DIFFERENCES BETWEEN THE ACTIONS OF ETHEPHYL AND ETHIMIZOLE ON WATER AND SODIUM TRANSPORT THROUGH ISOLATED BIOLOGICAL MEMBRANES

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Like antidiuretic hormone (ADH), theophylline, and caffeine, the compounds ethimizole and ethephyl in concentrations of 1 μmole/ml increase the osmotic permeability of the wall of the frog's urinary bladder. Unlike ethimizole and theophylline, ethephyl decreases the transport of water when stimulated by ADH. Diaphylline and ethimizole potentiate, while ethephyl reversibily inhibits active sodium transport by cells of the frog's skin.

Xanthine derivatives, especially throphylline, have a marked effect on the excretion of water and ions by the kidney [9]. Theophylline not only improves the hemodynamics of the kidneys but also exerts a direct influence on the reabsorption of water and ions in the tubules [4, 5] and also on the transport of water and sodium through isolated biological membranes [6, 7, 10].

The object of the present investigation was to study the effect of certain alkyl derivatievs of omidazole dicarboxylic acid on isolated biological membranes. These derivatives were: ethephyl (bis-ethylamide of imidazole-4,5-dicarboxylic acid) and ethimizole (bis-methylamide of 1-ethyl-ethylimidazol-4, 5-dicarboxylic acid), synthesized in the Department of Pharmacology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, on the basis of the caffeine molecule [1]. Experiments on rats showed that these compounds affect the excretion of water and sodium by the kidneys [2].

EXPERIMENTAL METHOD

Experiments were carried out on male winter frogs (Rana temporaria). Permeabilities to water was measured on the isolated urinary bladder [6]. The bladder was filled on the side of its mucous membrane with Ringer's solution diluted 1:10 with distilled water, and immersed on the side of the serous membrane in Ringer's solution. Osmotic permeability was determined from the volume of water passing along the osmotic gradient during 30-min intervals and converted into milligrams per cm^2 bladder surface per minute. In some experiments to investigate the dynamics of the changes in permeability the water transport was measured at 5 min intervals. Active sodium transport by the frog's skin cells was determined by a modified method of measurement of the short-circuited current [3]. The results were aggregated for the series of experiments and subjected to statistical analysis on a Promin' 2M computer [8].

EXPERIMENTAL RESULTS

Like theophylline and caffeine, the compounds ethephyl and ethimizole, when added to the fluid on the side of the serous membrane in a concentration of 1 μmole/ml, caused a tenfold increase in the osmotic

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TABLE 1. Changes in Osmotic Permeability (in mg H_2O/cm^2 per minute) of the Frog Urinary Bladder Wall (M±m)

Substance	n	Dose (in μmoles/ml)	Control (30 min)	Period I (30 min)	Period II (30 min)
Theophylline	8	10	0,023±0,005	1,05±0,14	0,73±0,1
	13	1	0,030±0,006	0,70±0,11	0,50±0,09
	22	0,1	0,031±0,01	0,19±0,04	0,14±0,02
Ethephyl	18	10	0,030±0,003	1,18±0,13	1,13±0,14
	10	1	0,016±0,004	1,10±0,17	0,47±0,11
	19	0,1	0,030±0,004	0,13±0,03	—
Ethimizole	6	10	0,056±0,01	0,79±0,15	0,76±0,13
	9	1	0,025±0,004	0,64±0,14	0,22±0,04
	18	0,1	0,033±0,007	0,17±0,04	—
Caffeine	5	10	0,030±0,01	0,65±0,11	0,52±0,06
	8	1	0,027±0,003	0,72±0,02	0,11±0,03
	7	0,1	0,040±0,01	0,07±0,03	—

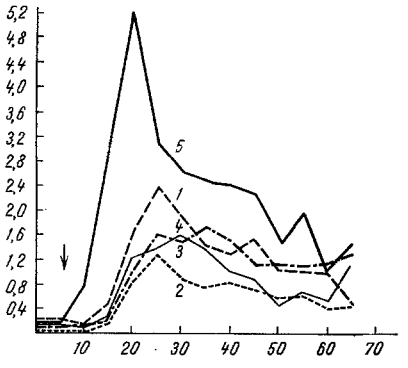

Fig. 1.

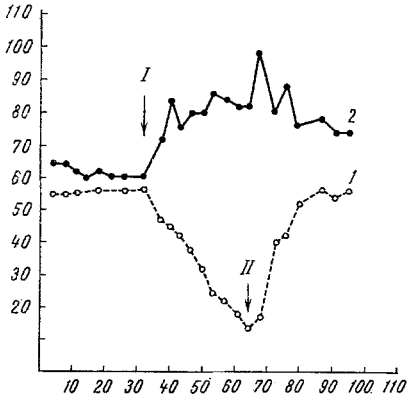

Fig. 2

Fig. 1. Dynamics of changes in osmotic permeability of the frog urinary bladder wall: 1) ethephyl; 2) ethimizole; 3) caffeine; 4) theophylline; 5) pituitrin. Ordinate, flow of water along osmotic gradient (in mg/cm^2-min); abscissa, time of investigation (in min). Arrow indicates addition of compound to solution on side of serous membrane fo the frog urinary bladder.

Fig. 2. Difference between actions of ethephyl and ethimizole on sodium transport through the frog skin. Ordinate, magnitude of short-circuited current (in μA/cm2); abscissa, time of investigation (in min). Arrow: I) addition of 1 μmole/ml of ethephyl (1) and ethimizole (2); II) change of Ringer's solution.

permeability of the wall of the frog's urinary bladder (Fig. 1). The dynamics of the change in permeability resembled in many respects the action of antidiuretic hormone (ADH): after a maximal increase in water transport, which occurred after 15-30 min, despite the presence of these compounds in the solution the water transport decreased (Fig. 1). The mechanism of this phenomenon remains unexplained despite frequent investigation [7]. A study of the dose–effect relationship showed that theophylline, ethimizole, and ethephyl produce a detectable increase in permeability to water in a concentration of 0.1 μmole /ml; (especially ethephyl). A further increase in the dose to 10 μmoles/ml leads to a significant increase in the flow of water only in experiments with theophylline (Table 1).

Since the experiments were carried out on isolated urinary bladder tissue the change in permeability was the result of the direct effect of these substances and was not produced indirectly through nervous or humoral factors. The increase in permeability to water could be connected with an influence on some of the

TABLE 2. Effect of Preliminary Treatment of Frog Urinary Bladder Tissue by Various Substances on Action of Pituitrin (M±m)

Substance tested	Dose (in μmoles/ml)	n	Pituitrin (0.2 i.u./ml)	n	Pituitrin (2.5 i.u./ml)
Control	—	10	0,70±0,16	6	2,61±0,28
Theophylline	1	8	1,54±0,21	5	2,59±0,32
	10	8	0,34±0,03	—	—
Ethimizole	1	5	1,61±0,2	4	2,47±0,1
Ethephyl	1	5	0,90±0,15	5	1,74±0,24
	10	15	0,43±0,03	—	—
Caffeine	1	8	0,94±0,15	5	2,14±0,15

Note: Pituitrin was added in all cases 60 min after preliminary treatment of the tissue with the corresponding substance.

mechanism on which ADH acts. To verify these possibilities the action of ADH was investigated after preliminary treatment of the urinary bladder tissue for 60 min with one of the four compounds. In a dose of 1 μmole/ml theophylline and ethimizole poteniated the action of pituitrin in a dose of 0.2 i.u./ml. These results are in complete agreement with those of experiments which showed that the action of ADH is potentiated by theophylline [10]. On the other hand, ethephyl and caffeine did not possess this action (Table 2). With an increase in the dose of theophylline and ethephyl to 10 μmole/ml a definite decrease in permeability was observed after the addition of pituitrin by comparison with the action of these compounds alone and also of pituitrin (Table 2). Finally, the action of a higher dose of ADH (2.5 i.u/ml), giving rise to a near-maximal increase in osmotic permeability, was investigated. The addition of this dose of ADH to bladder tissue after preliminary treatment with theophylline and ethimizole did not alter the response to the hormone, while ethephyl reduced the response to ADH slightly (Table 2). The action of ethimizole was thus similar to that of theophylline, whereas ethephyl, with the same direct action on osmotic permeability as these substances, differed from them in lowering reactivity to ADH.

Differences were found especially clearly between ethimizole and ethephyl in the experiments on the frog's skin. On addition of ethimizole in a dose of 1 μmole/ml on the side of the inner surface of the skin the sodium transport was increased from 74.8 ± 7.6 to 91.6±8.6 μA/cm^2 (n = 9). In view of the variability of the original values of the current the statistical analysis of these series of results was carried out by method of comparison of combinations with paried variables [8]. The changes in transport due to introduction of the substance were compared with the initial values of active transport in the same frog's skin. The increase in value of actively transported sodium ions was Δ± m 16.8±4.8 μA/cm^2 (P< 0.01). Diaphylline, which increased the current from 106.6±11.1 to 149± 15.6 μA/cm^2 (n = 10), acted similarly to ethimizole. Unlike ethimizole, ethephyl in a dose of 1 μmole/ml lowered the sodium transport from 73.2 ±6.4 to 22.2±3.0 μA/cm^2 (n = 15), Δ±m 51.0± 5.8 (P < 0.001). With a decrease in the concentration of ethephyl to 0.1 μmole/ml it had no effect on sodium transport: 76.0 ± 9.4 μA/cm^2 in the initial period and 74.0 ± 10.4 μA/cm^2 after the action of ethephyl (n = 5). The dynamics of the changes in sodium transport under the influence of ethephyl and ethimizole is shown in Fig. 2. It must be emphasized that the inhibitory action of ethephyl is reversible and the sodium transport is quickly restored after rinsing out the ethephyl.

The mechanism of action of theophylline is evidently based on inhibition of phosphodiesterase and accumulation of 3', 5'-AMP in the cell [10]. The similarity between the action of ethimizole and theophylline suggests that the distinctive structure of the ethimizole molecule, with an ethyl radical attached to the nitrogen atom of the imidazole ring and with more considerable structural changes in the remainder of the molecule (opening of the pyrimidine ring, transposition of CO and CH_3 groups), was not reflected significantly in the ability of ethimizole to react with phosphodiesterase or with some other enzyme with which tehophylline interacts. In the case of ethephyl, however, substitution of the CH_3 groups by C_2H_5 in the amide part of the molecule led to essential changes in its structure: Instead of activating dosium transport ethephyl began to inhibit it. The hypothesis that this molecular rearrangement is responsible for the change in the properties of ethephyl is based on comparison of the structures of ethephyl and theophylline, in which the imidazole moieties of the molecules are identical, and on comparison of ethimizole with each of the compounds mentioned. The results of these experiments on isolated membranes confirm the earlier hypothesis regarding the direct antidiuretic action of ethimizole on the renal tubules of rats and not its indirect action mediated through secretion of ADH by the neurohypophysis [2].

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