These results suggest that the receptors are proteins containing disulphide bonds, disruption of which alters their properties without rendering them ineffective.

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The effect of theophylline on sodium transport across frog-skin in the absence of chloride

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It is widely accepted (see Orloff & Handler, 1967) that neurohypophysial vasopressin stimulates the transport of water and sodium across various epithelia via increased intracellular production of cyclic 3′,5′-AMP, and that theophylline-induced stimulation of active sodium transport results from decreased degradation of endogenous 3′,5′-AMP, caused by inhibition of phosphodiesterase. Recently, this view has been challenged by the suggestions that the action of theophylline on sodium transport across frog-skin is secondary to an increased chloride permeability, and that phosphodiesterase inhibition may be unimportant (Cuthbert & Painter, 1968b; Cuthbert, Painter & Prince, 1969).

In the present experiments frog skins (*Rana temporaria*) were mounted between pools of Ringer solutions for measurement of short-circuit current (I_{SC}), open-circuit potential (E) and for calculation of total resistance (R), using standard techniques (Ussing & Zerahn, 1951).

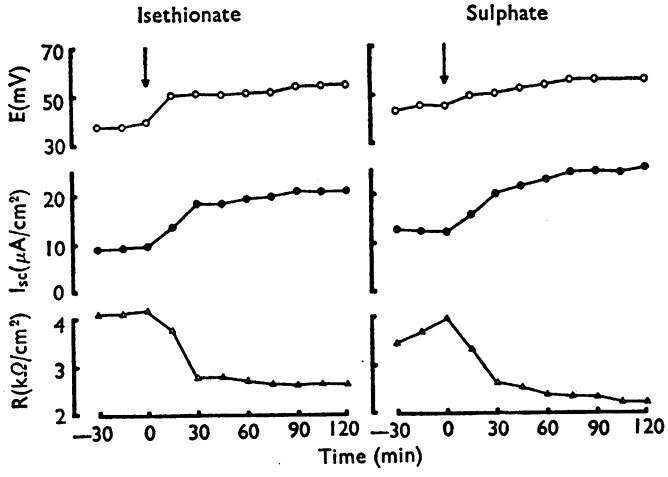

FIG. 1. Open-circuit potential difference (E), short-circuit current (I_{sc}) and resistance (R) in *Rana temporaria* skins mounted in, left, sodium isethionate Ringer, and, right, sodium sulphate Ringer, solutions. The arrows indicate the time of addition of theophylline ($2{\cdot}5 \times 10^{-3}$M) to the inner solution.

The electrolyte composition of the basic Ringer solution was: NaCl, 111 mM; KCl, 3 mM; $CaCl_2$, 1·8 mM; $NaHCO_3$, 2·38 mM. In other experiments chloride was replaced, completely, by various anions to make up sulphate, gluconate and isethionate Ringer solutions. In all Ringer solutions, sucrose was included to bring the measured osmolality to 224 m-osmole/kg H_2O. After an equilibration period, theophylline ($2{\cdot}5 \times 10^{-3}$M) was added to the inner bathing solution.

The stimulatory effect of theophylline on active sodium transport (measured as I_{sc}) across skins mounted in chloride Ringer was similar to that described by others (Baba, Smith & Townshend, 1967; Cuthbert & Painter, 1968a).

However, theophylline also caused clear changes—increased I_{SC} and decreased R—in skins mounted in sulphate, gluconate and isethionate Ringer solutions, i.e., in the absence of chloride. Typical examples are depicted in Fig. 1.

We conclude that the presence of chloride is not necessary to demonstrate the influence of theophylline on active sodium transport; and we consider it likely that the effect of theophylline in chloride-free solution is mediated via accumulation of cyclic 3′, 5′-AMP.

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The mechanism of potentiation of inotropic responses to phenylephrine by theophylline

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Phenylephrine (10–200 μg) sometimes produced small inotropic effects in the isolated perfused heart of the frog. Thirty to sixty minutes after the inclusion of theophylline hydrate (5 mM) in the perfusion fluid, all these doses of phenylephrine produced inotropic effects which were bigger than pre-existing ones and which could be blocked by propranolol ($3{\cdot}5 \times 10^{-6}$M). Iminazole (10–50 mM) prevented the inotropic effect of phenylephrine.

It seems unlikely that the potentiation of the inotropic effect of phenylephrine was due to inhibition of uptake, since the uptake site has a low affinity for phenylephrine (Iversen, 1964). Furthermore theophylline potentiated inotropic responses to tyramine (100–400 μg) in parallel with inotropic responses to phenylephrine, whereas cocaine ($2{\cdot}25 \times 10^{-5}$M) in the presence of theophylline potentiated the effects of phenylephrine but inhibited those of tyramine.