EFFECT OF OXYTOCIN ON ELECTRICAL POTENTIAL AND IONIC PERMEABILITY OF THE APICAL MEMBRANE OF FROG GALL BLADDER EPITHELIAL CELLS

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The effect of oxytocin on the intracellular Na^+ and K^+ concentrations, the level of the transmembrane potential difference, and the relative ionic permeability (P_{Na}/P_K) of the apical parts of the surface membrane of the epithelial cells was studied in experiments on the isolated frog gall bladder. In a dose of 20 milliunits/ml, oxytocin, added to the external incubation solution, reduced the transmembrane potential difference, increased the P_{Na}/P_K ratio, and led to a very small shift in the Na^+ and K^+ concentrations in the intracellular fluid. After exposure of the organ to the hormone for 30 min the membrane potential (MP) of the cells fell from 52.7 to 38.7 mV at $P < 0.001$ (negative charge inside the cell), whereas the value of P_{Na}/P was increased from 0.083 (control) to 0.175 (experiment) at $P < 0.001$. Meanwhile the intracellular Na^+ concentration was increased by 18.3 meq/kg and the K^+ concentration reduced by 5.2 meq/kg intracellular water. This shift in the intracellular concentrations of Na^+ and K^+ could result in an evident decrease of only 0.7 mV in MP whereas in fact MP fell by 14.0 mV. Consequently, the decrease in the transmembrane potential difference takes place as a result of an increase in P_{Na}/P_K under the influence of oxytocin. Electrogenic ion transport through the apical membranes of the frog gall bladder epithelial cells could not be detected.

KEY WORDS: gall bladder; epithelial cells; apical membrane; electrical potential; ionic permeability; oxytocin.

Investigations have shown that oxytocin, if added to the incubation solution on the side of the serous surface of the isolated gall bladder (GB) of fish [8], frogs [2], or rabbits [7], inhibits active transport of isotonic fluid outward from the cavity of the organ. Oxytocin also reduces Na,K-ATPase activity in the GB epithelial cells [3, 6] and increases their Na^+ and K^+ concentrations [4]. It has been postulated on the basis of these investigations that the inhibition of transport of isotonic fluid through the GB wall under the influence of oxytocin is due to its inhibitory action on the transport activity of Na,K-ATPase [3, 6].

However, the possibility cannot be ruled out that not only is Na,K-ATPase activity blocked by the hormone, but the mechanisms of ionic conductivity of the cell membrane also are altered.

In the investigation described below the membrane effects of oxytocin were studied, with special reference to its effect on the transmembrane potential difference of the apical part of the cell surface membrane and its relative permeability for Na^+ and K^+.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated GB of frogs. The membrane potential (MP) of the epithelial cells was measured and the Na^+ and K^+ concentrations were determined in the whole tissue and in the extracellular and intracellular water compartments of the GB wall. On the basis of the results the ratio between the Na^+ and K^+ permeabilities of the membrane (P_{Na}/P_K) was calculated by the Goldman–Hodgkin–Katz equation. The actual equation used for the calculation was:

$$E = 58 \lg \frac{P_K \cdot [K]_o + P_{Na} \cdot [Na]_o}{P_K \cdot [K]_i + P_{Na} \cdot [Na]_i},$$

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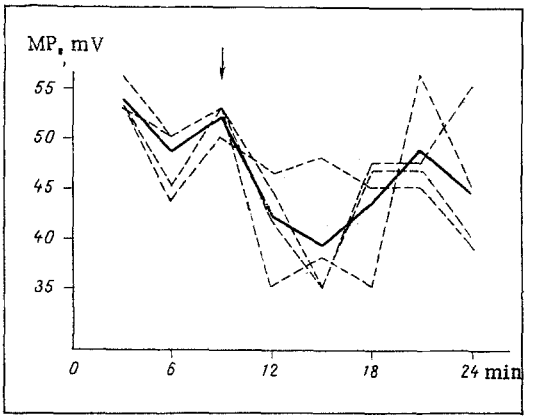

Fig. 1. Dynamics of changes in MP under the influence of oxytocin. Broken lines show mean results for each GB; continuous line represents mean MP for all experiments.

TABLE 1. Values of MP, P_{Na}/P_K, and r for Apical Membranes of Epithelial Cells of Isolated Frog GB in Control and after Exposure of GB to Oxytocin (20 milliunits/ml) for 30 min (M ± m)

Index	Control ($n=5$)	Oxytocin ($n=7$)	P
MP, mV	52,7±1,0	38,7±1,9	0,001
P_{Na}/P_K	0,083±0,004	0,175±0,012	0,001
r	0,97±0,02	1,01±0,03	0,2

where $[Na]_0$, $[K]_0$, and $[Na]_i$, $[K]_i$ denote the extracellular and intracellular Na^+ and K^+ concentrations respectively. According to the data of Mullins and Noda [11], this equation can be used to calculate whether any change takes place in MP under the influence of the electrogenic transport of ions by the cell. For this purpose the coefficient r to be introduced into this equation must be determined:

$$E = 58 \lg \frac{r \cdot [K]_0 + P_{Na}/P_K \cdot [Na]_0}{r \cdot [K]_i + P_{Na}/P_K \cdot [Na]_i}.$$

The content and concentration of Na^+ and K^+ in the tissue and cells of GB were determined as described previously [1, 4, 5]. MP was measured by an ordinary microelectrode technique. Glass electrodes filled with 3 M KCl solution had tips with a resistance of 20-30 MΩ and a potential of not more than 5 mV. The reference electrode was an Ag–AgCl electrode connected to the incubation medium by an agar bridge. The bridges consisted of polyethylene tubes (internal diameter 3 mm), filled with 4% agar–agar made up in Ringer's solution. MP was recorded by CRO (the VEKS-4m vectorelectrocardioscope was used) and a type KSP-4 automatic potentiometer. For the experiments to measure MP the GB wall was sectioned and fixed in the form of a membrane on a waxed cork slab with the mucous surface uppermost, after which the slab was fixed in an incubation chamber which was filled with 50 ml of Ringer–Bentley solution; the column of fluid did not reach higher than 1 mm above the surface of GB. The GB was incubated for 2 h. At the beginning of the experiment the MP level was measured in 15-20 cells, after which oxytocin (20 milliunits/ml) was added to the incubation medium and the measurements were repeated in the course of 30 min. The total duration of measurement of MP of the same cell was not more than 1 min.

EXPERIMENTAL RESULTS

When the microelectrode tip entered the cell the CRO beam jumped from the zero line and recorded the potential difference existing on the membrane at the moment of puncture. During the next few seconds the value of MP rose with fluctuations for 10-20 sec, after which it fell to its original level or remained a little higher than initially until the end of measurement. In most cells the value of MP remained stable for 1-3 min after puncture of the membrane, after which the membrane rapidly depolarized.

TABLE 2. Water Content and Na^+ and K^+ Concentrations in Intracellular Space of Isolated Frog GB in Control and after Exposure to Oxytocin (M ± m)

Index	Control ($n=9$)	Oxytocin ($n=9$)	P
$(H_2O)_i$	2,36±0,08	2,11±0,06	0,05
$[Na]_i$	67,8±3,5	96,1±4,0	0,001
$[K]_i$	101,6±4,2	96,4±3,3	0,5

Legend. $(H_2O)_i$ intracellular water content (in g/g dry residue of tissue), $[Na]_i$ and $[K]_i$ concentrations of sodium and potassium in intracellular fluid (in meq/kg intracellular water).

The mean value of MP at the moment of puncture of the membrane (the jump phase) was 36.0 mV (negative charge inside the cell), whereas in the hyperpolarization phase it was 52.7 mV.

During the action of oxytocin on GB regular changes were observed in the transmembrane potential difference (Fig. 1). MP began to fall 3-5 min after addition of the hormone to the incubation medium, and its average decrease after 15 min was by 8.1 mV ($P < 0.001$) and after 30 min by 14.0 mV ($P < 0.001$), i.e., by 26.5% (Table 1).

Besides a decrease in the MP level, a regular shift was observed in tissue water–electrolyte balance. Under the influence of the hormone the total sodium and potassium content in the GB wall was unchanged but the water content in it fell from 5.4 (control) to 4.7 g/g dry residue of tissue ($P < 0.001$). The volume of fluid in the extracellular compartment of the GB wall remained unchanged at 0.476 ml/g wet weight of tissue in the control and 0.456 ml/g in the experiment ($P < 0.5$). Consequently, the decrease in the water content in the GB tissue could only have resulted from the outflow of water from the intracellular space. Calculations show that the water content in the cells fell from 2.4 (control) to 2.1 g/g dry residue of tissue (oxytocin) ($P < 0.05$); under these circumstances there was a clear increase in the intracellular sodium concentration, but no significant change in the potassium level (Table 2).

Oxytocin had its most significant effect on the relative permeability of the surface membrane of the epithelial cells to Na^+ and K^+. For instance, in the control experiments the value of P_{Na}/P_K, calculated by the Goldman–Hodgkin–Katz equation, was 0.083. This indicates that the membrane permeability of these cells for Na^+ is one twelfth of that for K^+. Under the influence of oxytocin the P_{Na}/P_K ratio changed to 0.175, i.e., almost twice the control level. Consequently, the sodium permeability of the membrane increased twofold relative to its potassium permeability, and this was mainly responsible for the decrease in the MP level as a result of the action of the hormone on GB.

The increase in the intracellular Na^+ concentration while the value of P_{Na}/P_K remained unchanged could cause the MP level to fall by only 0.7 mV, whereas oxytocin actually reduced the potential by 14.0 mV.

It can tentatively be suggested that the decrease in MP was connected with the inhibitory action of oxytocin on the mechanism of electrogenic ion transport. However, an electrogenic component of MP for these cells is unlikely to exist, for the coefficient r was approximately equal to unity in both the experiment and the control (Table 1).

By its action on GB, oxytocin thus changes the permeability of the surface membrane of the epithelial cells, as a result of which the value of P_{Na}/P_K is doubled, with a consequent reduction in the transmembrane potential difference.

LITERATURE CITED

1. N. G. Kochemasova and M. S. Yaremenko, Fiziol. Zh. (Ukr.), No. 1, 129 (1965).
2. M. S. Yaremenko and I. A. Butusova, in: Proceedings of the 2nd All-Union Symposium on the Physiology and Pathology of Absorption in the Gastrointestinal Tract [in Russian], Odessa (1973), p. 148.
3. M. S. Yaremenko, I. A. Butusova, and O. N. Kharlamova, Fiziol. Zh. SSSR, No. 10, 1592 (1974).
4. M. S. Yaremenko and I. A. Butusova, Fiziol. Zh. SSSR, No. 2, 349 (1976).

5. M. S. Yaremenko and N. G. Kochemasova, Fiziol. Zh. SSSR, No. 6, 765 (1966).
6. M. S. Yaremenko and O. N. Kharlamova, Byull. Éksp. Biol. Med., No. 11, 10 (1974).
7. D. Gremaschi, B. Jiordana, C. Lippe, et al., Arch. Int. Physiol. Biochim., 76, 813 (1968).
8. J. M. Diamond, J. Physiol. (London), 161, 442 (1962).
9. L. J. Mullins and K. Noda, J. Gen. Physiol., 47, 117 (1963).