BBA 73114

The action of theophylline on the isolated skin of the frog (*Rana temporaria*)

Experimental evidence[1] suggests that the effect of antidiuretic hormone on the toad bladder involves an increased formation of cyclic AMP. As theophylline is an inhibitor of phosphodiesterase, this compound may be expected to have the same effect as antidiuretic hormone. Experiments by CUTHBERT AND PAINTER[2] indicate, however, that the actions of theophylline and antidiuretic hormone are not identical. They conclude that the two compounds do not necessarily act *via* the same mediator and suggest an alternative explanation for the action of theophylline.

The purpose of this communication is to describe the effect of theophylline on the basis of the frog skin model of USSING AND WINDHAGER[3] and the cyclic AMP theory. Ionic fluxes were measured isotopically on short-circuited skins[4]. The composition of the Ringer's solutions may be found elsewhere[3]. After a control period theophylline was added to both sides of the skins with a final concentration of 2 mM, which is twice the concentration used by CUTHBERT AND PAINTER[2], but as it is added to both sides, the actual cellular concentrations may be significantly greater in this study.

Like CUTHBERT AND PAINTER[5] we find that theophylline increases the permeability to Cl^- in chloride Ringer's. In 12 experiments the k_{trans} for Cl^- was increased by a factor of 3.4 (S.D. = ± 1.36). The possibility of glandular activity being responsible for the change in Cl^- outflux has been excluded by the observation that theophylline does not cause the appearance of any droplets in the gland outlets on the surface of frog skins mounted with a dry outside. These droplets appeared on the same skins as a result of epinephrine stimulation.

Table I shows that theophylline increases Na^+ fluxes in such a way that the

TABLE I

THE EFFECT OF THEOPHYLLINE ON Na^+ FLUXES ACROSS THE ISOLATED FROG SKIN (7 cm^2) IN CHLORIDE RINGER'S

The influx is calculated from the outflux and the short-circuit current. The outflux is measured with $^{24}Na^+$.

Na^+ fluxes (μequiv/7 cm^2 per h)				*Flux ratio*	
Influx before addition of theophylline	*Influx after addition of theophylline*	*Outflux before addition of theophylline*	*Outflux after addition of theophylline*	*Before theophylline*	*After theophylline*
9.69	17.00	0.36	1.70	27.0	10.0
9.56	19.93	0.38	2.01	24.9	9.9
6.60	16.28	0.41	1.35	16.1	12.1
2.31	15.37	0.70	0.81	3.3	19.0
6.68	14.36	0.33	1.55	20.0	9.5
8.34	22.14	0.14	1.99	59.1	11.2
8.71	14.29	0.32	1.60	27.2	8.9
7.02	15.46	1.06	3.89	6.6	4.0
11.76	18.44	0.56	1.26	21.0	14.6
8.27	14.18	0.95	2.82	8.7	5.0
9.19	13.94	0.26	1.25	35.4	11.2
6.50	11.08	0.54	1.03	12.0	10.8

outflux is increased relatively more than the influx, resulting in a drop in flux ratio.

These two findings explain the drop in potential difference across the skin, which was also observed by CUTHBERT AND PAINTER[2].

Using isoethionate Ringer's, CUTHBERT AND PAINTER found no increase in net Na^+ transport. This is contrary to our findings in sulfate Ringer's (Table II). In this Ringer's we do not find any increase in Na^+ outflux (Table III) or in Cl^- permeability.

TABLE II

THE EFFECT OF THEOPHYLLINE ON NET Na^+ TRANSPORT ACROSS THE ISOLATED FROG SKIN BATHED IN SULFATE RINGER'S, CALCULATED FROM THE SHORT-CIRCUIT CURRENT

Net Na^+ transport (μequiv/7 cm^2 per h)	
Before addition of theophylline	*After addition of theophylline*
8.02	12.12
6.15	11.00
7.46	12.68
4.85	9.33
8.20	13.42
12.65	15.67
10.80	13.80

TABLE III

THE EFFECT OF THEOPHYLLINE ON Na^+ OUTFLUX, MEASURED ON SHORT-CIRCUITED SKINS WITH $^{24}Na^+$, IN SULFATE RINGER'S

Outflux of Na^+ (μequiv/7 cm^2 per h)	
Before addition of theophylline	*After addition of theophylline*
0.45	0.52
0.78	0.76
0.48	0.51
0.66	0.58
0.66	0.53

TABLE IV

THE EFFECT OF THEOPHYLLINE ON Cl^- PERMEABILITY IN SULFATE RINGER'S CONTAINING 1 mM NaCl

Influxes are measured with $^{36}Cl^-$ on short-circuited skins.

Influx of Cl^- (nmoles/7 cm^2 per h)	
Before addition of theophylline	*After addition of theophylline*
187	123
23	23
141	148
9	10
230	220
260	205

These experiments show that the presence of Cl^- is not necessary for the effect of theophylline on the net Na^+ transport but only for the large increases in the passive ion movements. The experiments would be compatible with the following hypothesis: Theophylline induces an increase in cyclic AMP concentration by preventing its hydrolysis. By some unknown mechanism this leads to an increased Na^+ permeability

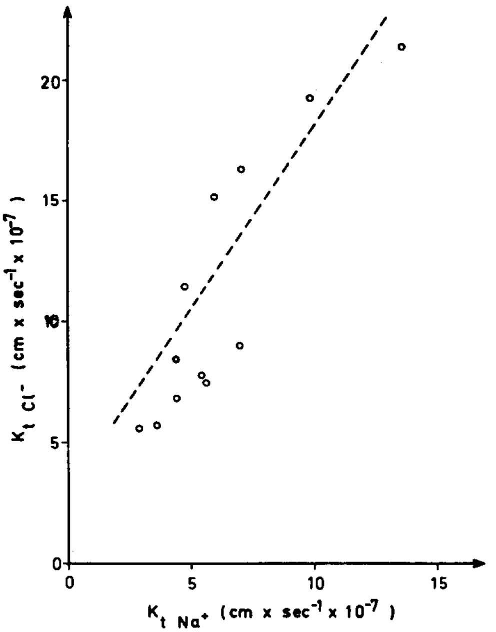

Fig. 1. The relation between the permeabilities of Na^+ and Cl^- in theophylline-treated skins. The broken line indicates the slope, which would be expected for free diffusion in water.

of the outward facing membrane of the frog skin. Na^+ enters the cells, and for electroneutrality reasons Cl^- also enters, mainly from the inside. This gives rise to an increased osmotic pressure which again leads to a swelling of the cells, which has in fact been shown to occur[6]. The stretching of the membrane, resulting from the swelling, results in the opening of passive paths for Na^+ and Cl^-. The relation between the passive permeabilities of the two ions in theophylline-treated skins (Fig. 1) indicates that this path has some resemblance to simple "holes".

It is, however, necessary to mention that the increase in net Na^+ transport induced by theophylline is larger in Cl^- than in SO_4^{2-}. This would be expected, because the increase in Na^+ permeability in Cl^- will be a result partly of cyclic AMP action and partly of the increase in permeability due to swelling. Only the first of these will be of significance in sulfate Ringer's. On the other hand, the results do not exclude some secondary effects of theophylline in Cl^- solutions. Seasonal and batchwise variation may then determine which of the actions of theophylline will be the most dominant.

The differences in the actions of theophylline and antidiuretic hormone with respect to passive permeabilities is under investigation. They may be due to an inborn limitation of the antidiuretic hormone response which is not present in the case of the nonphysiological theophylline activation.

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Received February 12th, 1970

Biochim. Biophys. Acta, 203 (1970) 579–582