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STIMULATION OF SODIUM AND OF CHLORIDE TRANSPORT IN EPITHELIA BY FORSKOLIN

A.W. CUTHBERT & J.A. SPAYNE

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

The diterpene, forskolin, is shown to produce a concentration-dependent, increase in short circuit current in two epithelial preparations, amphibian skin and rat colon. In the amphibian tissue the increase is sensitive to amiloride and due to an increase in electrogenic transepithelial sodium transport towards the serosal side. In the rat colon piretanide attenuated the forskolin effect, suggesting the terpene increases electrogenic transepithelial chloride transport towards the mucosal side. Half-maximal activation of both processes was achieved with concentrations of 1–3 μM, similar to those required to activate half-maximally the catalytic subunit of adenylate cyclase.

Introduction In intact tissues direct, reversible activation of the catalytic subunit of adenylate cyclase (ATP pyrophosphate-lyase, EC4.6.1.1) has not been possible until now. The diterpene, forskolin (7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxylabd -14-en-11-one) has been shown to activate adenylate cyclase by a direct action independently of receptors, guanyl nucleotides or the guanine nucleotide regulatory protein (Seamon, Padgett & Daly, 1981; Seamon & Daly, 1981). Using either membrane preparations or intact tissue slices, biochemical evidence for activation by forskolin was found in all 14 tissues examined. We believe this agent will become an important tool in pharmacological studies. Possibilities are, for example, to show that adenylate cyclase can generate cyclic nucleotide at sites within cells which can then evince a response. This type of test has a different information content compared with that gained from adding exogenous nucleotide. In other situations the use of forskolin may point to the involvement of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in responses where receptors, or hormones to activate them, are unknown. Furthermore, in instances where receptor numbers are regulated it may be possible to show whether receptor availability limits the response.

We are aware of only one other pharmacological study with forskolin showing cardioactive effects and made before the mechanism of action was known (Lindner, Dohadwalla & Bhattacharya, 1978). We have chosen to examine the effects of forskolin on two types of ion transporting epithelia and where cyclic nucleotides are thought to be involved in the transport processes. These are the sodium absorbing epithelium of amphibian skin (Orloff & Handler, 1967) and the epithelium lining the mammalian colon which secretes chloride in response to cyclic AMP (Frizzell, Field & Schultz, 1979).

Methods Short circuit currents (SCC) were recorded from isolated epithelia by standard techniques (see Cuthbert & Margolius, 1982). Concentration-response relationships were measured using epithelial pieces (0.6 cm^2) mounted in Ussing chambers. The muscle of the colon was stripped away before the tissues were mounted. Results are expressed as the increase in current ($\mu A\,cm^{-2}$) caused by exposure to a single concentration of forskolin. Usually 30 min or less were necessary for the response to be fully developed.

For conductance measurements in frog skin larger areas (9.6 cm^2) were used. To measure conductance the clamping voltage was set temporarily at 10 mV, alternately above and below zero, for 1.6 s every 40 s.

The bathing solutions used were (a) for frog skin: (mM) NaCl 110, KCl 2, $CaCl_2$ 1, Tris buffer (pH 7.6) 5 and glucose, 11.1 and (b) for rat colon: (mM) NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2 and glucose 11.1. The amphibian bathing solution was gassed with air and used at 20°C: the mammalian solution was gassed with 95% O_2–5% CO_2 and used at 37°C. Forskolin was dissolved in 95% alcohol. The amount of alcohol added to the bathing fluid was $<0.1\%$ and was without effect on SCC.

Results Forskolin was more effective in increasing SCC in frog skin when added to the mucosal bath than the serosal one. The diterpene is lipid soluble and it is likely that the corium which underlies the epithelium acts as a sink, reducing the amount of drug reaching the epithelium. Consequently all the results described here, for both tissues, refer to addition to the mucosal bath. In frog skin there was an initial delay, which was relatively invariant with concentration, before SCC began to increase. The response was complete within 30 min at low concentrations (0.1 μM) and at shorter times at higher concentrations. SCCs declined slowly (30 min) to their original values after several changes of bathing solution. The

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SCC increase was due to increased transepithelial movement of sodium, shown by the sensitivity of the response to amiloride added to the mucosal bath (Figure 1a). Half maximal increases of SCC were achieved with 1 μM forskolin (Figure 1c) compared with the value of 5–10 μM for cyclic AMP generation by cerebral cortical membranes (Seamon *et al.*, 1981). The concentration-response relationship is steep, but it is likely that partition effects complicate the relation between bath concentration and that at the cellular membranes. When percentage increases in SCC over basal current were plotted against con-

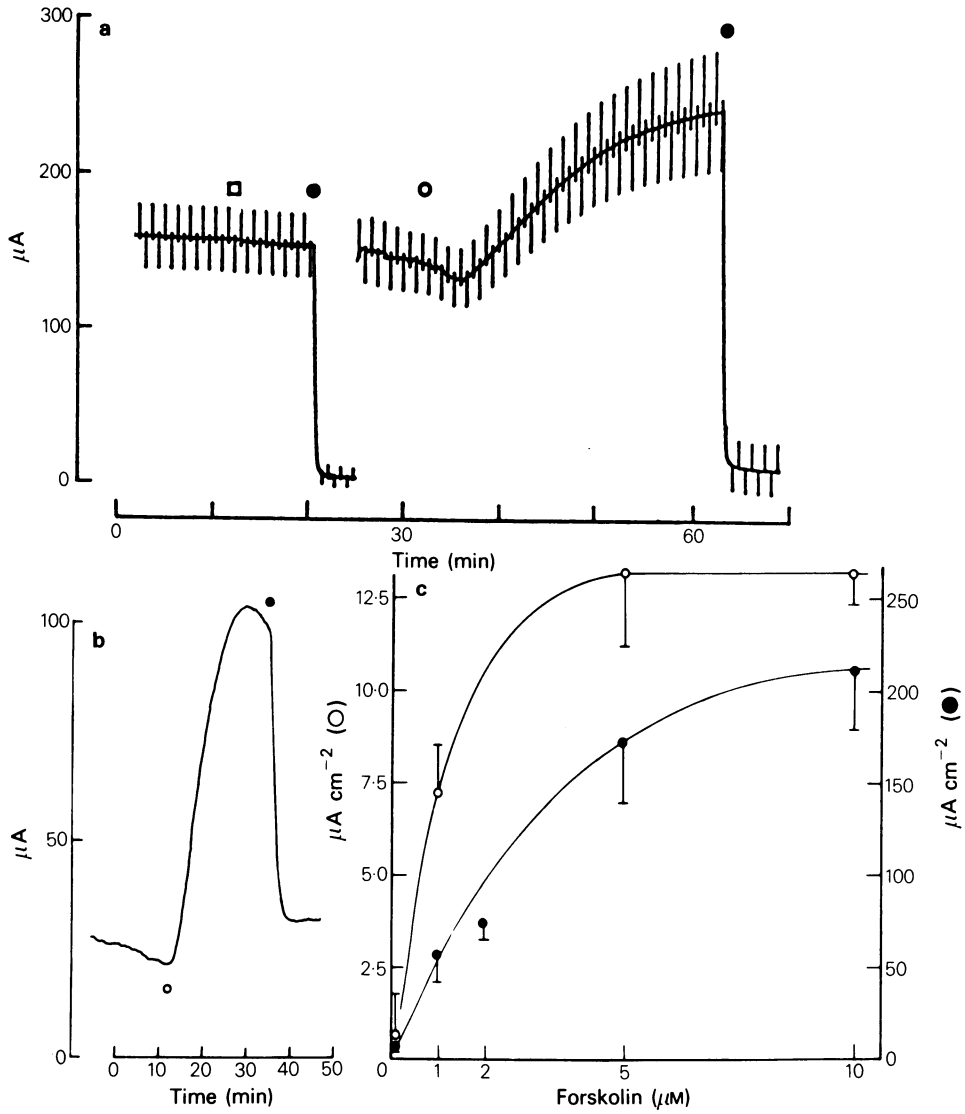

Figure 1 (a) Short circuit current (SCC) recording from frog skin (9.6 cm^2). Voltage was clamped at $\pm$ 10 mV every 40 s for 1.6 s. Drugs were added to the mucosal bath as follows: (●) amiloride 100 μM; (○) forskolin, 10 μM in 95% alcohol (10 μl) and (□) 10 μl 95% alcohol. Amiloride-sensitive conductance was 1.35 mS before and 2.1 mS after forskolin. (b) SCC recording from rat colon (0.6 cm^2). Forskolin, 5 μM was added to the mucosal bath (○) and piretanide, 2 mM (●) to the serosal bath. (c) Concentration-response relationships for forskolin measured in frog skin (○) and rat colon (●). Each tissue was exposed only once to forskolin. For frog skin each point shows mean and s.e. for 6 measurements. For rat colon each point shows mean and s.e. for either 4 or 5 measurements.

centration, a curve virtually identical to that illustrated was obtained. Forskolin increased both transepithelial and amiloride-sensitive conductance consistent with increased mucosal sodium permeability (Cuthbert & Shum, 1978). In the presence of forskolin a supramaximal concentration of amiloride failed to reduce conductance or SCC to the values found in the control condition. These discrepancies were, on occasion, quite considerable indicating that the diterpene probably has additional effects other than those on sodium permeability.

Forskolin caused a much more rapid increase in SCC when added to the rat colon compared to skin. The threshold concentration for an effect was similar (0.1 μM) and the half-maximally effective concentration was approx. 2–3 μM (Figure 1c). The rarity of the material did not allow us to establish whether concentrations above 10 μM can cause a further current increase, but the increase between 5 μM and 10 μM was relatively small and responses were not significantly different at these two concentrations.

Forskolin was able to cause quite remarkable changes in charge transfer, currents increasing from around 30 $\mu A\,cm^{-2}$ to values greater than 250 $\mu A\,cm^{-2}$. Support of the hypothesis that the increase in current is due to electrogenic chloride secretion from serosal to mucosal bath is gained from the effects of frusemide-like drugs (Figure 1b). Piretanide (2 mM) (Zeuthen, Ramos & Ellory, 1978) added to the serosal bath caused 61.7 ± 7.4% (mean ± s.e., $n = 8$) inhibition of the responses induced by high concentrations (5–10 μM) of forskolin.

Discussion We have shown for the first time that two very different ion transporting epithelia are stimulated by forskolin. The concentration of diterpene causing half maximal stimulation was similar in both tissues (1–3 μM) and similar to the concentration causing half maximal activation of adenylate cyclase (5–10 μM) (Seamon *et al.*, 1981).

There is considerable evidence to show that antidiuretic hormone, catecholamines, prostaglandins, acetylcholine and other agents interact with receptors located in the serosal surface of amphibian skin to generate cyclic AMP which results, eventually, in increased sodium permeability of the mucosal face. The effects of amiloride on conductance and SCC in this tissue are consistent with the view that forskolin increases sodium permeability, presumably via cyclic AMP.

In rat colon, bradykinin and kallidin are extremely potent agents for increasing SCC. This increase can be accounted for by an increase in net chloride flux towards the mucosal bath. The effect is attenuated (60%) by frusemide added to the serosal bath (Cuthbert & Margolius, 1982). Piretanide, a frusemide-like drug, has a similar attenuating action on the effects of forskolin. The effects of the kinins involve prostaglandin-dependent processes, which in turn may activate adenylate cyclase.

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