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# SHORT COMMUNICATION

## Polyphosphoinositides in insect muscle and sensory tissues

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THE POLYPHOSPHOINOSITIDES, phosphatidyl inositol phosphate and bisphosphate, have been found as trace lipids in yeast (TALWALKAR & LESTER, 1973), protozoans (PALMER, 1973), and mammals (FOLCH, 1949). In mammals, these lipids are thought to be particularly enriched in neural tissue where they have been shown to undergo changes in metabolism with stimulation of the tissue (see MICHELL, 1975; HAWTHORNE & WHITE, 1975). This supported the suggestion that the polyphosphoinositides play a specific role in nerve impulse propagation (KAI & HAWTHORNE, 1969; HENDRICKSON & REINERTSEN, 1971). Their precise function, however, remains unclear and their occurrence in non-neural tissues and cells may point to other biological roles.

We have previously shown that polyphosphoinositides are present in inner ear tissues of the guinea pig, where their metabolism is affected by ototoxic drugs (ORSULAKOVA *et al.*, 1976). In order to study polyphosphoinositides in simple sensory systems, we investigated their occurrence in insects. We report here the presence of polyphosphoinositides in crickets and moths and their distribution in various tissues of the moth.

### METHODS

The Noctuid moth, *Agrotis ypsilon*, and the cricket, *Acheta domestica*, were selected for these experiments. Insects were briefly immobilized in ether fumes and pinned to a platform under a microscope. Five microliters of carrier-free radioactive isotope ([$^{32}$P]orthophosphate or myo-[$^{3}$H]inositol, New England Nuclear, Boston, MA) were injected into the thorax of the insect. For studies of the entire insect, the moth or cricket was killed at a given time after injection by homogenization in 5% trichloroacetic acid or chloroform–methanol (2:1, v/v) with a Polytron homogenizer (Brinkman Instruments, Westbury, NY). For studies of the various moth tissues, the moths were killed by injection of 10 $\mu$l of glutaraldehyde into the thorax followed by microwave irradiation. The tissues were dissected into chloroform–methanol (2:1, v/v) to which a homogenate of guinea-pig brain was added as a source of carrier lipids. The tissues were then homogenized in a glass–glass homogenizer.

Lipids were extracted into acidified chloroform–methanol and separated by TLC (SCHACHT, 1976). Insect lipids other than the polyphosphoinositides were identified by cochromatography with commerical standards (Supelco, Bellefonte, PA). Polyphosphoinositides were identified by co-chromatography with non-labeled and $^{3}$H-labeled polyphosphoinositides prepared from guinea-pig brain. $^{32}$P-phospholipids on thin-layer chromatographic plates were located by autoradiography on X-ray films, scraped into vials, and counted by liquid scintillation spectrometry.

For analysis of the polyphosphoinositides content of the moth, 17 moths were homogenized as described above. Phosphatidyl inositol phosphate and bisphosphate were separated from the total lipid extract and from each other by column chromatography on immobilized neomycin (SCHACHT, 1978). Lipid phosphorus was determined after ashing (AMES & DUBIN, 1960).

### RESULTS AND DISCUSSION

Administration of [$^{32}$P]orthophosphate to a moth or cricket leads to rapid labeling of the polyphosphoinositides. In the cricket, 30 min after injection of isotope, 50% of the lipid labeling appears in phosphatidyl inositol bisphosphate and 15% in phosphatidyl inositol phosphate (Table 1). The rapid labeling of the polyphosphoinositides can also be demonstrated in the moth (Fig. 1). These phospholipids have the greatest initial rate of labeling, while the other quantitatively major lipids, including phosphatidyl inositol, incorporate $^{32}$P more slowly. This pattern of labeling is generally observed with polyphosphoinositides in other tissues. It is thought that the rapid $^{32}$P-labeling of these lipids is primarily due to a turnover of their monoesterified phosphate groups via kinase and monoesterase reactions and not due to *de novo* synthesis. The incorporation of [$^{3}$H]inositol into the phosphoinositides is consistent with the notion that such a phosphorylation-dephosphorylation cycle is also responsible for the $^{32}$P-labeling of the polyphosphoinositides in the moth. Three hours after injection of 20 $\mu$Ci of [$^{3}$H]inositol into the moth, 300,000 c.p.m. appeared in phosphatidyl inositol, 60,000 c.p.m. in phosphatidyl inositol phosphate, and 5000 c.p.m. in phosphatidyl inositol bisphosphate.

The phospholipid content of the moth was also analyzed. There were approx 20 $\mu$mol of total phospholipid/g wet weight of a moth (a single moth weighs about 220 mg),

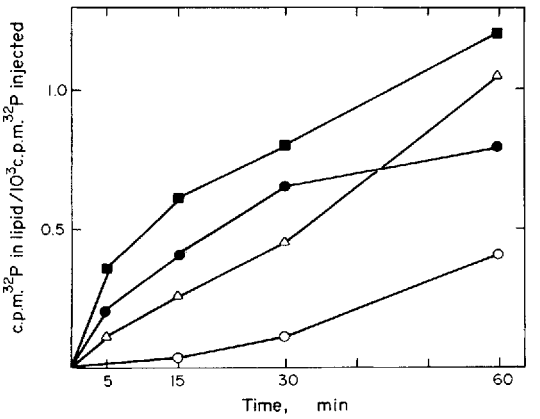


FIG. 1. Time course of $^{32}$P-incorporation into phospholipids of the moth. 100 $\mu$Ci $^{32}P_i$ (carrier-free) were injected into the thorax of a moth. Moths were killed and analyzed as described in Methods. Each point is the average of two experiments. ■, phosphatidyl inositol phosphate; ●, phosphatidyl inositol bisphosphate; △, phosphatidic acid, phosphatidyl inositol, and phosphatidyl serine; ○, phosphatidyl ethanolamine.

TABLE 1. POLYPHOSPHOINOSITIDE LABELING IN INSECTS

| | c.p.m. $^{32}$P-Phospholipid/insect (%of total labeled lipid) | |
|---|---|---|
| | Phosphatidyl inositol phosphate | Phosphatidyl inositol bisphosphate |
| Cricket* | 313,500 (15) | 1,023,000 (50) |
| | 296,300 (13) | 1,271,300 (55) |
| Moth tissues† | | |
| Antennae | 1000 (28) | 600 (17) |
| Ears‡ | 240 (25) | 480 (50) |
| Eyes | 460 (10) | 840 (19) |
| Proboscis | 380 (40) | 320 (34) |
| Flight muscle | 19,600 (15) | 44,700 (33) |

*56 $\mu$Ci $^{32}P_i$ (carrier-free) were injected into the thorax of a cricket. Insects were killed 30 min later and analyzed as described in Methods. Three crickets were pooled for each analysis, values are from two experiments.

†120 $\mu$Ci $^{32}P_i$ (carrier-free) were injected into the thorax of a moth. Moths were killed 15 min later and analyzed as described in Methods. Four sensory tissues were pooled for analysis, but data is expressed per insect. Values are from a single experiment, a second experiment gave similar results.

‡Scoloparium and nodular sclerite combined (KILIAN & SCHACHT, 1977).

35 nmol of phosphatidyl inositol phosphate, and 20 nmol of phosphatidyl inositol bisphosphate. Thus, polyphosphoinositides make up 0.3% of the total phospholipids in the moth.

Labeled polyphosphoinositides were further investigated in various tissues of the moth. They were found in all the sensory structures analyzed (eye, ear, antennae, and proboscis) and in flight muscle (Table 1). After 15 min of labeling, the polyphosphoinositides represent between 31% and 73% of total $^{32}$P-lipids in these tissues. The ratio of labeled phosphatidyl inositol phosphate to phosphatidyl inositol bisphosphate varies among the tissues, but this may not represent true differences because of the rapid post-mortem degradation of the polyphosphoinositides. The fixation conditions used here were optimal for analysis of the auditory organ and different fixation procedures may yield different labeling ratios for the other tissues. The remaining parts of the insect (head, thorax, and abdomen) contained the bulk of the $^{32}$P-polyphosphoinositides, but these segments were not further dissected.

To the best of our knowledge, this is the first time that polyphosphoinositides have been demonstrated in insects as well as in sensory structures other than the ear. Similarly, their presence in flight muscle is interesting, since polyphosphoinositides have not been detected in mammalian skeletal muscle (DAWSON & EICHBERG, 1965). They are present in smooth iris muscle (ABDEL-LATIF *et al.*, 1977).

In conclusion, polyphosphoinositides become rapidly labeled with $^{32}$P and are widely distributed in a variety of insect tissues. For studies of the physiological functions of the polyphosphoinositides, insects may be attractive models since their organs are often far simpler and more easily accessible than mammalian organs and experiments are subject to fewer restraints, such as anesthesia. Therefore, insects lend themselves well to experiments correlating biochemical and electrophysiological or behavioural data. We are currently investigating the role of polyphosphoinositides in auditory tissues in the moth.

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