PALMITIC ACID OXIDATION BY SECRETING SUBMANDIBULAR GLAND TISSUE *IN VITRO*

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1. Introduction

The induction of secretion by secretagogues, such as epinephrine or acetylcholine, is accompanied by a marked increase in respiration in rat parotid [1] and submandibular [2] salivary glands. Blockage of respiration with inhibitors of oxidative phosphorylation prevents secretion [3] indicating that respiration-produced energy is required for proper secretion. The extract nature of the relationship between respiration and secretion is still speculative, however, it has been postulated that the "extra" energy derived from elevated respiration is used for secretory granule–plasma membrane interactions [4, 5].

While increased glucose oxidation has been reported for stimulated salivary gland slices [1, 2], neither normal nor increased fatty acid oxidation has been adequately demonstrated for such preparations, even though one group of investigators has suggested the possibility that much of the increased respiration during secretion could come from fatty acid oxidation [3]. Very recent studies in this laboratory have shown that isolated submandibular gland mitochondria readily oxidize palmitic acid [6], definitely demonstrating that this tissue can utilize fatty acids for energy production.

Studies of fatty acid involvement in secretion with tissue slice preparations are complicated by the fact that Ca^{2+} is required in the medium for adequate secretion [7, 8], yet Ca^{2+} is a potent precipitant of fatty acids. This problem was overcome in the present series of experiments by using a two stage incubation wherein the fatty acid was incorporated into the tissue before the addition of Ca^{2+} and stimulant.

The results indicate that palmitic acid can be oxidized to CO_2 by submandibular gland slices and that this oxidation rate is increased during secretion.

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2. Materials and methods

Radiochemicals were obtained from New England Nuclear (USA). Epinephrine bitartrate and Tes buffer were purchased from Calbiochem (USA).

2.1. *Preparation of 1-^{14}C-palmitate-labelled slices*

For each preparation 6 to 8 male rats of the Long–Evans strain were killed by decapitation and the submandibular glands quickly excised, cleaned and, after removal of the closely-adhering sublingual glands, placed in Ca^{2+} free Krebs–Ringer saline [9] containing 5 mM inosine [10] and buffered with 25 mM Tes (pH 7.4) at room temp. Glands (1.5–2 g) were then cut into 10–20 mg pieces with a razor blade and all transferred to 4 ml fresh buffer. 1-^{14}C-palmitate (in 5 μl acetone) was added to give an approx. conc. of 10^{-5} M and activity of 0.5–1.5 μCi. After thorough gassing with O_2, the flask was stoppered and incubated at 37°. Fig. 1 shows the disposition of the label among the lipids on continued incubation. For most oxidation studies a time period of 30 min was used to label the slices. At this period there was no measurable conversion of palmitic acid to any non-fatty acid materials except CO_2. The medium was decanted and the slices washed with several changes of buffer and re-incubated in 4 ml of buffer for 10 min. After a further

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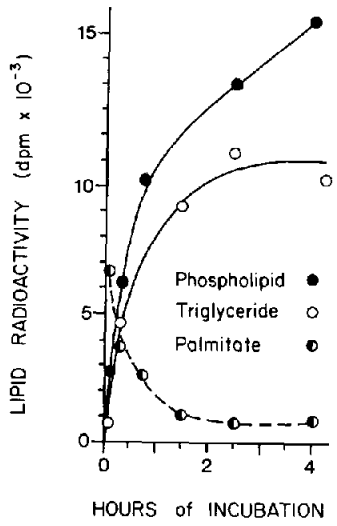

Fig. 1. Accumulation of radioactivity in lipids of submandibular gland slices during prolonged incubation in the presence of 1-^{14}C-palmitic acid.

washing, the slices were placed on a buffer-dampened filter paper contained in a petri dish. After gentle blotting, the tissue slices were divided into 100 (±3) mg portions, each of which was placed in 1 ml of Krebs–Ringer saline containing Ca^{2+} and buffered with Tes. Epinephrine was added as indicated in the text. Each vessel was then gassed with O_2, stoppered with serum stoppers fitted with disposable polypropylene centre wells (Kontes Glass Co., USA) and incubated at 37° for varying periods of time.

2.2. *Measurement of $^{14}CO_2$ production*

Reactions were terminated by injection of 0.4 ml 4 N $HClO_4$ into the medium followed by 0.3 ml hyamine hydroxide into the centre well. Incubation was then continued for 2 hr after which the centre wells were placed in scintillation vials containing 3 ml methanol and 10 ml of scintillation fluid was added (Liquifluor, Nuclear Chicago, USA). The measured radioactivity was corrected for quenching and adjusted to 100% efficiency.

2.3. *Measurements of the secretory response*

The extent of secretion and possible changes in response to epinephrine was measured in samples separate from those used for $^{14}CO_2$ collection because

Table 1
Secretion from submandibular gland slices.

Incubation conditions	NANA in medium (nmoles)	Protein in medium (μg)
No additions (controls)	18.8 ± 1.2	3240 ± 56
50 μM epinephrine added	35.5 ± 1.8	3780 ± 109

Slices (2–3 g) were incubated for 30 min at 37°, in Ca^{2+} free Krebs–Ringer saline buffered with 25 mM Tes pH 7.4, then rinsed several times with fresh media and re-incubated in 200 mg lots in 1 ml of fresh buffer containing Ca^{2+} for 2 hr. The medium was clarified by centrifugation and analyzed for *N*-acetylneuraminic acid (NANA), after hydrolysis, and for protein. Figures are mean ± S.E.

$HClO_4$ partially destroys both sialoprotein and lipid. At the termination of the final incubation the medium was decanted, centrifuged and the clear supernatant fluid used for the estimation of sialoprotein [11] and protein [12]. The tissue slices were rinsed with isotonic saline several times, then homogenized in 50 mM KCl and the lipids extracted [13]. The purified lipids were separated and analysed as reported previously [14, 15].

3. Results

Fig. 2 shows that palmitic acid was readily oxidized by submandibular gland slices and that added epinephrine markedly increased this oxidation. Table 2 indicates that fatty acid from both phospholipid and triglyceride, as well as the free fatty acid in the slice

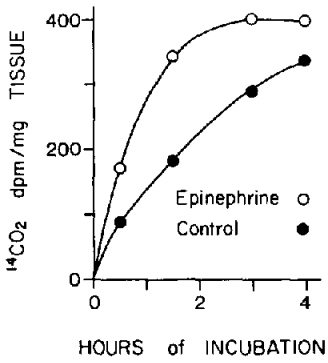

Fig. 2. Production of $^{14}CO_2$ from submandibular gland slices labelled for 30 min with 1-^{14}C-palmitic acid.

Table 2
Labelling of lipids in submandibular gland slices by 1-^{14}C-palmitic acid.

	dpm in lipid/mg fresh tissue		
	At end of 30 min pulse	At termination of second incubation (2 hr)	
		Control	+ 50 μM epinephrine
Triglycerides	624	321	229
Phospholipids	619	596	483

(cf. fig. 1), were utilized. As would be expected, triglycerides were the most affected by the lipolytic action of epinephrine [16]. Submandibular salivary gland has been shown to contain appreciable lipase activity [17] whose activation could be mediated through epinephrine.

The addition of unlabelled glucose (0.1–1.0 mM) to the palmitate oxidation system either during pulse labelling or during the final incubation had no measurable effect on $^{14}CO_2$ production (unreported results). However, ^{14}C-glucose oxidation was inhibited by pretreatment of slices with unlabelled palmitic acid (table 3) and this inhibitory effect appeared to be more pronounced on epinephrine-treated (secreting) slices.

4. Discussion

The present results demonstrate that submandibular gland can oxidize fatty acid for energy production and that this oxidation is increased during epinephrine-induced secretion. While glucose has little apparent influence on fatty acid oxidation, the presence of fatty acids appears to depress glucose oxidation especially during secretion. These findings strongly suggest that energy production from fatty acid oxidation is preferred by this tissue during secretion.

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Table 3
Effect of palmitic acid on $^{14}CO_2$ production from UL-^{14}C-glucose *in vitro*.

Type of slices	Additions	dpm in $^{14}CO_2$/mg fresh tissue/3 hr	
A	none	2261	(100)
A	50 μM epinephrine	3776	(167)
B	none	1873	(83)
B	50 μM epinephrine	2587	(114)

Conditions: 1–2 g fresh slices (each slice about 15 mg) were incubated for 10 min in Krebs–Ringer saline (no Ca^{2+}), rinsed several times with fresh buffer and then divided into two parts. A) Slices were incubated a further 10 min, then rinsed and transferred to fresh medium containing UL-^{14}C-glucose (approx. 2 μCi) and incubated 5 min. After thorough washing the slices were divided into 100 mg portions each of which was placed in 1 ml fresh buffer (+Ca^{2+}). After 3 hr of incubation, the released $^{14}CO_2$ was collected and measured. B) Identical to (A) except that the initial 10 min incubation medium contained 0.1 mM palmitic acid. Results are mean of 3 experiments. Parentheses indicate percent.

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