# ARACHIDONIC ACID METABOLISM IN THE SALT SECRETING (RECTAL) GLAND OF SQUALUS ACANTHIAS: ROLE OF CYTOCHROME P-450 DEPENDENT METABOLISM

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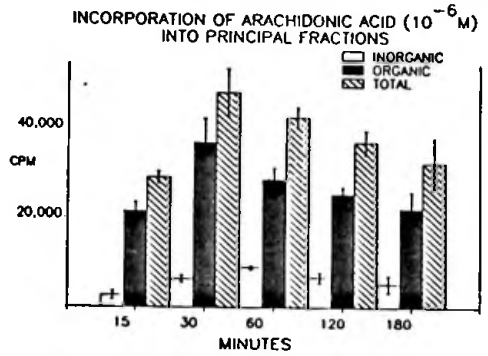
It is now well established that oxygenated metabolites of arachidonic acid serve as autocoids that modulate cellular responses in different organs to a wide array of humoral and neurohumoral stimuli. We have determined that the rectal gland secretes chloride actively and that this process is mediated by a cyclic AMP-dependent mechanism (Stoff et al., J Exp Zool 199:443-448, 1977). Our studies indicate that vasoactive intestinal peptide (VIP) stimulates chloride transport in the gland by a cyclic AMP dependent mechanism (Stoff et al., Am J Physiol 237: F138-144, 1979). We have also determined that this secretagogue is present in the plasma of the shark as well as contained in peptinergic fibers which innervate the gland (Stoff et al., Am J Physiol 255: R212-216, 1988). The present study is focused on the interaction between VIP action and the potential modulatory influence of arachidonic acid metabolism. To characterize this relationship, we investigated the incorporation of AA into cell lipids and the release of metabolic products after stimulation with the secretagogue VIP. In additional studies, the functional response to arachidonic acid and inhibitors of AA metabolism were studied in the isolated perfused rectal gland preparation.

Arachidonic acid metabolism was assessed by three different methods: 1) $^{3}$H-AA uptake and distribution into principal lipid fractions was determined in freshly prepared tissue slices of rectal gland. Glands were perfused for 5 min with dogfish Ringer solution (DRS) to remove blood, sliced into transverse slices (<0.5 mm) with a Stadie-Riggs microtome and incubated with AA for varying periods of time at two different substrate concentrations, $10^{-4}$ and $10^{-5}$ M. Lipids were extracted by organic solvents, separated by TLC, visualized by iodine vapor and quantitated by beta scintillation counting. 2) Intracellular lipid pools were labelled by $^{3}$H-AA at equilibrium conditions. The release of radioactive products into the medium and redistribution into lipid fractions were determined after stimulation with VIP, forskolin, and calcium ionophore. 3) Isolated rectal glands were perfused *in vitro* with VIP in the presence and absence of arachidonic acid and inhibitors of cyclooxygenase, lipoxygenase and epoxygenase (cytochrome P-450 NADPH-dependent monooxygenase) metabolism. Glands were perfused for a total of 90 min with DRS containing VIP ($3 \times 10^{-9}$M) and fatty acid free BSA (0.005-0.01%). Three, 30 minute clearance periods were calculated from 10 min collections and the results are expressed as µEq/h/gWW. Clearance periods I and III are VIP alone and period II is the experimental period containing VIP and the agent studied.

In the first series of experiments, the time course of $^{3}$H-AA uptake was measured. Uptake was initially determined into organic and nonorganic solvent extractable compartments. Uptake was rapid, reached a peak by 15 min and then declined slightly reflecting efflux of labelled substrate or metabolite (Figure 1). The organic phase accounted for five-fold more accumulation of radioactivity compared to the inorganic or aqueous phase. To further define the principal lipids which were labelled, the organic extract was fractionated by TLC. These studies demonstrate that the phospholipid compartment was the most dominant lipid and rapidly incorporated isotope with more than 25% of the label recovered in

this fraction within 15 min (Figure 2). Over the next 30 min there was increased activity in several compartments which comigrated with triglyceride standards. These different triglyceride fractions probably reflect hydrolysis products of triglycerides. After 3h of uptake more than 50% of the precursor label was found in triglycerides, suggesting that this class of lipid may provide an important storage form of metabolic energy in the rectal gland cell.



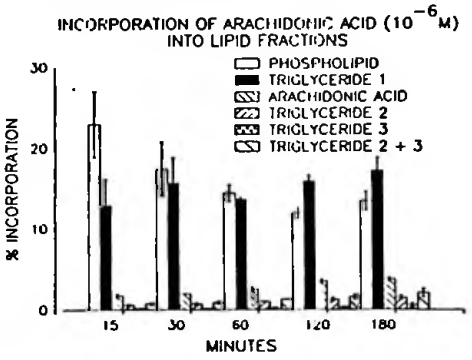




**FIGURE 1.** **FIGURE 2.**

Rectal gland slices were loaded with $^{3}$H-AA, $10^{-5}$ M for 60 min. Slices were washed free of extracellular $^{3}$H-AA and then incubated in the presence and absence of VIP $10^{-6}$ M for varying periods of time. Slices were removed and the media and cells were analyzed for radioactive products. VIP inhibited the release of radioactive products into the media and conversely there was an increase radioactivity recovered in the cell (Figure 3). These changes were noted within 2 min of exposure to hormone and persisted for more than 60 min. To further define this process, the cell lipid pools were characterized by TLC. VIP increased the accumulation of label into the phospholipid compartment while diminished activity was noted in the principal triglyceride fraction-1 (Figure 4). These results suggest activation of phospholipid metabolism and possible suppression of triglyceride synthesis or inhibition of triglyceride hydrolysis. No changes were seen in other lipid compartments including arachidonic acid, triglyceride 2 and 3, and cholesterol esters.

To characterize the signal mechanism which may mediate the VIP- induced inhibition of release of $^{3}$H-AA products, the effects of forskolin an activator of adenylate cyclase was studied. Forskolin ($10^{-5}$M) had no effect on the release of radiolabelled products. Since VIP action on ion transport may be mediated by intracellular calcium concentration, the calcium ionophore A23817 ($10^{-5}$M) was tested. No effect of the ionophore was found.

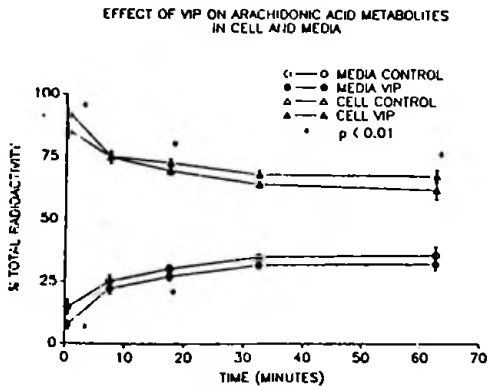



**FIGURE 3.**

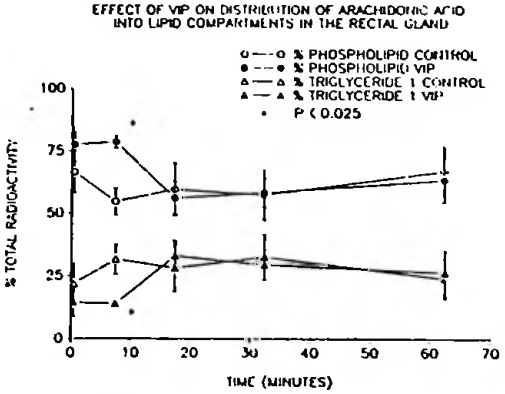



**FIGURE 4.**

Rectal glands were perfused with VIP in the presence and absence of AA and inhibitors of each of the principal metabolic pathways of conversion of AA to oxygenated metabolites were studied.

| EXPERIMENTAL CONDITION (n) | PERIOD I | PERIOD II | PERIOD III |
|---|---|---|---|
| ARACHIDONIC ACID ($10^{-4}$M) (9) | 1195±201 | 1130±259 | 1003±264 |
| INDOMETHACIN ($10^{-4}$M) (3) | 1581±384 | 1850±397 | 1134±263 |
| NDGA ($10^{-5}$M) (4) | 823±111 | 539±109' | 269±50' |
| KETOCONAZOLE ($10^{-5}$M) (6) | 1180±184 | 753±118' | 750±146' |

'll values are Mean±SEM and are expressed as µEq/h/gWW. VIP is present in all periods at 3 x $10^{-8}$M. Experimental agents are present only in Period II. * p < 0.01. Statistical significance was calculated by paired t-test.

VIP increased the chloride secretory rate 10-20 fold above usual basal levels of ≈50-100 µEq/h/gWW. AA had no consistent effect on the secretory response under the experimental conditions. Indomethacin, an inhibitor of the cyclooxygenase pathway stimulated $Cl^-$ secretion but did not reach significance, in part due to the small number of experiments. This effect was reversible as noted by the decline in period III. Nordihydroguaiaretic acid (NDGA), an inhibitor of the lipoxygenase pathway, and ketoconazole, an inhibitor of the epoxygenase pathway, inhibited the VIP response 35% and 36% respectively. Both these responses were irreversible.

These data indicate that the rectal gland incorporates AA into multiple lipid storage pools. Phospholipid incorporation is most active compared to triglyceride fractions and cholesterol esters. VIP alters this process by enhancing incorporation into the phospholipid compartment at the expense of triglycerides and also inhibits the release of radioactive products into the media. We are attempting to identify these products at the present time.
The lack of a functional effect of arachidonic acid on VIP stimulated $Cl^-$ secretion suggests that the release of endogenous AA from phospholipid storage pools is not rate limiting in the production of local autacoids. An alternative possibility is that AA decomposed during the perfusion experiments because of the high $O_2$ tension present in the perfusate. Additional studies will be necessary to investigate these possibilities.

The experiments with inhibitors of AA metabolism indicates that inhibition of cyclooxygenase is stimulatory while inhibition of the lipoxygenase and cytochrome P-450 NADPH dependent monooxygenase is inhibitory. These data indicate that these pathways are active in the rectal gland and produce products which modulate transport. The results are consistent with the possibility that products of the lipoxygenase and epoxygenase pathways generate stimulatory modulators of ion transport. In preliminary studies, we have found direct evidence for cytochrome P-450 NADPH activity in homogenates of the rectal gland. Further studies are required to directly measure these AA products and test their effects directly on hormone stimulated ion transport.

In conclusion, these studies demonstrate that AA is rapidly incorporated into cell lipid pools and that this process is regulated by VIP through a non-cyclic AMP mechanism. The enhanced turnover of the phospholipid compartment and stimulation by VIP suggests this may be an important target of VIP action, possibly through activation of phosphoinositol metabolism. Evidence is presented which supports the generation of lipoxygenase and epoxygenase metabolites of arachidonic acid which may stimulate chloride transport in the rectal gland. (These studies were supported in part by the NIEHS-SCOR to MDIBL and a BRSG from the University of Massachusetts Medical School to JSS.)