HYPERPOLARIZATION OF THE FROG SKIN BY FRUSEMIDE

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Application of frusemide to the outer surface of the frog skin causes a rapid and reversible increase in the potential difference by 50 ± 5.6 mV. The effect of frusemide is not observed if the chloride of the Ringer's solution is replaced by sulfate. Frusemide evidently reduces the chloride permeability of the apical surface of frog skin cells.

KEY WORDS: frusemide; frog skin; electrodermal potential; anionic permeability.

Frusemide is the most powerful modern diuretic and it acts by increasing the sodium excretion by the kidney [2]. Since it had no effect on active sodium transport by the skin of Rana temporaria [3] but potentiated sodium excretion, the increase in the sodium excretion by the kidney could be caused by the primary action of frusemide on chloride permeability.

The investigation described below was carried out to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on the abdominal skin of the frog Rana temporaria. The potential difference was measured with a high-ohmic LPU-01 potentiometer, using calomel electrodes connected by agar bridges with the Ringer's solution. The magnitude of the short-circuit current was determined by the method of Ussing and Zerahn [9] with the apparatus described by the writer previously [1]. Frusemide (Hoechst) was used in the experiments.

EXPERIMENTAL RESULTS

Application of frusemide in concentrations of up to 1 mg/ml to the inner surface of the frog skin caused no change either in the potential difference (Fig. 1) or in the short-circuit current [3]. If, however, a few milliliters of the same solution were applied to the outer surface of the frog skin, after 10-15 sec the potential difference started to rise and it reached 65 mV after 1-2 min (Fig. 1).

In 18 experiments on the frog skin the effect of one dose or of different concentrations of frusemide applied consecutively to the outer surface was investigated. Frusemide in a dose of 0.01 mg/ml did not change the potential difference and hyperpolarization appeared clearly in response to application of 0.1, 0.5 and, in particular, 1 mg/ml frusemide. In this concentration it increased the potential difference on the average from 60.1 to 110.2 mV (n = 13), the increase varying from 24 to 100 mV – $\Delta \pm m = 50.1 \pm 6.5$ ($P < 0.001$).

Substances reducing the sodium permeability of the apical membrane also reduced the potential difference and short-circuit current [6]. Since frusemide did not affect the short-circuit current (Fig. 2) and the potential difference was increased, it might be supposed that the diuretic inhibits chloride permeability on the outer surface of the cells (or in the region of intercellular contacts, perhaps) and would be ineffective if chloride was absent from the solution. After replacement of the chloride of the Ringer's solution by sulfate, the potential difference increased but frusemide no longer induced hyperpolarization (Fig. 2). Replace-

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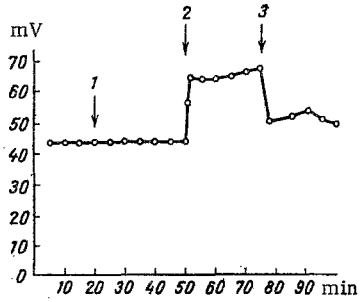

Fig. 1

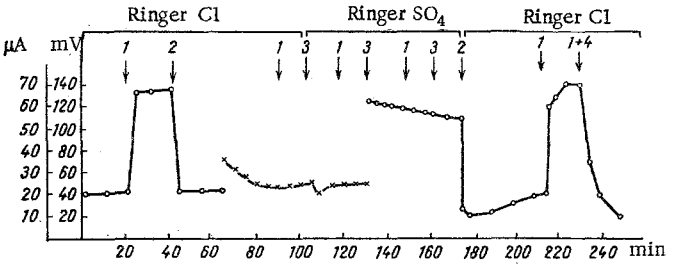

Fig. 2

Fig. Difference between effect of frusemide on potential difference through frog's skin depending on site of application of diuretic. Ordinate, potential difference (in mV); abscissa, time (in min). 1) Addition of 1 mg/ml frusemide to Ringer's solution in contact with inner surface of frog's skin; 2) Ringer's solution in contact with outer surface of frog's skin replaced by Ringer's solution with frusemide; 3) pure Ringer's solution in contact with outer surface of skin.

Fig. 2. Effect of frusemide acting on outer surface of skin on potential difference and short-circuit current. Ordinate, short-circuit current (in μA), potential difference (in mV); abscissa, time (in min). Circles – potential difference; crosses – short-circuit current. 1) Application of 1 mg/ml frusemide to outer surface of skin; 2) rinsing preparation with fresh Ringer-chloride solution; 3) the same, with Ringer-sulfate solution applied to both sides of the frog skin; 4) nystatin (1 mg/ml) applied to outer surface of skin. Whole experiment carried out on a single frog skin preparation.

ment of the sulfate of the Ringer's solution by chloride reduced the potential difference to its original level, and frusemide again increased the potential difference. Consequently, hyperpolarization of the frog skin through the action of frusemide was due to an increase in resistance as a result of a decrease in chloride permeability. In this case the nonspecific increase in permeability of the apical plasma membrane for various ions and, in particular, for chloride ought to abolish the effect of frusemide. Nystatin has such an action; it increases the ionic permeability of the apical cell membrane without disturbing the ability of the cells to transport sodium and potassium [4]. Application of nystatin to the outer surface of the skin (Fig. 2) abolished the hyperpolarizing action of frusemide.

The relationship between ionic permeability and the activity of ion pumps differs in the skin of different species of amphibians, and this may explain the conflicting nature of the data for the effect of frusemide on the short-circuit current and potential difference in toads and frogs [3, 7, 8]. The constantly reproducible, very rapid change in potential difference under the influence of frusemide (Fig. 2), similar to the action of substances that reduce ionic permeability, differs from the slower effect of sodium pump inhibitors in the frog skin [4]. This accordingly suggests that frusemide may inhibit chloride permeability in the frog skin and not the chloride pump, which is supposed to be inhibited in the renal tubules of rabbits by this diuretic [5].

Experiments on the frog skin thus indicate that frusemide induces hyperpolarization with no change in the short-circuit current, i.e., it selectively increases membrane resistance, evidently for chloride, for it has no action in the presence of sulfate. The increased excretion of sodium produced by frusemide may possibly be caused by a decrease in chloride permeability and inability of the tubular cells to reabsorb sodium under these conditions.

LITERATURE CITED

1. V. V. Ivanov and Yu. V. Natochin, Fiziol. Zh. SSSR, No. 1, 122 (1968).
2. Yu. V. Natochin, Ann. Rev. Pharmacol., 14, 75 (1974).
3. Yu. V. Natochin and E. A. Lavrova, Byull. Éksperim. Biol. i Med., No. 4, 63 (1974).
4. Yu. V. Natochin, V. T. Bakhteeva, and E. A. Lavrova, in: The Biophysics of Membranes [in Russian], Kaunas (1973), p. 484.
5. M. Burg, L. Stoner, J. Cardinal, et al., Am. J. Physiol., 225, 119 (1973).

6. J. Eigler and J. Crabbé, in: Renal Transport and Diuretics, Berlin (1969), p. 195.
7. J. Eigler, H. Carl, and H. H. Edel, Klin. Wschr., 44, 417 (1966).
8. W. Herms and K. E. Hofmann, Arch. Exp. Path. Pharmak., 251, 355 (1965).
9. K. Ussing and K. Zerahn, Acta Physiol. Scand., 23, 110 (1951).