Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Dario Cremaschi, Silvio Hénin, Marina Calvi

Inhibition of NaCl-$NaHCO_3$, pump by high levels of Na^+ salts in Rabbit Gall Bladder epithelial cells

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **50** (1971), n.2, p. 216–220.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1971_8_50_2_216_0>

Fisiologia. — *Inhibition of* $NaCl—NaHCO_3$ *pump by high levels of* Na^+ *salts in Rabbit Gall Bladder epithelial cells* (*). Nota di DARIO CREMASCHI, SILVIO HÉNIN e MARINA CALVI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — L'Anfotericina B permeabilizza ai sali di Na^+ la membrana plasmatica mucosale delle cellule epiteliali in cistifellea di coniglio. Inibisce inoltre il trasporto netto isosmotico di acqua dal lume alla serosa dell'organo. Dagli esperimenti riportati risulta che la permeabilizzazione della membrana determina un innalzamento del livello dei sali di Na^+ in cellula e l'eccesso di tali sali causa l'inibizione della pompa $NaCl—NaHCO_3$ e conseguentemente del trasporto di acqua.

INTRODUCTION

Diamond [1] has reported that isosmotic net water transport, in " in vitro " rabbit gall bladder, attains its maximal value between 125 and 250 mOsm. of the perfusion fluids. Larger osmolarities cause an inhibition. Therefore the transport is reduced in gall bladder perfused by normal Krebs solution (290 mOsm.). As fluid transport is isosmotic in the whole osmolarity range, its modifications seem to be due not to water permeability variations, but to a changed $NaCl—NaHCO_3$ pump activity. Similar data have been obtained by other Authors [2].

Under these experimental conditions two parameters are simultaneously varied, i.e. osmolarity and NaCl concentration, and it is difficult to discriminate between the effects of the former and the latter.

In previous papers [3, 4] we have reported that Amphotericin B increases ionic permeability of mucosal plasma membrane in the epithelial cells and consequently endocellular level of Na^+ salts. Hence this antibiotic can be used as a tool to detect, during perfusion with normal Krebs solution, with constant extracellular osmolarity, the effect of the modified intracellular Na^+ salt concentration on the pump.

This paper reports the results of such experiments.

METHODS

Experiments were carried out on gall bladder of conventional rabbits, excised from the animal and washed free of the bile with bicarbonate Krebs–Henseleit solution (NaCl 118 mM; $NaHCO_3$ 24,9 mM; KCl 4,7 mM; $CaCl_2$ 2,5 mM; $MgSO_4$ 1,2 mM; KH_2PO_4 1,2 mM).

(*) Lavoro eseguito nell'Istituto di Fisiologia Generale dell'Università di Milano, con finanziamento da parte del Ministero della Pubblica Istruzione – Roma (art. 286 T.U. 31/81933).

(**) Nella seduta del 20 febbraio 1971.

In the first set of experiments the organ was opened and the tissue layer was put between two lucite chambers, each filled with 6 ml of Krebs perfusion fluids, bubbled with 95 % O_2 + 5 % CO_2, pH 7,4, at 27° C.

Electrical resistance was measured by passing a constant direct current through the tissue by two Ag—AgCl electrodes (connected with a power supply in series with a galvanometer) and measuring the constant new value of potential difference (p. d.) across the tissue. Ag—AgCl electrodes were fixed at 35 mm constant distance from each other. P. d. measures were carried out with calomel electrodes connected with a Keithley 155 microvoltmeter. The salt bridges used (saturated KCl bridges in agar 3 %) were fixed at 6 mm constant distance from each other approaching the middle of the tissue; they were in contact with perfusion fluids only during the measure time. The solution resistance between the bridges in the absence of the tissue was subtracted from the total resistance previously determined.

After 4 control periods (5 min each) Amphotericin B (40 γ/ml) was added to the mucosal medium and resistance modifications were detected.

In a second set of experiments the gall bladder was everted, cannulated and perfused on both sides in Krebs solution, to determine net water transport by gravimetric method [3].

As in the former set of experiments, after 4 control periods (10 min each) Amphotericin B was added to the mucosal medium (40 γ/ml) and net water transport modifications were measured.

When transport was completely abolished, mucosal and serosal krebs solutions were replaced by a Na^+ free Krebs medium (Tris/Tris HCl isotonically substituted for NaCl and $NaHCO_3$, pH 7.4). This solution was bubbled by 100 % O_2 and added with the polyene (40 γ/ml). After 20 min the gall bladder was perfused again in the Amphotericin-Krebs medium.

RESULTS AND DISCUSSION

Amphotericin B is known to act on its substrates, increasing passive permeability to electrolytes and non-electrolytes [5, 6, 7, 8]. In gall bladder too a similar action on thiourea permeability has been detected [3]. The reported decrease in electrical resistance (fig. 1) emphasizes an increase in the passive permeability of ions also.

In particular this effect is to be refered to an enlarged Na^+ salt permeability [4].

An inhibition in isosmotic net water transport, besides the effect on passive permeability, is observed in gall bladder (see fig. 2 and ref. [3]). Therefore a polyene action on NaCl—$NaHCO_3$ pump is required to account for this phenomenon and it is likely to be an indirect one, supported by the antibiotic effects on passive permeability.

The sequence of the phenomena is likely to be the following:

1) increase in permeability of the mucosal plasma membrane of the epithelial cells.

2) increase in salt (Na^+ salts as reported in ref. [4]) intracellular level.

3) inhibition of net water transport.

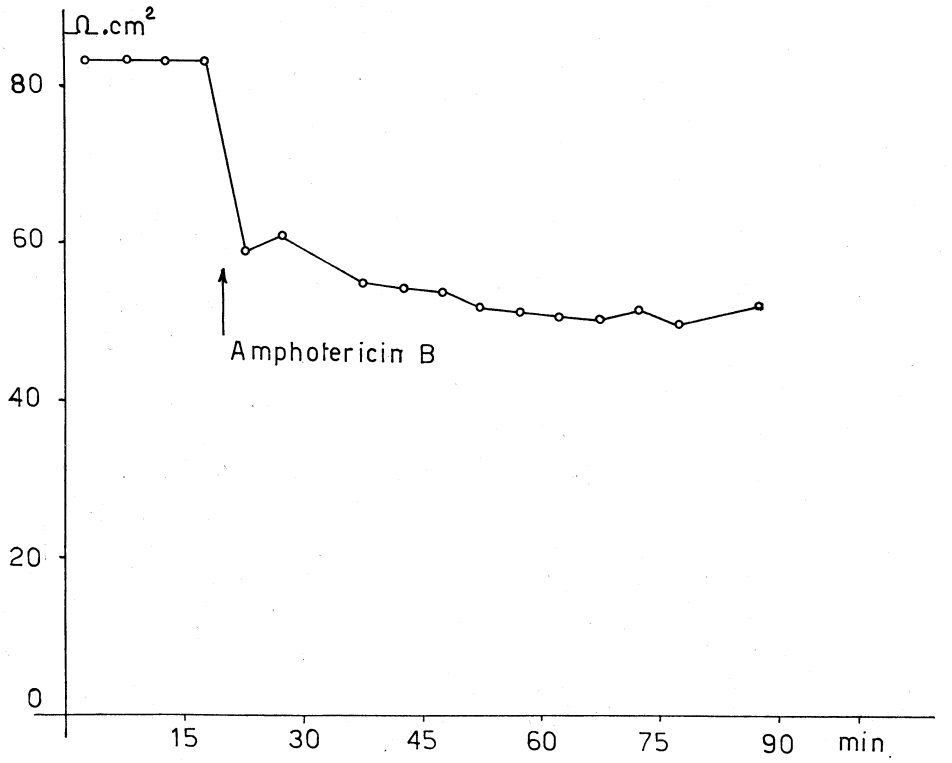

Fig. 1. – Effect of Amphotericin B treatment on transepithelial electrical resistance (representative among 7 experiments).

Ordinate: electrical resistance (Ω cm^2); *Abscissa*: time (min).

The excess of substrate (Na^+ salts) in the cell would result in an inhibition of Na^+ salt pump and consequently of water transport (see also ref. [2]).

If so, the action on permeability must occur before that on net water transport. Moreover, water transport, inhibited by the antibiotic, must be reactivated over some time, by lowering Na^+ salt intracellular level by some experimental procedure. Table I shows the consistence of the first assumption. The half-time of the electrical resistance decrease (3.5 ± 0.8 min in 7 exps) is much shorter than the half-time of water transport abolishment ($36.7 \pm \pm 7.5$ min in 9 exps).

TABLE I.

Half-time of Amphotericin B *effects on electrical resistance and net water transport in rabbit gall bladder.*

Half-time (min.) of electrical resistance decrease (means ± S.E.)	Half-time (min.) of net water transport decrease (mean ± S.E.)
3.5 ± 0.8 (7) (*)	36.7 ± 7.5 (9) (*)

(*) Number of experiments.

The experiment reported in fig. 2 supports the second hypothesis. The lowering of Na^+ salt intracellular level (by perfusing the tissue in Na^+ free solution over 20 min) reactivates water transport. Similar results have been obtained in other experiments. The final gall bladder weight increase might

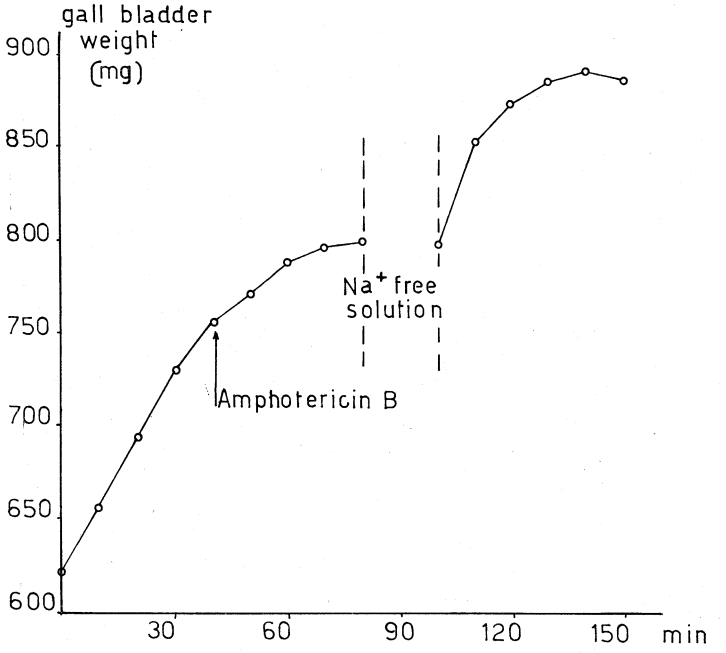

Fig. 2. – Isosmotic net water transport: inhibition by Amphotericin B and reactivation after perfusion in Na^+ free solution (representative among 9 experiments).

Ordinata: gall bladder weight (mg); *Abscissa*: time (min).

be due to a cellular swelling, rather than to a true transepithelial transport, if, during Na^+ free perfusion, cells have been shrunk. This possibility may be ruled out because no weight change has been observed during Na^+ free medium incubation ($+0.09 \pm 1.53$ mg cm^{-2} in 20 min, 8 exps).

In conclusion, an excess of the intracellular Na^+ salts seems to inhibit Na^+ salt pump.

Moreover such a pump under normal perfusion conditions seems to work at about its maximal level or to be partially inhibited.

References

[1] Diamond J. M. (1964) – « J. Gen. Physiol. », *48*, 15.
[2] Frederiksen O. and Leyssac P. P. (1969) – « J. Physiol. », *201*, 201.
[3] Cremaschi D., Montanari C., Simonič T. and Lippe C. (1971) – « Arch. Int. Physiol. Bioch. », *79*, 33.
[4] Cremaschi D., Hénin S. and Calvi M. (1971) – « Arch. Int. Physiol. Bioch. » in press.
[5] Kinsky S. C., Luse S. A. and Van Deenen L. L. M. (1966) – « Fed. Proc. », *25*, 1503.
[6] Lichtenstein N. S. and Leaf A. (1965) – « J. Clin. Invest. », *44*, 1328.
[7] Lippe C. (1968) – « J. Mol. Biol. », *35*, 635.
[8] Andreoli T. E. and Monahan M. (1968) – « J. Gen. Physiol. », *52*, 300.