to equilibrate with a small pool that is readily broken down after addition of 5-hydroxytryptamine. The ability of $10\mu\text{M}$ -5-hydroxytryptamine to deplete over 80% of the newly labelled phosphatidylinositol over a 2h period appears to result from equilibration of this label preferentially with a small hormone-responsive pool. It is difficult to estimate the size of this hormone-sensitive pool, but some indication of its size is apparent from the recovery experiments. Resynthesis of only 1.7pmol of phosphatidylinositol in a gland whose total content is about 135pmol was sufficient to give approx. 50% restoration of hormone-responsive $^{45}\text{Ca}^{2+}$ flux in glands previously desensitized by incubation with a high concentration of 5-hydroxytryptamine. Resynthesis of not more than 9pmol was sufficient to maximally restore Ca^{2+} flux. The hormone-sensitive pool of phosphatidylinositol may thus be a small fraction of the total pool.

The inactivation of Ca^{2+} flux during prolonged stimulation with high doses of 5-hydroxytryptamine may result from depletion of phosphatidylinositol in this small hormone-sensitive pool. The phosphatidylinositol content of this pool is expected to decline because 5-hydroxytryptamine not only stimulates the hydrolysis of phosphatidylinositol (Fain & Berridge, 1979), but it simultaneously inhibits the resynthesis of this phospholipid (Berridge & Fain, 1979). During prolonged stimulation, however, there was no evidence for any decrease in total phosphatidylinositol, whether measured by the decrease in phosphorus content (Table 1) or loss of radioactivity from uniformly labelled phospho-

tidylinositol (Table 2), which again suggests that the hormone-sensitive pool is a small fraction of the total pool of phosphatidylinositol. The lack of any effect of 5-hydroxytryptamine on the total phosphatidylinositol pool could also be explained if there is some other phosphorus-containing substance that is soluble in chloroform/methanol, which co-migrates with phosphatidylinositol and whose turnover is unaffected by 5-hydroxytryptamine. However, the most likely phospholipid contaminant is phosphatidylserine, which was readily separated from phosphatidylinositol in the chromatographic system by using silica gel impregnated with magnesium acetate. The inactivation of Ca^{2+} flux during prolonged stimulation may thus depend on the loss of phosphatidylinositol from the small hormone-sensitive pool.

The location of this hormone-sensitive pool has not been established, but is probably made up of the phosphatidylinositol positioned in the basal plasma membrane of the secretory cells, which normally faces the blood. All the 5-hydroxytryptamine receptors are presumably located on this basal plasma membrane, which constitutes a small fraction (5%) of the total plasma membrane. Most of the plasma membrane is located on the secretory side of the cells facing the lumen, where there are extensive infoldings forming secretory canaliculi (Oschman & Berridge, 1970). We postulate that newly synthesized phosphatidylinositol equilibrates with the small pool in the basal plasma membrane that contains the hormone receptors and is exposed to the blood. As yet, however, there is no direct evidence as to the site at which phosphatidylinositol is hydrolysed in the presence of hormone.

The failure to observe a decrease in total phosphatidylinositol content in the blowfly salivary gland under conditions in which 5-hydroxytryptamine caused an 80% breakdown of newly synthesized phosphatidylinositol is in contrast with the situation in some mammalian tissues. In the albatross salt gland and the mouse pancreas a high concentration of acetylcholine caused a 40% drop in total phosphatidylinositol and an increase in phosphatidic acid (Hokin-Neaverson, 1974, 1977). In the rat parotid gland Jones & Michell (1974) observed a 33% decrease in total phosphatidylinositol content after only 5min exposure to 1mM-acetylcholine. This occurs despite the finding that addition of muscarinic cholinergic agonists to the rat parotid gland results in an increase in incorporation of [^3P]P_i into phosphatidylinositol.

The rat parotid gland shows a different response from the blowfly salivary gland after incubation with [2- ^3H]inositol. Keryer & Rossignol (1978) washed parotid glands previously incubated for 2h with radioactively labelled inositol for 20min in buffer without inositol and then examined the fate

of labelled phosphatidylinositol over the next hour. The addition of carbamoylcholine increased the accumulation of labelled phosphatidylinositol at 20 min, but by 60 min the amount of labelled phosphatidylinositol had returned to control values. The finding of an increase rather than a decrease in labelled phosphatidylinositol is in striking contrast with what is seen in the salivary gland. Perhaps the newly made phosphatidylinositol in the rat parotid gland does not rapidly equilibrate with the pool that is broken down after the addition of hormones. The rapid increase in labelled phosphatidylinositol suggests that the intracellular pool of inositol is relatively large and the breakdown of unlabelled phosphatidylinositol results in a stimulation of phosphatidylinositol formation.

Studies on the hormonal regulation of phosphatidylinositol turnover in blowfly salivary glands appear to be facilitated by several unusual features that are not present in most tissues. The intracellular pool of inositol is quite small and rapidly equilibrates with inositol in the medium (Fain & Berridge, 1979). The newly made phosphatidylinositol, whether labelled with [^{32}P]P_i or [^3H]inositol, rapidly equilibrates with a small pool of phosphatidylinositol, which is hydrolysed by hormones. The third feature is that the synthesis of phosphatidylinositol is actually inhibited by 5-hydroxytryptamine (Berridge & Fain, 1979). Thus the blowfly salivary gland offers several unique features that facilitate studies on the

role of phosphatidylinositol breakdown in hormone-induced gating of Ca^{2+} . The present results suggest that only a small fraction of the total salivary gland phosphatidylinositol is involved in 5-hydroxytryptamine-induced Ca^{2+} gating.

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