Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Dario Cremaschi, Silvio Hénin, Marina Calvi

Action of Posthypophyseal hormones on Cholesterol Content of the Mucosal Plasma Membrane in Toad Urinary Bladder

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **49** (1970), n.5, p. 323–328.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1970_8_49_5_323_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1970.

Fisiologia. — *Action of Posthypophyseal hormones on Cholesterol Content of the Mucosal Plasma Membrane in Toad Urinary Bladder* (*). Nota di DARIO CREMASCHI, SILVIO HÉNIN e MARINA CALVI, presentata (**) dal Socio Corrisp. V. CAPRARO.

RIASSUNTO. — Gli ormoni postipofisari incrementano la permeabilità all'acqua, urea e Na^+ della membrana plasmatica mucosale nell'epitelio di vescica urinaria di rospo. Scopo di questo lavoro è di portare alcune dimostrazioni riguardo a una interferenza del colesterolo in questa azione. Si è incubata la vescica con una soluzione di Ringer–colesterolo radioattivo (—1T, 1.2 T, —4 ^{14}C) e si è misurata la velocità dello scarico di radioattività dalla membrana. Il trattamento ormonale incrementa la velocità di scarico in funzione della dose e l'effetto sullo scarico sembra correlato con l'effetto sulla permeabilità osmotica dell'acqua.

INTRODUCTION

Posthypophyseal hormones are known to enhance water, urea and Na^+ permeability of the mucosal barrier in the epithelial cells of Toad bladder and of other tissues [1], [2], [3], [4], [5], [6]. This effect seems to be due to an adenylcyclase stimulation and to an increase in 3′, 5′ cyclic AMP cellular pool [7], [8], [9]. Many hypotheses have been advanced to explain the hormonal action on the membrane.

In 1958 Ginetzinsky suggested that a hyaluronidase secretion and its action on mucopolysaccharides of intercellular cement are the permeability enhancing factors in the kidney [10]. However, Leaf did not find any hyaluronidase action on Toad bladder [2]. In 1965 Bentley suggested a Ca^{++} displacement by the hormones from the mucosal membrane [11]. Rosenbloom pointed out the possible presence of a lecithin-lisolecithin cycle near the membrane affected by the hormones [12]. Leaf suggested the transformation of water from an ice-like structure to a liquid one [13].

Recently cholesterol relevance in the structure and permeability of artificial and natural membranes has been emphasized by many authors [14], [15], [16], [17], [18]. Particularly, Finkelstein observed a correlation between cholesterol and water permeability [19]. On this basis we suggest here a possible interference of cholesterol with the mechanism of action of posthypophyseal hormones.

METHODS

Urinary bladders of female Toads (*Bufo bufo*) excised from the pithed animal were used.

In a first set of experiments the two half bladders were opened and the tissue layer was put between two lucite chambers and perfused with Ringer

(*) Lavoro eseguito nell'Istituto di Fisiologia generale dell'Università di Milano.

(**) Nella seduta del 14 novembre 1970.

solution bubbled with air at room temperature (25–27° C). The apparent exposed area was 0.63 cm^2· 10 μCi/ml of cholesterol —1 T (10.4 Ci/mM, Radiochemical Centre) or —1.2 T (47 Ci/mM, NEN) or —4 ^{14}C (30 Ci/mM, Sorin) in benzene solution mixed with Ringer fluid to a steady solution were present in the perfusion fluid of the mucosal chamber (10 μl of benzene/ml of Ringer solution). After a 4 hour incubation period serosal and mucosal perfusion fluids were removed and renewed with cholesterol-free Ringer solution. This operation was repeated every 20 minutes and the discharge rate of the radioactivity from the tissue was measured throughout 10 periods. Pituitrin Park and Davies (50 mU/ml) was present in the serosal fluid of one of the two half bladders in IV, V, VI, VII periods.

Potential differences after incubation and treatment were measured in several experiments to control a possible benzene toxicity.

In a second set of experiments the control hemibladder was not opened, but cannulated and perfused in Ringer solution. After 1 hour equilibration

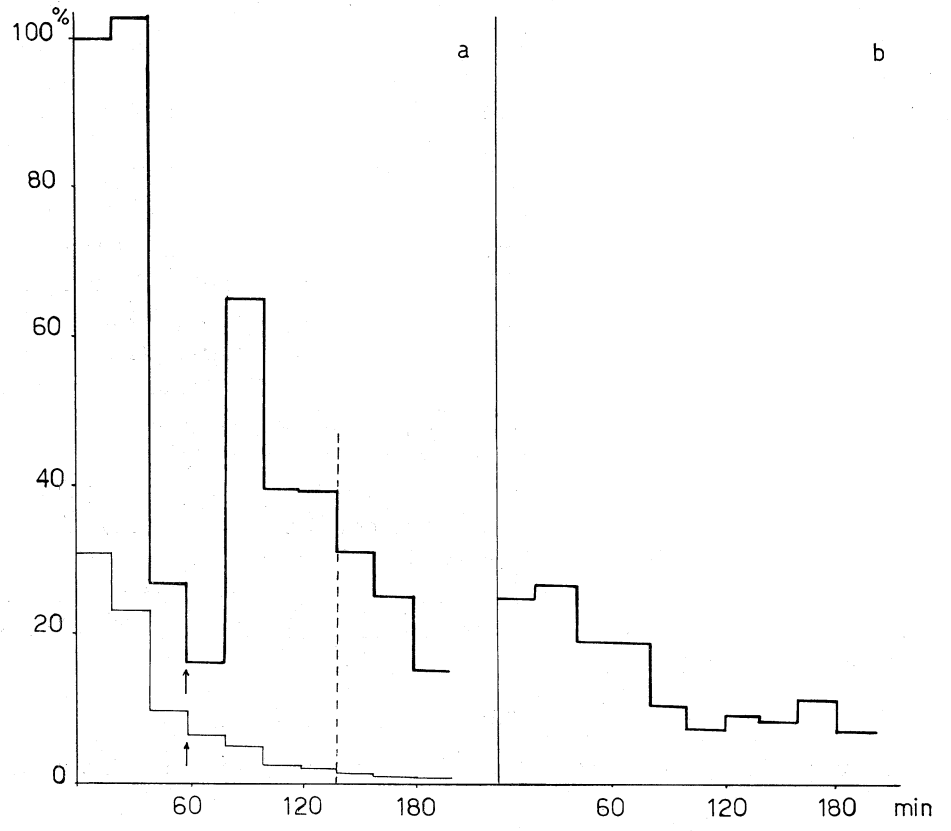

Fig. 1. – Radioactivity discharge from the tissue after a 4 hour incubation period. Ordinata: the discharge is reported in per cent and the first 20 minute period mucosal discharge of hemibladder a) is taken as a reference value. Abscissa: time in minutes.

a) ——— mucosal radioactivity discharge; ——— serosal radioactivity discharge. The arrow stands for the beginning of treatment (50 mU/ml) and the dashed line for the end. *b)* Control hemibladder: mucosal discharge.

the mucosal fluid was changed and replaced by a 1 : 10 diluted Ringer solution. Net water transport was measured under this osmotic gradient by a precision blance every 10 minutes without and with Pituitrin 50 mU/ml in the serosal medium. The transport rate was expressed in $\mu l\ cm^{-2}\ h^{-1}$ considering the bladder as a sphere. The ratio between the maximum of transport during treatment and the transport before treatment was determined.

The Ringer solution used in all the experiments had the following composition: NaCl 110 mM, KCl 2 mM, $CaCl_2$ 2.5 mM, Na_2HPO_4 3.7 mM, NaH_2PO_4 1.7 mM, pH = 7. Radioactivity was measured by a Tri-Carb scintillation spectrometer (Packard 3003). Experiments were carried out in May and June.

RESULTS

Fig. 1 reports one of the fifteen experiments carried out. The tissue was incubated with cholesterol — 4 ^{14}C. The discharge of the radioactivity from the mucosal and serosal sides of the first hemibladder is shown in *a*).

TABLE I.

Transepithelial electric potential in every hemibladder before and after hormonal treatment (50 mU/ml).

The two hemibladders have been incubated in Ringer cholesterol over 4 hours.

EXP. N.	Transepithelial electric potential (mV)	
	before treatment	after treatment
1 *a*	4,0	4,9
1 *b*	1,7	—
2 *a*	5,2	8,8
2 *b*	2,0	—
3 *a*	3,2	3,4
3 *b*	5,8	—
4 *a*	3.5	4.7
4 *b*	3,0	—
5 *a*	16.7	—
5 *b*	14.7	—

A strong increase in the mucosal discharge is evident after hormonal treatment (see the arrow), whereas the serosal one seems to be unaffected. The mucosal discharge in the control hemibladder (*b*) shows no significant kinetic modification. Table I reports that a transepithelial electric potential difference

of several mV is always detectable after incubation in Ringer-cholesterol benzene solution. The potential increases after treatment.

The mucosal discharge of a bladder first treated with 50 mU/ml (first arrow) and after with 100 mU/ml (second arrow) is shown in fig. 2. A higher discharge is remarkable with 100 mU/ml.

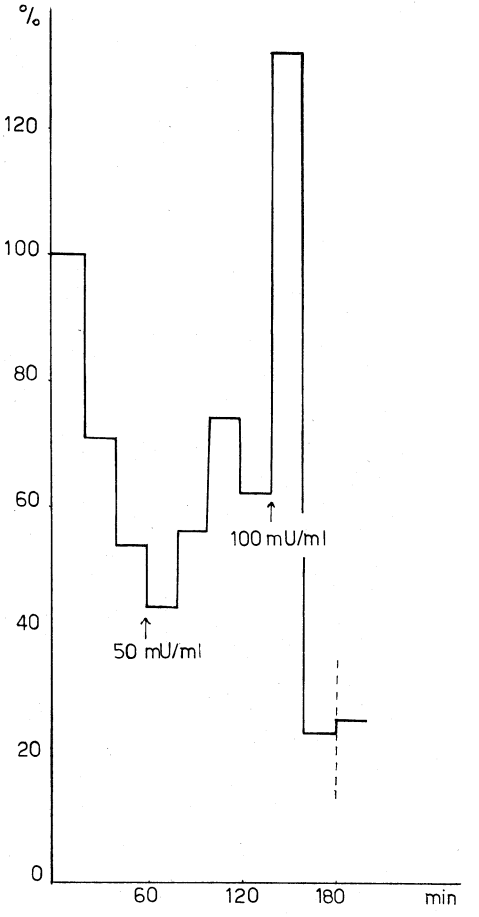

Fig. 2. – Radioactivity mucosal discharge (ordinata). Hormonal treatment with 50 mU/ml and 100 mU/ml (see the arrows). The dashed line shows the end of treatment. Discharge is reported in per cent and the first 20 minute period discharge is taken as a reference value. Abscissa: time in minutes.

The values of net water osmotic fluxes of the 5 experiments in which this measure was carried out are given in fig. 3 *a*. The diagram reports the flux before, the maximal flux during and the restored basal flux after hormonal treatment. Correspondent mucosal discharges of the radioactivity in related hemibladders are shown in fig. 3 *b* as in fig. 1. A good correlation between hormonal effect on water flux and hormonal effect on discharge is focused.

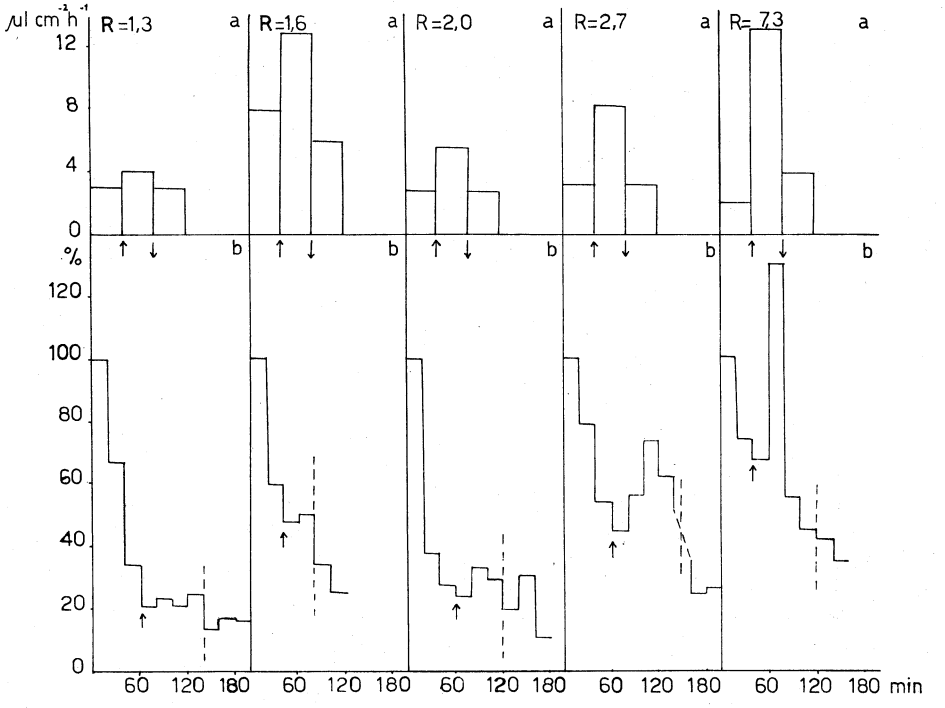

Fig. 3. – Osmotic water fluxes (*a*) in the first hemibladder and radioactivity discharge (*b*) in the second hemibladder. The arrows stand for the beginning of treatment (50 mU/ml) and the dashed lines or the inverted arrows for the end. R is the ratio between the maximal osmotic water flux during treatment and the basal one before treatment. Abscissa: time in minutes (for *b* only).

DISCUSSION

In the incubation period labelled cholesterol is likely to distribute itself in many cell types and cellular compartments of the tissue. Since only the mucosal barrier of the absorbing cells seems to be affected by hormonal action, modifications in mucosal discharge after treatment should be related to specific modifications of this barrier turn-over. Fig. 1 shows such a modification and supports an interference of hormonal action with the membrane cholesterol. It is noteworthy that hormonal treatment causes only alterations in the mucosal discharge kinetics of the radioactivity, whereas the serosal discharge is unaffected. The mucosal discharge is unmodified in control hemibladder.

As benzene (10 µl/ml) is present in the incubation solution, potential measurements to control the functionality of the preparation were carried out after the incubation period and, in the treated hemibladders, after hormonal treatment. Table I shows that potential differences of some mV are present and they increase after treatment. These results support a good activity of Na^+ pump in spite of benzene presence.

Since higher effects would be obtained with higher hormonal concentrations, discharge modifications were tested with 50 mU/ml and 100 mU/ml. In fact, fig. 2 shows a larger discharge increasing hormonal concentration.

Finally a good correlation of the hormonal action on mucosal discharge of radioactivity and on net water osmotic flux is observed in fig. 3.

All reported results support a hormonal action on cholesterol of mucosal plasma membrane. Conversely, they do not point out whether cholesterol is only displaced by hormonal transmitters or is chemically destroyed.

We are carrying out chromatographies of the labelled discharged substance to elucidate this fundamental point.

REFERENCES

[1] P. J. BENTLEY, « J. Endocrinol. », *17*, 201 (1958).
[2] A. LEAF, « J. Gen. Physiol. », *43*, suppl. 1, 175 (1960).
[3] A. LEAF and R. M. HAYS, « J. Gen. Physiol. », *45*, 921 (1962).
[4] R. H. MAFFLY, R. M. HAYS, E. LAMDIN and A. LEAF, « J. Clin. Invest. », *39*, 630 (1960).
[5] J. ANDERSON and R. W. S. TOMHNSON, « J. Physiol. », *177*, 133 (1965).
[6] P. J. BENTLEY, « J. Endocrinol. », *21*, 161 (1960).
[7] J. S. HANDLER, R. W. BUTCHER, E. W. SUTHERLAND and J. ORLOFF, « J. Biol. Chem. », *240*, 4524 (1965).
[8] J. ORLOFF and J. S. HANDLER, « J. Clin. Invest. », *41*, 702 (1962).
[9] J. ORLOFF and J. S. HANDLER, « Am. J. Med. », *36*, 686 (1964).
[10] A. G. GINETZINSKY, « Nature », *182*, 1218 (1958).
[11] P. J. BENTLEY, « Life Sciences », *4*, 133 (1965).
[12] A. A. ROSENBLOOM and P. ELSBACH, « Am. J. Physiol. », *214*, 161 (1968).
[13] R. M. HAYS and A. LEAF, « J. Gen. Physiol. », *45*, 905 (1962).
[14] J. S. O'BRIEN, « J. Theoret. Biol. », *15*, 307 (1967).
[15] C. LIPPE, « J. Mol. Biol. », *39*, 669 (1969).
[16] J. DE GIER, J. C. MANDERSLOOT and L. L. M. VAN DEENEN, « Bioch. Biophys. Acta », *150*, 666 (1968).
[17] K. R. BRUCKDORFER, R. A. DEMEL, J. DE GIER and L. L. M. VAN DEENEN, « Bioch. Biophys. Acta », *183*, 334 (1969).
[18] R. A. DEMEL, S. C. KINSKY, C. B. KINSKY and L. L. M. VAN DEENEN, « Bioch. Biophys. Acta », *150*, 655 (1968).
[19] A. FINKELSTEIN and A. CASS, « Nature », *216*, 717 (1967).