THE EFFECT OF ACETYLCHOLINE AND ATROPINE ON THE LABELING OF SMOOTH AND ROUGH MEMBRANES FROM OVINE SUBMAXILLARY GLANDS *

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Received 23 September 1969

1. Introduction

Secreted proteins, including glycoproteins, are believed to be synthetized in vesicular membrane structures of the rough endoplasmic reticulum and subsequently transported and extruded via a complex mechanism including several energy linked steps and possibly involving a continuous restructuration of rough and smooth membrane. This process has been mainly studied on the pancreas [1–4] but also on glycoprotein secreting cells, for instance plasmocytes [5, 6] and goblet cells [7]. In a previous paper [8] concerning the mucin secreting ovine submaxillary gland, rough and smooth membranes (including the Golgibodies) have been separated and fractionated and the time course dependent labeling of the mucin synthetized, as well as of the membranes themselves, has been studied.

On the other hand it is known that acetylcholine greatly enhances the rate of phospholipid labeling in a variety of secreting tissues including the pancreas [9, for early references see 10] and salivary glands [11, 12]. The aim of the present work is to provide preliminary data concerning the labeling of discrete rough and smooth membrane fractions (and of their phospholipids) from ovine submaxillary gland and to study the effect of both acetylcholine and atropine on this phenomenon.

* This work is part of a Doctoral Thesis (Doctorat ès-Sciences) to be submitted by one of the authors (B.R.) in the near future.

2. Materials and methods

Ovine submaxillary glands were dissected immediately after the animals have been slaughtered (we are indebted to the CNRZ, Jouy-en-Josas, for this material), kept at 0°, sliced and incubated according to the procedure previously described [8, 13] within one hour after the death of the animal. After 90 min incubation in the presence of various radioactive precursors, the slices were collected, homogenized at 0–4° with an ultraturrax homogenizer [8] and a first fractionation was performed by centrifuging (1000 *g*, 0°) a procedure which eliminates unfractionated cell material, nuclei, and a considerable proportion of the endoplasmic reticulum which in this tissue cannot be easily disrupted. A second centrifugation at 105.000 *g* yields microsomes which are still contaminated by mitochondria [8]; this fraction (referred to as "total microsomes") is processed as follows: (a). fractionation of total microsomes on a discontinuous sucrose gradient (1.11M; 0.957M; 0.636M; 0.335M) [14] yields three discrete fractions of smooth membranes, and a sediment containing rough membranes and mitochondrial material [8]; (b) total lipids (including phospholipids) are extracted either from the total microsomes or from the various rough and smooth fractions according to a slight modification of the method of Garbus [15]: to account for the presence of high concentrations of sucrose, a ratio $CH_3OH/CHCl_3$ of 3/1 (v/v) has been adopted instead of the 2/1 (v/v) ratio of the original procedure. Preliminary data, using silica gel chromatography, showed that under these conditions 80% of the radioactivity incorporated from ^{14}C-glucose were

Table 1
Microsomal radioactivity from ^{14}C-glucose

	Expt.	Control	Acetylcholine 2 X 10^{-3}M Eserine 10^{-4}M	Acetylcholine 2 X 10^{-3}M Atropine 10^{-4}M Eserine 10^{-4}M
Microsomal fraction (protein content mg)	1	20	22	19
	2	22	25	-
Total microsomal fraction radioactivity (cpm)	1	265.000	275.000	283.000
	2	321.000	186.000	-
Radioactivity (cpm per mg protein)	1	13.250	12.500	14.900
	2	14.600	11.450	-

Table 2
Distribution of radioactivity from total microsomes after lipid extraction by $CHCl_3/CH_3OH$ (1 : 3). Precursor ^{14}C-glucose. For details of the extraction procedure, see text.

		Control	Acetylcholine 10^{-3}M Eserine 10^{-4} M	Acetylcholine 2 X 10^{-3}M Atropine 10^{-4}M Eserine 10^{-4}M
Aqueous phase	cpm	6.600	7.100	7.850
	% stimulation	-	+ 7.5	+ 19
Organic phase	cpm	12.900	28.500	21.700
	% stimulation	-	+ 120	+ 63
Precipitate	cpm	113.000	109.000	122.000
	% stimulation	-	−3.5	+ 8

Table 3
Specific radioactivity of phospholipids from rough and smooth membranes. Precursor ^{14}C-glucose.

	Rough membranes		Smooth membranes	
	Control	Acetylcholine 2 X 10^{-3}M Eserine 10^{-4}M	Control	Acetylcholine 2 X 10^{-3}M Eserine 10^{-4}M
cpm per mg phospholipid	2.900	3.560	728	1.230
% stimulation	-	+ 23	-	+ 69

Table 4
Microsomal radioactivity from ^{32}P-phosphate.

	Expt.	Control	Acetylcholine 2×10^{-3}M Eserine 10^{-4}M	Acetylcholine 2×10^{-3}M Atropine 10^{-4}M Eserine 10^{-4}M
Microsomal protein (mg)	1	13,65	14,3	12,70
	2	9	10	-
Radioactivity of fraction (cpm)	1	79.500	145.000	89.500
	2	30.400	50.500	-
Radioactivity cpm per mg protein	1	5.830	10.150	7.050
	2	3.380	5.050	-

Table 5
Specific radioactivity of phospholids from rough and smooth membranes (precursor ^{32}P-phosphate). For details of the fractionation procedure, see text.

	Expt.	Rough membranes			Smooth membranes		
		Control	Acetylcholine 2×10^{-3}M Eserine 10^{-4}M	Acetylcholine 2×10^{-3}M Atropine 10^{-4}M Eserine 10^{-4}M	Control	Acetylcholine 2×10^{-3}M Eserine 10^{-4}M	Acetylcholine 2×10^{-3}M Atropine 10^{-4}M Eserine 10^{-4}M
cpm per mg phospholipid	1	5.450	10.600	6.650	4.000	-	4.150
	2	2.720	5.160	-	1.360	3.000	-
	3	11.700	23.500	-	9.750	18.000	-
% Stimulation	1	-	+ 94	+ 22	-	-	+ 4
	2	-	+ 90	-	-	+ 120	-
	3	-	+ 101	-	-	+ 85	-

located in phospholipidic material. Proteins have been determined by the method of Lowry et al. [16]; total phosphate according to Fiske and Subbarrow [18] or Berenblum and Chain [18]. The radioactivity of the samples were counted on a Nuclear Chicago Model C 115 low background counter. The radioactive precursors used were U-^{14}C-glucose and $^{32}P(NaH_2PO_4)$ both from CEA, Saclay, France*.

* We are indebted to the CEA for participating financially in the expenses involved in the purchase of the radioactive compounds.

3. Results

3.1. *Labeling from ^{14}C-glucose*

Table 1 shows that when ^{14}C-glucose is used as a percursor, heavy labeling occurs in the total microsomes. No stimulation of the labeling was found in the presence of acetylcholine + eserine nor in the presence of acetylcholine + eserine + atropine. It has been shown in a previous paper [8] that under these conditions a considerable percentage of the radioactivity was incorporated into submaxillary mucoprotein, glycogen and a fraction which dialized after laurysulfate treatment and contained phospholipids. Table 2 refers to 50% of the microsomal material

from experiment 1 (table 1) and shows that when total lipids are extracted by the modified Garbus procedure from this material a considerable stimulation of the labeling of lipidic material in the presence of acetylcholine occurs in the organic phase, whereas the stimulation in the aqueous phase is inconspicuous and no stimulation occurs in the precipitate (mostly denatured proteins and glycogen). Atropine, when incubated simultaneously with acetylcholine inhibits its effect to a considerable extent. A fraction of the material from experiment 2 (table 1) has been refractionated in order to separate rough and smooth membranes and the lipids have been extracted from both fractions according to the procedure described above. Table 3 shows that the labeling of smooth membrane lipid appears to be stimulated by acetylcholine to a greater extent than the labeling of lipids within the rough membranes.

3.2. *Labeling from ^{32}P-phosphate*

Contrasting with what has been found concerning the use of ^{14}C-glucose as a precursor, the labeling of total microsomes from ^{32}P-phosphate appears to be strikingly stimulated by acetylcholine even on the total microsomes without any further extraction or fractionation procedure as shown by table 4. Atropine nearly suppresses the stimulation. According to preliminary experiments, the greater part of this phosphate is incorporated into phospholipids. Table 5 shows that in this case the percentage stimulation of phospholipid labeling is nearly equal in smooth and rough membranes and that atropine acts in the same way on both fractions. Finally fig. 1 shows the detailed labeling of the four discrete zones obtained when fractionating total microsomes by the discontinuous gradient mentioned above. As found previously [8] one of these zones (referred to on the figure as zone 1) seems to have a very low turnover rate and is almost unlabeled.

4. Discussion

The results reported in the present paper are in complete agreement with what has been observed for other tissues or in other species [9, 10]. It must be mentioned however that Eichberg and Karnovsky [19] working on the same material found that neither the glucose and oxygen consumption, nor the glycoprotein secretion were stimulated by acetylcholine. On the other hand stimulation of incorporation of label from ^{14}C-glucose into phospholipidic material has to our knowledge never been reported previously. Hokin et al. [9, 20] reported high turnover rates for phosphatidic acids and phosphatidylinositols in membrane material from the pancreas. Hence it may be postulated that ^{14}C-label from glucose might be located mainly in glycerol and inositol but this

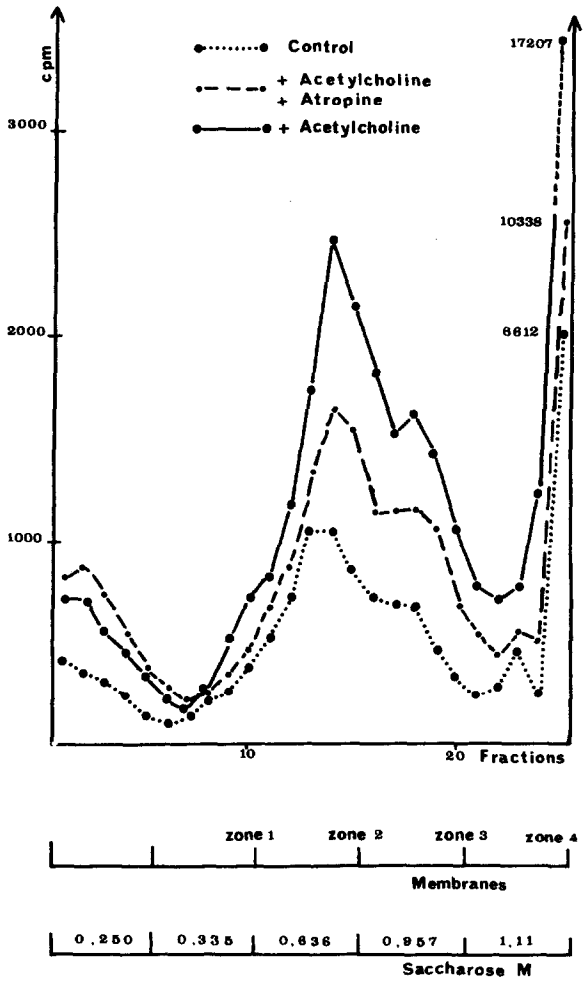

Fig. 1. The three other zones viz zone 2 and 3 (smooth membranes) and 4 (rough membranes) are all heavily labeled by phosphate; stimulation of this labeling by acetylcholine and inhibition of the stimulation by atropine occur in these zones.

hypothesis remains to be confirmed. In addition two points deserve special attention with reference to the results described above: first it may be noticed that specific radioactivity of phospholipids deriving from either of the two radioactive precursors is always significantly higher in the rough than in the smooth membranes. Anologous results have been mentioned by Glauman and Dallner [21] but it is not known presently if they reflect a higher turnover rate of the rough membranes or a precursor product relationship between the two fractions. Secondly it must be mentioned that in a recent work Hokin [9] found that the response of pigeon pancreas slices to acetylcholine was twofold. At low doses (10^{-7}M) protein secretion was enhanced and phospholipid turnover stimulated; at high doses (10^{-4}M) no further increase of protein excretion excretion was observed whereas the stimulation of phospholipid biosynthesis exhibited a continuous increase to very high rates indeed. In the present work high levels of acetylcholine have been employed and it remains to be seen if the twofold effect of acetylcholine on pigeon pancreas, which has been discussed by Hokin [9] applies to the submaxillary gland material as well.

Acknowledgements

We are greatly indebted to Doctor F.Meyer for his stimulating suggestions at the onset of this work.

The present experiments have been performed thanks to a financial support from the C.N.R.S. (Era no. 33).

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