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Addition of cAMP to Mucosal or Serosal Medium Induces Different Actions on *Necturus* Gallbladder

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Open tip and Cl^--selective microelectrodes were used to study the effects of cAMP on apical membrane potential (V_a), fractional voltage ratio (f_a) and intracellular chloride activity (a^i_{Cl}) in *Necturus* gallbladder under open-circuit conditions. In the presence of cAMP in the mucosal medium V_a depolarized from -68 ± 5 mV in control conditions to -56 ± 5 mV and f_a decreased from 0.56 ± 0.15 in control conditions to 0.15 ± 0.02. Concomitantly a^i_{Cl} fell from 15 ± 2 mM to 8 ± 3 mM, a value close to its electrochemical equilibrium activity. These results differ markedly from those obtained when cAMP was added to serosal medium and indicate that cAMP elicits different transport mechanisms whether it is added to the serosal medium or to the mucosal medium.

Key words: cAMP, Cl^--selective microelectrodes, Membrane potential, *Necturus* gallbladder, Open tip microelectrodes.

In a previous paper (1) the action of 6 mM cAMP on the transport mechanisms of *Necturus* gallbladder was studied. A decrease in the intracellular activities of Na^+ and Cl^- was found, whereas transapical membrane potential was not affected. It was concluded that cAMP action on *Necturus* gallbladder inhibit NaCl coupled electroneutral transport, i.e.: only an antiabsorptive effect of cAMP was observed. In the same tissue DUFFEY *et al.* (2) found membrane depolarization, a drop of the ratio of the apical membrane resistance to the basolateral membrane resistance and an increase in the transepithelial electrical resistance after addition of 8-Br-cAMP. Recently, PETERSEN and REUSS (5) have confirmed these previous observations of DUFFEY *et al.* (2). They concluded that the main action of cAMP induces a dominant Cl^- conductance in the apical membrane, and that there is no need to postulate an effect of cAMP on coupled electroneutral transport. Because of these discrepancies a re-examination of transport

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mechanisms during exposure to cAMP has been made.

Materials and Methods

Necturus maculosus specimens were obtained from Graska Biological Supplies (Oshkosh, WI, U.S.A.). They were kept in a large acquarium at 4°C. Animals were killed by a blow on the head. The gallbladder was removed and mounted as a flat sheet in a divided chamber (4) at room temperature (23°C). The mucosal and serosal sides of the gallbladder were perfused continuously and independently. The perfusion system permitted the solution bathing the mucosal or the serosal side of the gallbladder to be changed rapidly. The control Ringer's solution contained, in mM: 100 NaCl, 2.5 KCl, 1 $CaCl_2$ and was buffered to 8.2 with 5 mM Tris-Cl. Both perfusion solutions were continuously bubbled with 100 % O_2. Adenosine 3′,5′-cyclic-monophosphoric acid (cAMP; Sigma) was added, at a concentration of 6 mM, to the mucosal or to the serosal bathing medium. When this was done, the pH was adjusted to 8.2 with Tris. The cAMP solution prepared in this way was 12 mM hypertonic with respect to control Ringer's solution. It was established by separate experiments that an increase in the osmolarity of the mucosal or serosal medium of 12 mM, did not affect the electrophysiological characteristics of the tissue.

The transepithelial potential difference (V_t), the transepithelial electrical resistance (R_t), the transapical potential difference (V_a), the fractional voltage ratio (f_a) and the intracellular chloride activity were measured following the technique described in detail by GARCÍA-DÍAZ *et al.* (4).

Individual experiments were performed as follows: after mounting the tissue under open-circuit conditions in the chamber, 60 min were allowed for the establishment of a steady-state under control conditions. Microelectrodes were mounted perpendicularly to the tissue on a micromanipulator (MM 33, Narishige, Japan). Initially, the micromanipulator was used to position the microelectrode close to the tissue. Final movement of the cell impalement with the microelectrode was accomplished with a piezoelectric positioning device (PZ 501, Inchworm, Burleigh Inst.). Following a minimum of five acceptable impalements with conventional microelectrodes one of these microelectrodes was kept inside a cell. Then the mucosal or the serosal solution was changed to that containing 6 mM cAMP. When the microelectrode potential reached a stable value, the control Ringer's solution was reintroduced. The microelectrode was kept inside the cell until a new stable value was obtained. In this way a microelectrode was kept inside a cell for as long as 50 min. After this, the same protocol was used with Cl^--selective microelectrodes.

Results

When cAMP (6 mM) was added to the serosal bathing medium no significant effect on the electrophysiological parameters studied was obtained. When it was present in the mucosal side it depolarized V_a by 12 mV (table I). This effect became apparent 2 min after cAMP addition to mucosal bathing medium. When V_a became steady, approximately 5 min after cAMP addition, the mucosal solution was replaced by control solution and V_a reversed to the same value existing before adding cAMP. However, this repolarization process was slower than the depolarization one induced by cAMP. Sometimes as much as 40 min were necessary before a new steady-state could be achieved. A dra-

Table I. *Effect of 6 mM cAMP on electrophysiological parameters of* Necturus *gallbladder* ($\bar{x} \pm$ S.E.M.).
N is the number of tissues studied. Paired t-test was used.

Group	N	R_t (Ω cm^2)	f_a	V_t (mV)	V_a (mV)
1) Control	5	180 ± 15	0.46 ± 0.11	1.1 ± 0.4	−65 ± 6
+ 6 mM cAMP (serosal side)	5	180 ± 15	0.46 ± 0.10	1.1 ± 0.4	−65 ± 5
2) Control	4	170 ± 20	0.56 ± 0.15	1.6 ± 0.3	−68 ± 5
+ 5 mM cAMP (mucosal side)	4	170 ± 20	0.15 ± 0.02*	2.1 ± 0.3*	−56 ± 5*

* $p < 0.01$.

matic decrease in the apical voltage ratio was produced simultaneously to the V_a depolarization. The recovery of the apical voltage ratio after cAMP removal from bathing medium followed a time-course similar to that of V_a. No significant changes in transepithelial resistance were observed during the approximately 5 min in which the tissue was exposed to cAMP. A small but significant increase (0.5 mV) in the transepithelial potential was produced. Cl^- intracellular activity was measured by means of Cl^- selective microelectrodes. Cl^- activity decreased from a control value of 15 ± 2 mM to 8 ± 3 mM under the presence of cAMP in the mucosal side. This effect became steady within about 5 min after addition of cAMP and reversed slowly after removal of cAMP from the bathing medium. In control conditions Cl^- accumulates inside the cell about 2.7 times its electrochemical equilibrium value and after the addition of cAMP it fell to a value that does not significantly differ from the expected value for electrochemical equilibrium.

Discussion

The results presented in this study for the action of cAMP added at a concentration of 6 mM to the serosal side of the tissue is in good agreement with the results presented in a previous paper (1). V_a and f_a remained unaffected by the presence of cAMP in the serosal side. In a previous study (1) Na^+, K^+ and Cl^- intracellular activities were measured and it was concluded that cAMP when added to the serosal side of the tissue inhibits NaCl coupled electroneutral transport. The results presented in this paper for the action of cAMP when added to mucosal side agree with the previous study of DUFFEY *et al.* (2) and that of PETERSEN and REUSS (5), who found a depolarization of V_a and a decrease in the apparent ratio of apical to basolateral membrane resistance, after addition of cAMP to the mucosal side of the tissue. PETERSEN and REUSS (5) concluded that the main action of cAMP is to increase Cl^- permeability of the apical membrane which can explain the inhibition of fluid absorption produced by theophylline in this tissue and the fall of a^i_{Cl} to its electrochemical equilibrium value without any need to postulate an effect on the coupled NaCl entry mechanisms. However, in the light of the present and previous studies (1, 2, 5) one must differentiate between two different actions elicited by cAMP on *Necturus* gallbladder. One occurs when cAMP is added to the serosal bathing medium where

cAMP inhibits NaCl coupled transport, as it does in the intestine, this being the so-called anti-absorptive effect (3). The other one occurs when cAMP is added to mucosal medium where the main effect of cAMP is to increase apical Cl^- conductance. Field (3) has stressed the importance of an increase in the apical Cl^- conductance in the secretory effect elicited by cAMP in the intestine. Whereas in the intestine the antiabsorptive and secretory effects of cAMP are simultaneously produced, in the gallbladder, inhibition of NaCl coupled transport or increase in the apical Cl^- conductance is produced depending on the bathing solution where cAMP, or its derivatives, are added. The origin of this dual action of cAMP in *Necturus* gallbladder remains unclear.

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Resumen

Se estudia el efecto del AMPc sobre la diferencia de potencial transapical (V_a), el voltaje fraccional (f_a) y la actividad intracelular de cloro (a^i_{Cl}), utilizando microelectrodos convencionales y ión-selectivos, en la vesícula biliar del *Necturus* en condiciones de circuito abierto. Al añadir AMPc a la disolución de incubación mucosal V_a sufre una despolarización de -68 ± 5 mV a -56 ± 5 mV y f_a disminuye desde $0{,}56 \pm 0{,}15$ a $0{,}15 \pm 0{,}02$. Simultáneamente a^i_{Cl} cae de 15 ± 2 mM a 8 ± 3 mM, valor muy cercano a su actividad de equilibrio electroquímico. Esos resultados difieren notablemente de los que se obtienen cuando el AMPc se añade a la disolución de incubación serosal, e indican que el AMPc actúa sobre mecanismos de transporte diferentes cuando se añade a uno u otro lado del tejido.

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