EFFECT OF ACETYLCHOLINE ON THE PITUITRIN-INDUCED OSMOTIC FLOW OF WATER THROUGH THE FROG BLADDER WALL

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An attempt was made by means of acetylcholine (ACh) and other cholinergic compounds to analyze the role of the paracellular pathway in ADH-dependent water transport through the frog bladder wall. ACh and the other cholinomimetics (ChM), in concentrations of 10^{-3}M, inhibited the osmotic flow of water stimulated by pituitrin. ACh had a similar action on the osmotic flow stimulated by cyclic AMP and theophylline. The antipituitrin effect was not reproduced by nicotine or by powerful muscarinic ChM [methylfurmethide, dioxalan (F-2268)] and was not prevented by nicotinic and muscarinic cholinolytics (atropine, oxyphenonium, hexamethonium, flaxedil). However, the antipituitrin effect was completely abolished by anticholinesterases (antiChE) with different types of action, in concentrations of 10^{-3}-10^{-6}M (neostigmine, physostigmine, GT-42, armin, acridine iodomethylate). The results rule out any connection between the mechanism of the antipituitrin effect of ACh and other cholinergic compounds and contraction of the smooth muscles and subsequent joining of the intercellular spaces. It is suggested that the effect of ACh is connected with cholinesterase activity. A possible role for phosphoinositides in the change in permeability of the frog bladder wall to water is also postulated.

KEY WORDS: antidiuretic hormone; acetylcholine; osmotic flow of water; urinary bladder of amphibians.

An important problem in the study of the mechanism of action of antidiuretic hormone (ADH) is the relationship between the trans- and paracellular pathways of ADH-dependent water transport. Convincing evidence exists that water moves both through the cell and through the paracellular spaces [4, 8, 10, 11]. However, the order of activation of these two pathways and the degree of their contribution to water transport have not yet been explained. The isolated amphibian urinary bladder, widely used in physiology as a test object for the study of the action of ADH, unlike the renal tubules, has the additional advantage that the intracellular spaces in its epithelium join together under the influence of acetylcholine (ACh), which induces contraction of smooth muscles [8, 9].

Consequently, ACh could be a means of blocking the paracellular spaces and of analyzing their role in ADH-dependent water transport. On the basis of the results of previous investigations the writers postulated that the transport of water from the apical membrane to the basolateral membrane takes place with the aid of a cytoplasmic system of microtubules, which either direct the flow of water or cause dilatation of the intercellular spaces [1].

In this investigation an attempt was made to block these spaces by means of ACh and other cholinergic compounds causing contraction of smooth muscles. Since cholinergic substances have been shown, in experiments on various objects, to inhibit the formation of cyclic AMP [12], it was also necessary to rule out this mechanism of depression of the ADH effect, since cyclic AMP is an intracellular mediator of the action of ADH [13].

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana temporaria) in the fall and winter, by Bentley's method [7]. Sensitivity of the individual groups of animals to pituitrin varied strongly, and for that reason half of the urinary bladder was used as a control and the other half for the experiments. To begin with, when necessary,

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TABLE 1. Effect of Cholinergic Drugs on Stimulated Osmotic Flow of Water through Frog Bladder Wall

Test substance	Concentration of drug, M	Number of animals	Loss of bladder wt. per min (in % of initial wt.) resulting from action of		P†
			50 milliunits/ml pituitrin	test substance + 50 milliunits/ml pituitrin	
Acetylcholine	10^{-6}	8	0,43±0,02	0,38±0,04	>0,1
	10^{-3}	22	0,46±0,02	0,19±0,02	<0,001
Butyrylchloline	10^{-3}	17	0,73±0,06	0,43±0,05	<0,001
Propionylcholine	10^{-3}	10	0,68±0,06	0,48±0,04	<0,001
Carbachol	10^{-3}	7	0,35±0,04	0,27±0,07	>0,05
Nicotine	10^{-3}	8	0,66±0,08	0,54±0,09	>0,05
Dioxalan (F-2268)	10^{-5}	9	0,58±0,07	0,50±0,09	>0,1
	10^{-4}	7	0,41±0,05	0,40±0,07	>0,1
	10^{-3}	15	0,45±0,07	0,35±0,04	>0,1
Methylfurmethide	10^{-3}	8	0,77±0,06	0,70±0,06	>0,05
Trimethylammonium	10^{-3}	18	0,58±0,07	0,59±0,05	>0,1
Acetylcholine*	10^{-3}	10	0,21±0,02	0,03±0,005	<0,001

*Osmotic flow of water stimulated by $0.5 \cdot 10^{-2}$M cyclic AMP and 10^{-6}M theophylline.
†P denotes significance of difference between mean control and experimental values (here and in Tables 2 and 3).

TABLE 2. Effect of Cholinolytics on Inhibition by ACh of Pituitrin-Stimulated Osmotic Flow of Water through Frog Bladder Wall

Test substance	Concentration of drug, M	Number of animals	Loss of bladder wt. per min (in % of initial wt.) resulting from action of		P
			10^{-3} M ACh + 50 milliunits/ml pituitrin	test substance + 10^{-3} M ACh + 50 milliunits/ml pituitrin	
Atropine	10^{-3}	9	0,21±0,03	0,17±0,02	>0,05
Oxyphenomum	10^{-5}	8	0,12±0,03	0,19±0,05	>0,05
Hexamethonium	10^{-3}	8	0,18±0,03	0,10±0,02	>0,05
Flaxedil	10^{-3}	9	0,21±0,02	0,18±0,02	>0,05

preincubation was carried out with anticholinesterase (antiChE) or cholinolytic (ChL) drugs for 30 min, after which ACh or cholinomimetics (ChM) were added to the incubation medium also for 30 min, when pituitrin was added. The osmotic flow of water was estimated from the loss of weight of the bladder during the 60 min after addition of pituitrin.

EXPERIMENTAL RESULTS

As Table 1 shows, ACh (10^{-3}M) significantly inhibited the effect of pituitrin. ACh had a similar effect on the flow of water stimulated by a combination of cyclic AMP and theophylline. The inhibitory action of ACh was the same if added to the external medium or introduced into the cavity of the bladder (the loss of weight of the bladder in the course of 1 min, expressed as a percentage of the initial value, was 0.15 ± 0.03 and 0.19 ± 0.02 respectively). All the salts used (iodide, bromide, chloride), in concentrations of 10^{-3}M, had the same action: 0.27 ± 0.03, 0.23 ± 0.07, and 0.31 ± 0.04. Nicotinic ChM (butyryl- and propionylcholine) had an inhibitory action, although nicotine was ineffective (Table 1). Carbachol and also methylfurmethide and dioxalan (F-2268), with a powerful muscarinic ChM action [3], were inactive. The inhibitory action of ACh was insensitive both to muscarinic (atropine, oxyphenonium) and to nicotinic cholinolytics (hexamethonium, flaxedil) (Table 2).

The results of the use of antiChE drugs with different types of action was unexpected (Table 3). Compounds of this group sharply reduced the effect of ACh. By themselves they had no action either on the spon-

TABLE 3. Effect of AntiChE Drugs on Inhibition by ACh of Osmotic Flow of Water Stimulated by Pituitrin through Frog Bladder Wall

Test substance	Concentration of drug, M	Number of animals	Loss of bladder wt. per min (in % of initial wt.) resulting from action of		P
			10^{-3}M ACh + 50 milliunits/ml pituitrin	test substance + 10^{-3} MM ACh + 50 milliunits/ml pituitrin	
Neostigmine	10^{-3}	30	0,25±0,02	0,50±0,04	<0,001
	10^{-4}	8	0,17±0,03	0,55±0,05	<0,001
Physostigmine	10^{-3}	8	0,29±0,05	0,65±0,07	<0,01
GD-42	10^{-6}	14	0,30±0,02	0,67±0,05	<0,001
Armin	10^{-6}	10	0,34±0,04	0,57±0,08	<0,001
Acridine iodomethylate	10^{-6}	8	0,37±0,04	0,83±0,08	<0,001

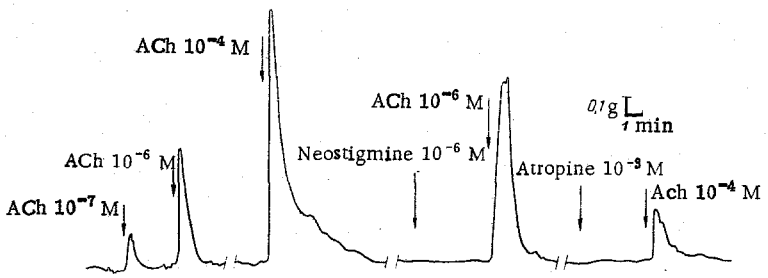

Fig. 1. Action of ACh, atropine, and neostigmine on frog bladder. Contractions recorded isometrically by method of Shelkovnikov et al. [6]. Arrows indicate time of action of drugs.

taneous loss of weight of the bladder or on the osmotic flow of water stimulated by pituitrin. Hydrolysis products of ACh – choline chloride (10^{-3}–10^{-1} M), acetic acid and its activated form, acetylcoenzyme A (10^{-3}M) – did not inhibit the effect of pituitrin (0.42 ± 0.02 and 0.42 ± 0.03, 0.57 ± 0.08 and 0.63 ± 0.06, 0.52 ± 0.06 and 0.58 ± 0.07).

What is the mechanism of the antipituitrin effect of ACh and ChM? Contraction of smooth muscles and joining of the intercellular spaces seems unlikely for the following reasons: 1) The antipituitrin effect of ACh is not prevented by atropine, whereas the joining of the intercellular spaces is sensitive to atropine [10]; 2) the effect was not reproduced by powerful muscarinic ChM; 3) the antipituitrin effect was inhibited (and not stimulated) by antiChE drugs.

Investigation of the effect of ACh on the smooth muscles of the frog bladder revealed different pharmacological characteristics: a low threshold dose of ACh and inhibition by atropine. In addition, contraction was potentiated by the antiChE compound neostigmine in a concentration of 10^{-6}M (Fig. 1).

Inhibition of cyclic AMP formation as the cause of the effect of ACh can be rejected on the grounds of inhibition by ACh of the osmotic flow of water induced by cyclic AMP (Table 1).

Finally, inhibition of the action of pituitrin may be due to cyclic guanosine monophosphate (cyclic GMP), formed under the influence of ACh and behaving as an antagonist of cyclic AMP [15]. However, cyclic GMP (10^{-4}M) likewise did not inhibit the effect of pituitrin (0.72 ± 0.10 and 0.62 ± 0.15).

The ability of hydrolyzable ChM (butyryl- and propionylcholine) to inhibit the action of ACh, despite the complete inactivity of carbachol and nicotine, and also the paradoxical effect of antiChE drugs may point to an important role of interaction between the cholinergic drugs and cholinesterase in the genesis of the observed effect. Since choline, a common component of the ChM and possessing antipituitrin action, is itself inactive, this suggests that the observed inhibition of the ADH-dependent flow of water through the bladder wall depends on activation of cholinesterase (ChE) by ACh [2]. An example of an effect taking place through

activation of ChE and inhibited by antiChE, is the ability of ACh to induce hydrolysis of triphosphoinositides [5]. Cyclic AMP, on the other hand, facilitates the phosphorylation of diphosphoinositides. An important role is ascribed to phosphoinositides in the change induced by ACh in the permeability of plasma membranes for ions, and permeability for water may perhaps also be connected with the state of the phosphoinositides. The antipituitrin effect of ACh may thus be localized on the apical membrane, where in the modern view the ADH-dependent barrier for water transport is located [10].

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