The effect of theophylline on chloride permeability and active chloride transport in various epithelia

SIR,—Antidiuretic hormone (ADH) and theophylline are known to stimulate sodium transport across frog skin in the absence of electrical, chemical or osmotic gradients (Baba, Smith & Townshend, 1967). Fig. 1 shows the increase

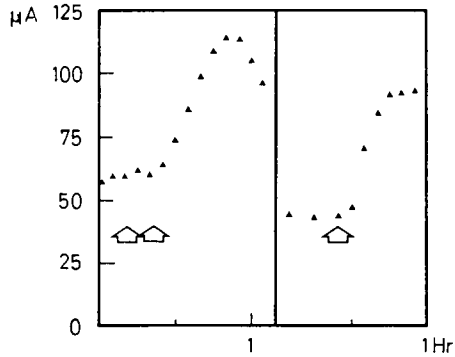

FIG. 1. Short circuit current measurements in 4 cm^2 pieces of frog abdominal skin (*R. temporaria*) bathed on both sides by frog Ringer. Left. At the arrows 130 and 260 mU/ml of arginine vasopressin were added to the solution bathing the inside of the skin. Right. At the arrow 1×10^{-3}M theophylline was added to the inner bathing solution.

in short circuit current (a measure of sodium transport [Ussing & Zerahn, 1951]) across abdominal skin of *Rana temporaria* caused by ADH and theophylline. The similarity of these responses has led to the idea for both frog skin (Baba and others, 1967) and other transporting epithelia (Orloff & Handler, 1967) that these two substances act through a final common pathway. The suggested common mediator is cyclic-3′,5′-AMP (Baba & others, 1967; Orloff & Handler, 1967) which, it is thought, increases the permeability of the outer facing membranes of the cells to sodium ions, thus supplying the actively pumping sites at an increased rate. It is proposed that endogenous levels of cyclic-3′,5′-AMP

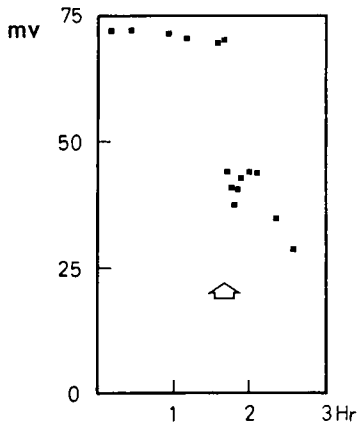

FIG. 2. The transepithelial potential of frog skin (*R. temporaria*) bathed in normal Ringer solution. At the arrow 1×10^{-2}M theophylline was added to the inner bathing solution.

are raised by activation of adenyl cyclase by ADH, or by prevention of destruction of the cyclic nucleotide by inhibiting phosphodiesterase with theophylline.

However, ADH and theophylline affect the frog skin potential in opposite ways. ADH causes an increase in the transepithelial potential, whereas the transepithelial potential falls on treatment of skins with theophylline. Fig. 2 shows the effect of theophylline on the skin potential of abdominal skin from *R. temporaria*. There is an immediate, precipitous fall followed by a somewhat slower fall. This effect of theophylline was dose dependent over the range 10^{-2} to 10^{-4}M. The increase in skin potential caused by ADH is understandable in terms of an increased permselectivity of the outer facing membranes to sodium ions (Civan, Kedem & Leaf, 1966), causing the outer surface to become more negative with respect to the inside. It was shown in further experiments that the fall in skin potential caused by theophylline did not depend on the presence of either sodium or potassium ions in the bathing fluid. The response did depend on a high concentration of chloride ions in the external medium, for instance when the skin was bathed in iso-osmotic choline chloride. It was also shown that the fall in skin potential resulted from changes occurring in the outer facing membranes of the skin even though the theophylline was applied to the inside of the skin. This was detected by using the technique described by Steinbach (1933). It is clear that if theophylline increases the permeability of the outer facing membranes to chloride ions then the movement of these ions down their chemical gradient generates a potential across the membrane such that the outer surface of the skin is less negative, thus the skin potential is reduced or may even be reversed.

The increase in sodium transport across the skin caused by theophylline results not from alterations in permeability of the membranes to sodium ions, but to chloride ions. In the open-circuited condition chloride ions exert an anion-drag on the movement of the actively transported species (Leaf, 1965). Even under short-circuited conditions anion-drag is still present, although to a lesser extent, since local potential gradients must still exist in a membrane with a mosaic of sodium and chloride permselective sites.

The actions of theophylline have been further investigated in two other actively transporting epithelia where active transport of chloride has been demonstrated. When the toad bladder (*Bufo marinus*) is bathed on both sides by Ringer solution deficient in potassium ions the transmembrane potential (serosal side positive) falls to zero and eventually reverses. Under these conditions a negative short circuit current is required to reduce the skin potential to zero.

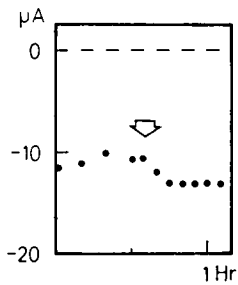

FIG. 3. Negative short circuit current measurements for 4 cm² of toad bladder (*B. marinus*). The bladder was bathed in potassium-free frog Ringer gassed with a mixture of 95% O_2 - 5% CO_2. At the arrow theophylline (1×10^{-3}M) was added to the inner bathing solution.

The negative short circuit current is equivalent to the mucosal to serosal active transport of chloride (Finn, Handler & Orloff, 1967). Theophylline applied to the serosal side of the bladder increased the extent of the negative short circuit current, as shown in Fig. 3.

Removal of sodium ions and reduction of chloride concentrations to low levels (2 m-equiv.) in the solution bathing the outside surface of the skin of *R. pipiens* abolishes sodium transport and exposes chloride transport (Martin, 1964; Martin & Curran, 1966). As in the toad bladder, active chloride transport was associated with a reversed potential and a negative short circuit current. In this case too, theophylline caused an increase in the negative short circuit current as shown in Fig. 4. The effects of theophylline on chloride transport

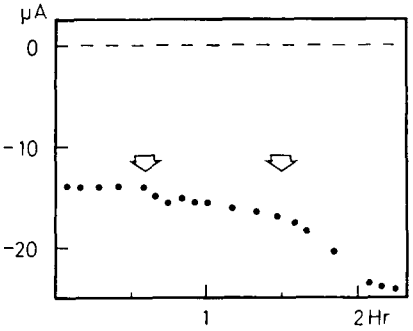

FIG. 4. Negative short circuit current measurements for 4 cm² frog skin (*R. pipiens*). The outer bathing solution was 2 mM potassium chloride, and the inner bathing solution was 2 mM potassium chloride and 56 mM sodium sulphate. Both solutions were made iso-osmotic with frog Ringer by addition of sucrose. At the first arrow 1×10^{-4}M theophylline and at the second arrow 1×10^{-3}M theophylline was added to the inner bathing solution.

are weak but chloride transport itself is not particularly marked. In the skin of *R. pipiens* chloride transport is only 5% of the sodium transport (Martin & Curran, 1966), while in the toad bladder sodium and chloride transport are mutually exclusive and it is suggested that the sodium and chloride ions use the same transfer system (Finn & others, 1967). The effects of theophylline on skin potential are, by comparison, dramatic. Perhaps the rate-limiting process in chloride transport is the chloride pumping, rather than access of chloride ions to the pumping sites. This differs from sodium transport across the epithelia where, quite clearly, the entry of sodium ions to the pumping sites limits the rate of sodium transport. Alternatively, chloride pumping in *R. pipiens* in the absence of external sodium ions may be restricted by cation drag.

The effects of theophylline on skin potential and negative short circuit current under the conditions described are consistent with the view that this agent increases the chloride permeability of the outer facing membranes. It also seems likely that the effect of theophylline on sodium transport in these various epithelia is due to the removal of anion-drag normally exerted on the sodium cation.

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Adrenergic receptors in the ruminal wall of sheep

SIR,—Ruminants suffering from infectious diseases often show a reduction in rumen motility, especially during the fever period. This effect can also be observed after injection of infusion fluids contaminated with pyrogens, or purified lipopolysaccharide from Gram-negative bacteria (Miert, 1966). We considered the possibility that the reduction in rumen motility after an injection with lipopolysaccharide is the result of sympathetic stimulation or adrenaline release (Miert, 1968). This led to a study of the adrenergic receptor in the rumen.

We know from the literature (summarized by Habel, 1956), that in the unanaesthetized ruminant with intact vagi, adrenaline inhibits rumen motility. In unanaesthetized vagotomized sheep it caused a single slow contraction of reticulum, rumen and abomasum. An intravenous injection of adrenaline in the anaesthetized goat results also in a contraction of the rumen.

Adrenaline on isolated strips of ruminal wall inhibited or stimulated the contractions (Dussardier & Navarro, 1953; Sanford, 1958). Duncan (1954) noted that the most characteristic effect of adrenaline on strips from the rumen abomasum and omasum of sheep was brief inhibition followed by contractions. With low doses, inhibition was the main effect, with high doses only strong contractions were observed. Dussardier & Navarro (1953) gave particular attention to the effects of adrenaline on strips of the abomasum. They noted that contractions from adrenaline could not be suppressed by atropine in concentrations which were sufficiently high to antagonize the action of acetylcholine. The adrenergic blocking agent 883F [2-(diethylaminomethyl)-1,4-benzodioxan], however, inhibited the motor effect of adrenaline.

For our experiments, strips of 7 × 2 cm were taken from the dorsal ruminal sac of sheep, immediately after slaughtering. These were transported in cooled Tyrode solution. In the laboratory, the serosa and mucosa layers were removed and the muscular layer placed in a bath with 50 ml of Tyrode solution without glucose, at 37° and aerated with an oxygen 95% and carbon dioxide 5% mixture. Recordings of the contractions were made isotonically on a kymograph (Stücklin, 1951). For specific α-receptor stimulation we chose oxymetazoline hydrochloride (Mujic & Rossum, 1965; Rossum & Mujic, 1965), and for specific β-stimulation we used isoprenaline hydrochloride, both at a concentration in the bath fluid of 0·2 μg/ml. Other agents were adrenaline hydrochloride (0·2 μg/ml), dibenamine hydrochloride (2 μg/ml), pronethalol hydrochloride (8 μg/ml) and Du 21445 [1-isopropyl-amino-3-(2-methylthiophenoxy)-propanol-2], also a strong β-blocking agent (2–4 μg/ml). The time intervals between the drug administration was usually about 30–40 min. After each response the bath fluid was renewed several times.