

ELECTRON MICROSCOPIC CYTOCHEMISTRY FOR DEMONSTRATION OF SODIUM FLUORIDE SENSITIVE ADENYL CYCLASE IN NORMAL HUMAN EPIDERMIS

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Abstract. Adenyl cyclase activity in normal human epidermis was detected by an electron microscopic cytochemical technique. The sodium fluoride sensitive receptors were demonstrated on the outer sheath of the cell membranes. Adenyl cyclase activity was demonstrated in the basal cells and in the 4-5 lower layers of the Malpighian cells, while the superficial layers, stratum granulosum and stratum corneum showed no activity. The findings confirm and extend previous biochemical studies and provide a clue to the conception of the adenyl cyclase enzyme as a transmembrane arranged system.

Key words: Adenyl cyclase; EM cytochemistry; NaF-activation; Hormonal action

Hormonal regulation of cellular metabolic processes mediated by the nucleotide, cyclic adenosine 3',5'-monophosphate (cAMP)—the second messenger function of cAMP—has been intensively investigated during the last decade, and accumulating evidence indicates that cyclic nucleotides play a major role in controlling cellular metabolic pathways including cell proliferation and differentiation (8, 10). cAMP is formed intracellularly by hormonal stimulation of the enzyme adenyl cyclase (AC), which catalyses the conversion of adenosine triphosphate (ATP) to cAMP and pyrophosphate (PPi). The adenyl cyclase enzyme system is believed to consist of two subunits, a hormone-sensitive receptor subunit and a catalytic subunit, the latter being activated by sodium fluoride (NaF) (7).

The presence of AC enzyme systems activated by various hormones has been established in most tissues, including skin, by biochemical methods using homogenized tissues and cell fractions (14). These methods made determination of total AC activity possible but were unsuitable for demonstra-

tion of the location of AC, both in respect to distribution between different cell types present in the tissue and the subcellular location.

The aim of this study was to demonstrate the exact location of AC in normal human epidermis using a method previously applied to rat nephrons (9), rat liver tissue (6), islets of Langerhans (3) and fungus (12).

MATERIAL AND METHOD

We adopted the method of Reik et al. (6), originally used in liver tissue experiments. The basis of the cytochemical reaction was the precipitation of a heavy lead salt (Pb^{2+})

Table I. *The technique used for fixation, incubation and counterstaining*

	S.m.	Pb^{2+}	ATP	NaF	Lead precipitation
Control 1	+	+	—	—	No
Control 2	+	+	+	—	No
AC activation with NaF	+	+	+	+	Yes

Fixation: 1.5% glutaraldehyde in Veronal acetate buffer, pH 7.4, with 220 mM sucrose for 1 hour at 4°C

Incubation:

S.M.: 80 mM Tris (hydroxymethyl) aminomethane (Tris maleate buffer, pH 7.4; sucrose, 220 mM; Mg sulfate 7 H₂O 4 mM; theophylline, 2 mM

Pb^{2+} : Lead nitrate, 4.8 mM

ATP: Adenosine triphosphate disodium salt, 0.5 mM

NaF: Sodium fluoride, 12.5 mM

At 30°C for 30 minutes.

Postfixation: 1% osmic acid

Embedding: Epon 812 after LUFT

Counterstain: 4% uranyl acetate



Fig. 1. Electron micrograph depicting adenyl cyclase activity as electron-opaque precipitates on the epidermal cell

membranes (arrows). The outline arrow indicates the area of higher magnification shown in Fig. 2. $\times 8250$.

of P_i produced from ATP by the action of adenyl cyclase. These electron-opaque precipitations are easily visualized by the electron microscope.

Punch biopsies, 4 mm in diameter, were obtained from clinically normal skin of two healthy volunteers. After fixation of the biopsies for 60 min at 4°C in 1.5% glutaraldehyde with 220 mM sucrose, the specimens were cut into slices, 0.1–1.0 mm thick. The fixation proceeded for half an hour in the same solution. The slices were then washed thoroughly with Veronal acetate buffer (pH 7.4) and 220 mM sucrose for 2 hours at 4°C. Before incubation,

a brief wash (10 min) with 80 mM Tris hydroxymethyl-aminomethane (Tris) maleate buffer (pH 7.4) with 220 mM sucrose was interposed. The incubation was performed at 30°C for 30 min. The incubation media are shown in the table (Table I).

Incubation was stopped by washing in Tris maleate buffer (pH 7.4) and 7.5% sucrose at 4°C for 10 min. After osmification, the slices were embedded in Epon 812.

Ultrathin sections were counterstained in a 4% aqueous solution of uranyl acetate and then studied under a Siemens electron microscope (Elmiskop 1A).

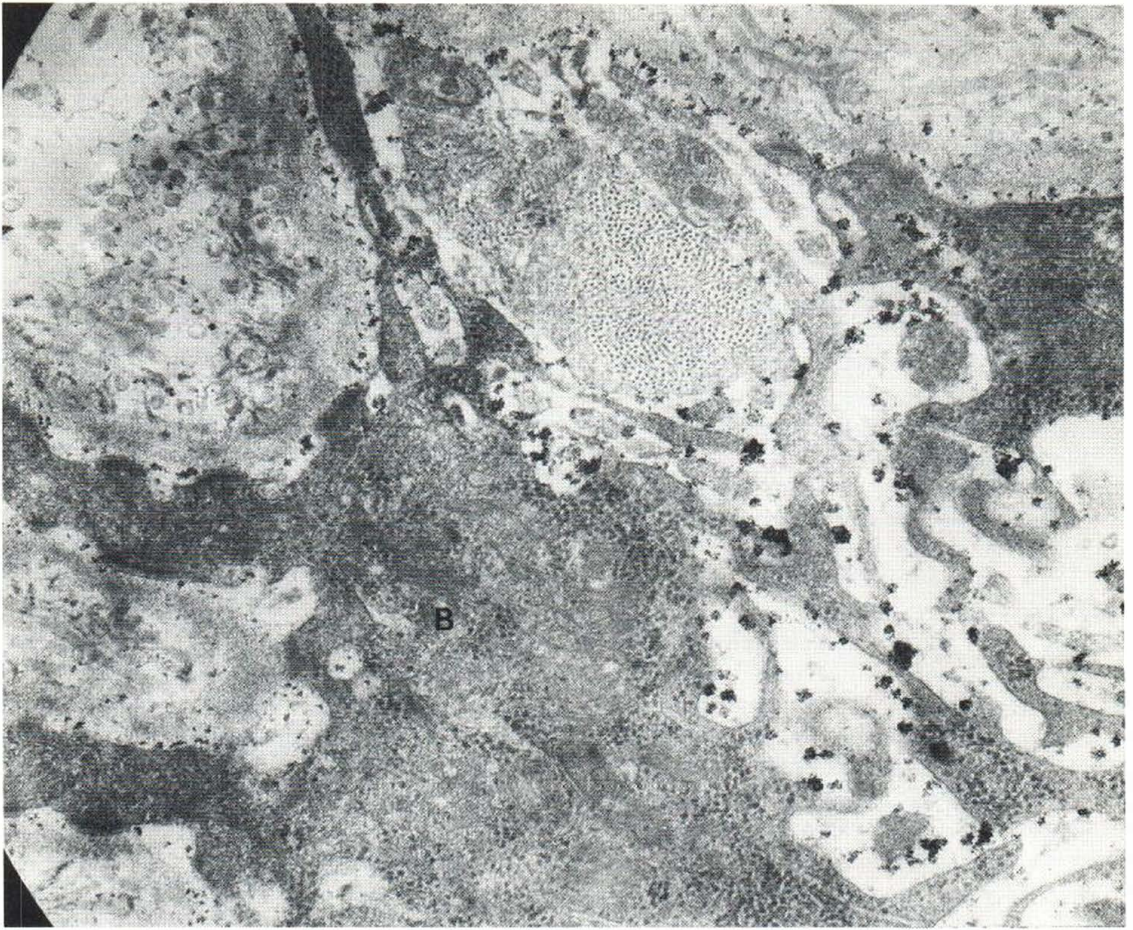


Fig. 2. A higher magnification of an area of Fig. 1, showing the lead precipitates on the cell membranes of epidermal basal cells (B). $\times 45\,000$.

RESULTS

NaF-stimulated AC activity was electron microscopically visualized as dense granular precipitates of lead-PPi complexes.

The precipitates were always located on the outer sheath of the cell membranes and in the extracellular spaces close to these membranes. Occasionally, some epidermal cells contained precipitates connected with mitochondria; otherwise no lead complexes were found inside the cells.

Adenyl cyclase activity was demonstrated on the basal cells and in the 4–5 lower layers of the Malpighian cells after stimulation (Figs. 1, 2). No activity was observed in the superficial layers of the squamous cells or in the stratum granulosum and

stratum corneum. In the stained areas the reaction products were not spread uniformly, but appeared in foci of epidermal cells with a dense distribution of lead granules on cells in the centre of the foci and fewer granules on cells in the peripheral zones of these foci. Unidentified dendritic cells with precipitates were also found occasionally.

In the experiments, the lead precipitates on the epidermal cell membranes could be demonstrated only in the presence of NaF and ATP in the standard medium (Table 1). When the skin was incubated in the lead (Pb^{2+}) containing standard, no reactions were observed (control 1). When both lead and ATP were included (control 2), an electron-dense reaction product was occasionally seen

in relation to mitochondria, and, furthermore, isolated precipitates were demonstrated as an inconstant finding under these conditions (control 2). The distribution throughout the slice, however, was random and differed markedly in location and density from the reactions present when NaF was included.

DISCUSSION

Understanding of the mechanisms of hormonal action in skin requires a knowledge of the precise location of the enzyme AC in the cutaneous tissue. Previously, adenylyl cyclase has been demonstrated in skin by biochemical methods (13). Hormonal stimulation of AC has rendered it possible to detect cAMP formation, but has failed to determine the location of AC and the type(s) of cell(s) from which cAMP was derived.

To establish the subcellular location of AC, various techniques have been used to demonstrate that AC activity is located predominantly on the cell membrane (1, 4, 16). Our findings of the AC activity located on the outer cell membrane thus confirm and extend these previous studies and provide a clue to the conception of the adenylyl cyclase system as a transmembrane arrangement mediating information from the extracellular environment to the intracellular compartments.

The intracellular precipitates, occasionally found close to mitochondria, were denser when AC activator (e.g. NaF) was supplemented to the incubation medium than was the case in controls. Since the method applied is semiquantitative the apparently denser reaction products in relation to mitochondria after stimulation cannot be considered specific for AC, even though a few investigations have revealed AC activity in intracellular structures including mitochondria (5, 11). As in Reik et al.'s study on liver tissue, the lead precipitates on epidermal cells were found only on the outer sheaths of the cell membrane or in the adjacent extracellular space. As suggested by Reik (6), this may indicate that AC is releasing its two products (PPi and cAMP) to the opposite surface of the plasma membrane.

The presence of various receptors has been established in epidermis. Our method visualizes the AC activity by demonstrating that lead precipitates are located on cells in the lower 4–5 cell layers of normal human epidermis when stimulated with NaF. Furthermore, the present findings demon-

strate the absence of AC in the superficial Malpighian cells, in stratum granulosum and stratum corneum, which indicates loss of receptors when basal cells are transformed to keratinocytes. This may further implicate a decrease in hormonal ability to influence the metabolic pathways in maturing keratinocytes.

The reasons for the focal, cluster-like appearance of enzyme activity in epidermis with varying density of lead precipitates in groups of epidermal cells are unexplained. The epidermal cells with dense granular staining did not differ from those with scant precipitates in structural characteristics. Hypothetically, a relation to the mitotic cell cycle could be suggested, since the appearance and disappearance of hormonal receptors on cell surfaces are phenomena known to occur (2, 15). The theoretical possibility may exist that, immediately after cell division, epidermal cells possess a maximum number of receptors (the strongly stained cells by electron microscopy), thus ensuring high intracellular levels of cAMP. As the mitotic cell cycle proceeds, a gradual decrease in numbers of receptors takes place (the cells stained slightly by electron microscopy), leading to a gradual decrease in the intracellular level of cAMP, thereby bringing the influence of cAMP to bear on cell division. In this connection it is worth noting that an apparent decrease in the number of epidermal adrenergic beta-receptors has been demonstrated in psoriasis, a disorder with increased epidermal cell turnover (17). However, although the suggested relations between focal staining and mitotic cycle may be a useful working hypothesis, an exact interpretation must await further elucidation.

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