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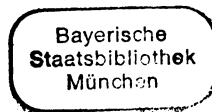
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ASYMMETRY OF ACETYLCHOLINESTERASE AND ACETYLCHOLINE RECEPTOR IN INTACT SECRETORY VESICLES FROM ADRENAL MEDULLA

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ABSTRACT

Highly purified secretory vesicles from adrenal medulla, isolated by differential and density gradient centrifugation using isotonic gradient material (PercollTM), contain acetylcholinesterase. The enzyme was latent in isolated secretory vesicles i.e. acetylcholinesterase was inaccessible to added substrate. The enzyme activity became patent after addition of detergent or in hypotonic media. Hypotonic treatment or specific lysis of the vesicles with Mg^{2+}/ATP in the presence of a permeant anion resulted in the release of soluble acetylcholinesterase from the vesicular content. Membrane-bound enzyme sedimented with the membranes. Binding of α -bungarotoxin could only be observed when secretory vesicles were lysed.

It is concluded, that the acetylcholine receptor as well as the membrane-bound form of acetylcholinesterase are localized on the inner surface of the secretory vesicle membrane, which becomes the outer surface of the cell membrane during exocytosis. Concomitantly the soluble form of acetylcholinesterase present within secretory vesicles is released into the extracellular fluid.

KEYWORDS

Acetylcholinesterase; acetylcholine receptor; secretory vesicles; adrenal medulla.

INTRODUCTION

During exocytosis secretory vesicle contents are released into the extracellular fluid and their limiting membranes are incorporated into the cell membrane. In this way the inside of the secretory vesicle membrane becomes the outside of the cell membrane. The existence of cell membrane components on the inner surface of secretory vesicle membranes would be consistent with a biogenetic relationship between the secretory vesicle and the cell membrane. In this report we describe evidence for the location of such cell membrane components, the acetylcholinesterase and the acetylcholine receptor of the chromaffin cell, within secretory vesicles.

RESULTS AND DISCUSSION

Secretory vesicles from adrenal medulla, isolated by differential cen-

trifugation, were further purified on a continuous self generating Percoll™ gradient. An osmolality of 420 mosmol/kg was maintained throughout the procedure. In this way highly purified secretory vesicles were obtained that leaked intravesicular adrenalin only slowly (Gratzl, Krieger-Brauer and Ekerdt, 1981). The vesicles were found to contain acetylcholinesterase with a specific activity of about 14 nmoles substrate hydrolyzed per min and mg protein (=total activity, determined in the presence of 0.2% Triton X 100). With no detergent added and in media of an osmolality of 420 mosmol/kg enzyme activity amounted only to about 4% of the total activity (Fig. 1).

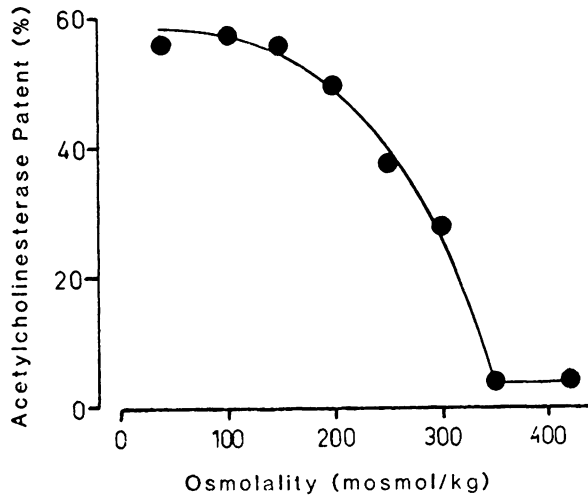


Fig. 1. Latency of acetylcholinesterase of isolated secretory vesicles. The vesicles were incubated for 5 min at room temperature in sucrose media of different osmolalities. The enzyme activity was determined without detergent added or with 0.2% Triton X 100 present (100%).

Reduction of the osmolality results in an increase of the enzyme activity. Below 200 mosmol/kg, an osmolality at which the vesicles have depleted all their intravesicular adrenalin (Gratzl, 1980), no further increase in enzyme activity could be detected (Fig. 1). During hypotonic treatment (200 mosmol/kg) of secretory vesicles about one third of the total acetylcholinesterase was released into the supernatant (Table 1), while the remaining activity sedimented with the membranes. Obviously a membrane-bound pool of acetylcholinesterase exists within secretory vesicles along with the soluble pool which could also be released from the vesicles by 2 mM Mg^{2+} /ATP in the presence of KCl, but not in the presence of sucrose (Table 1). Lysis of secretory vesicles as a consequence of an ATP driven proton pump in the presence of a permeant anion has been described earlier (Casey and Colleagues, 1976). Recently we have detected, that the membrane-bound form of acetylcholinesterase can be distinguished from the soluble enzyme by its electrophoretic mobility (Gratzl, Krieger-Brauer and Ekerdt, 1981).

Table 1 RELEASE OF ACETYLCHOLINESTERASE FROM ISOLATED SECRETORY VESICLES

Medium	Osmolality (mosmol/kg)	Acetylcholinesterase in Supernatant (%)
Sucrose	420	3.3
KCl	420	4.7
Sucrose, Mg ²⁺ /ATP	420	2.6
KCl, Mg ²⁺ /ATP	420	23.6
Sucrose	200	32.6

Isolated secretory vesicles were incubated for 30 min at 37°C in 20 mM Mops, pH 7.0, 1 mM EGTA and sucrose or KCl to obtain the final osmolality as indicated. The concentration of Mg²⁺ and ATP was 2 mM. The mixtures were centrifuged for 10 min at 130000 g_{av} in a Beckman Airfuge™ and the enzyme activity was determined in the supernatant. Total enzyme activity (=100%) was determined in the presence of 0.2% Triton X 100.

The experiments described so far show, that secretory vesicles from adrenal medulla contain two types of acetylcholinesterase, both sequestered by the vesicular membrane from the surrounding medium. One type is membrane-bound, but faces the intravesicular space, the other type is part of the vesicular content. In the following we provide evidence, that also the acetylcholine receptor is arranged in an asymmetric way in the secretory vesicle membrane, namely on the inner surface. α -bungarotoxin did not bind to intact secretory vesicles. However, when the vesicles were lysed in the presence of the venom, specific binding was observed (Fig.2).

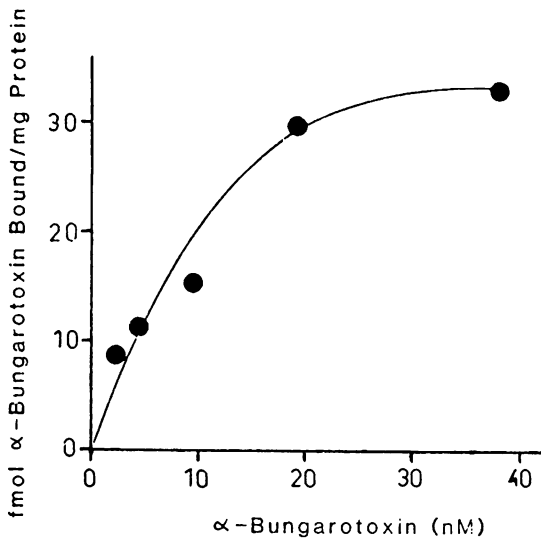


Fig. 2 Binding of [³H] α -bungarotoxin to secretory vesicles.

Isolated secretory vesicles were lyzed hypotonically (final osmolality 170 mosmol/kg) in the presence of different concentrations of [^3H] α -bungarotoxin or [^3H] α -bungarotoxin plus a constant amount of unlabelled α -bungarotoxin ($5 \times 10^{-7}\text{M}$). Incubation was continued for 45 min at room temperature. Membranes were separated from the medium in a sucrose step gradient. The radioactivity of the material recovered from the gradient was determined by liquid scintillation counting. Specific binding was obtained from the difference between the amount of [^3H] α -bungarotoxin bound in the absence and the presence of unlabelled α -bungarotoxin.

At the concentration of 40 nM specific binding of α -bungarotoxin was approximately saturated and the number of binding sites was 33 fmole/mg membrane protein. Saturation of α -bungarotoxin binding sites of cell membranes isolated from adrenal medulla was observed at 4 nM and the number of sites was 190 fmole/mg membrane protein (Wilson and Kirshner, 1977).

Concerning the distribution and the transport of acetylcholinesterase and acetylcholine receptor, interesting parallel observations have been made with chromaffin cells as well as with myoblasts. Both types of cells in culture release soluble acetylcholinesterase into the medium (Rotundo and Famborough, 1980a, Rotundo and Famborough, 1980, Mizobe and Livett, 1980). In myoblasts, newly formed acetylcholinesterase and acetylcholine receptors were found localized within intracellular organelles. Although acetylcholinesterase is secreted in a Ca^{2+} -dependent manner by stimulated adrenal glands, the mechanism of this process remained obscure (Chubb and Smith, 1975a, Chubb and Smith, 1975b, Somogyi and Colleagus, 1975). Since it has been shown now, that acetylcholinesterase (as well as acetylcholine receptor) is present within adrenal medullary secretory vesicles, an exocytotic pathway is likely to occur.

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