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The effect of auranofin on the colonic transport of Na^+ and fluid in the rat

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Auranofin in the mucosal fluid caused a dose-dependent inhibition of fluid and Na^+ absorption by everted sacs of rat colon. Serosal auranofin was without effect. (Na^+ + K^+)ATPase activity of homogenates of mucosal scrapes of rat colon was inhibited by auranofin in a dose-related manner, while Mg^{2+}-ATPase activity was little affected. These actions of the drug on colonic transport mechanisms could contribute to the diarrhoea associated with auranofin therapy.

Auranofin (*S*-(triethylphosphoranediylaurio)-1-thio-β-D-glucopyranose 2,3,4,6-tetraacetate) is a gold compound that can be administered orally in the treatment of rheumatoid arthritis. One of the common side-effects experienced by patients receiving this compound is a disturbance of bowel function, ranging from loose stools to diarrhoea (Heuer & Morris 1982). It has been shown that auranofin inhibits the absorption of nutrients, Na^+ and fluid by the small intestine (Hardcastle et al 1984) and this could contribute to the bowel symptoms described. However, disturbances in small intestinal function do not necessarily lead to diarrhoea, since the colon has a considerable capacity for compensation (Binder 1979). This aspect of colonic activity could be compromised if, in addition to its effects in the small intestine, auranofin also inhibited absorption in the colon. The present investigation was therefore designed to assess the effects of auranofin on the absorption of Na^+ and fluid by rat colon.

Methods

Experiments were carried out on male albino rats (Sheffield strain, 230–250 g). These were allowed free access to food (diet 86, Oxoid, London) and water. They were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.).

Measurement of intestinal transport. The transport of fluid and Na^+ was determined by the everted sac technique (Wilson & Wiseman 1954), using the entire colon. Each sac was filled with 0·75 ml fluid (serosal fluid) and incubated for 30 min at 37 °C in 25 ml fluid (mucosal fluid). The incubation fluid was Krebs bicarbonate saline (Krebs & Henseleit 1932) equilibrated with 95% O_2/5% CO_2 and containing additions as indicated. Auranofin was initially dissolved in ethanol and control sacs were exposed to an equivalent volume of this vehicle. Fluid transport was measured gravimetrically and the volume of fluid taken up by the sac, the mucosal fluid transport (MFT), is expressed as ml g^{-1} initial wet weight in 30 min. At the end of the incubation period the serosal fluid was collected and the intestinal sac was deproteinized with 5% trichloroacetic acid and homogenized. The Na^+ content of the final serosal fluid and gut homogenate was analysed using a Corning flame photometer (Model 430). Na^+ uptake was determined by subtracting from the total Na^+ content of the serosal fluid plus gut homogenate at the end of the incubation, an estimate of the initial Na^+ content of the sac (gut + serosal fluid). The initial Na^+ content of the serosal fluid was calculated from the initial serosal volume (0·75 ml) and its Na^+ concentration. The initial Na^+ content of the gut wall was determined in separate experiments and the mean value (75·3 ± 2·4 (10) μmol g^{-1} initial wet weight) used. Na^+ uptake is expressed as μmol g^{-1} initial wet weight in 30 min.

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Measurement of ATPase activity. The entire colon was removed from an anaesthetized rat and washed through with 0·9% saline. The colon was laid on a glass slab and opened along the mesenteric line. The mucosal surface was then gently scraped using a glass microscope slide to obtain a sample of mucosal cells. The ATPase activity of the unfractionated cell homogenates was measured using the method of Jørgensen (1975), with minor modifications. The mucosal scrape was added to incubation buffer to give a protein concentration of 1·5 mg ml^{-1}, and homogenized by 10 strokes of a ground glass homogenizer. Protein content was determined by the method of Lowry et al (1951). The incubation buffer had the following composition (mM); $MgCl_2$ 10, KCl 20, EDTA 1, imidazole 60 and Tris 60, pH 5. Triton X-100 was present at a concentration of 0·0375%, since preliminary experiments had shown this to be the optimum detergent concentration for 'activation' of ATPase in the preparation used. The mucosal homogenate was incubated for 30 min at room temperature (20 °C) so that the (Na^+ + K^+)ATPase could be 'activated'. It has been suggested by Jørgensen (1975) that the detergent activation of (Na^+ + K^+)ATPase is due to exposure of latent enzyme sites in the preparation. After the 'activation' period, 25 µl aliquots of the solution were transferred to test tubes containing 1 ml 3 mM ATP in incubation buffer for enzyme assay. The tubes were incubated at 37 °C for 30 min and at the end of this time the reaction was terminated by the addition of 100 µl 50% trichloroacetic acid. Liberated inorganic phosphate was then assayed by Allen's (1940) modification of the Fiske & Subbarow (1925) method and enzyme activity was expressed as µmol Pi (mg protein)$^{-1}$ h^{-1}. For each experimental condition the incubation was carried out in the absence and presence of 10^{-3} M ouabain. (Na^+ + K^+)ATPase activity was taken as the difference between values obtained with and without ouabain. Auranofin was added to the incubation buffer to give the assay concentrations indicated and control experiments were performed using an equivalent volume of the ethanol vehicle.

Expression of results. Results are expressed as mean values ± 1 s.e.m. of the number of observations indicated. Significance was assessed using Student's *t*-test, paired or unpaired as appropriate.

Chemicals. Ethylenediaminetetraacetic acid (EDTA), Tris base and imidazole were obtained from the Sigma Chemical Co., St Louis, USA Auranofin was generously supplied by Smith Kline and French Research Limited, Welwyn, Hertfordshire, UK.

Results

Effect of auranofin on Na^+ and fluid transport. The presence of auranofin in the mucosal fluid reduced the absorption of both fluid and Na^+ by everted sacs of rat colon (Fig. 1). This effect was dose-dependent, with

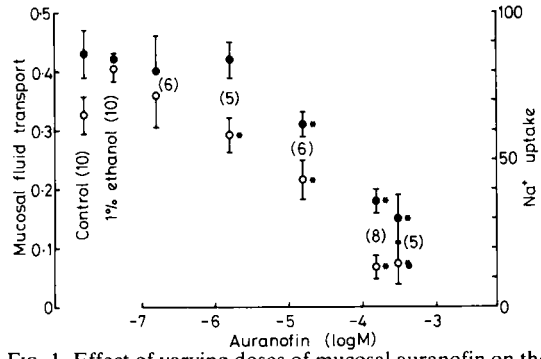

FIG. 1. Effect of varying doses of mucosal auranofin on the mucosal fluid transport (ml g^{-1} initial wet weight in 30 min; ●) and Na^+ uptake (µmol g^{-1} initial wet weight in 30 min; ○) by everted sacs of rat colon. Mucosal fluid transport is measured as the amount of fluid taken up by the sac from the mucosal fluid during the 30 min incubation. Na^+ uptake is calculated as the difference between the sum of the Na^+ content of the colonic homogenate and serosal fluid before and after incubation. Each value represents the mean ± 1 s.e.m. with the number of observations in parentheses. The values obtained with auranofin were compared with the ethanol controls and significance was assessed using an unpaired *t*-test. A significant difference ($P < 0·001$) is represented with an asterisk.

transport reduced to 50% control values at concentrations of 5·9 × 10^{-5} M for fluid absorption and 1·9 × 10^{-5} M for Na^+ uptake. The ethanol vehicle used to dissolve the drug did not affect significantly the transport of either fluid or Na^+ (Fig. 1). In contrast to the inhibitory actions of mucosal auranofin, serosal application of the drug was without effect ($P > 0·05$ for both fluid and Na^+ absorption).

Effect of auranofin on ATPase activity. The absorption of Na^+ by the colon depends on the activity of the Na^+ pump and this may therefore be the target for auranofin action. (Na^+ + K^+)ATPase activity can be used as an index of Na^+ pump function (Dahl & Hokin 1974) and when this was measured, it was found that auranofin caused a dose-dependent inhibition (Fig. 2), with a reduction to 50% control activity occurring at a concentration of 4·0 × 10^{-5} M. The ethanol vehicle did not significantly affect either (Na^+ + K^+)ATPase or Mg^{2+} ATPase activities. The maximum inhibition of (Na^+ + K^+)ATPase activity observed with auranofin was 79·8%, which is comparable to the 83·0% obtained in the case of Na^+ uptake by colonic sacs. The action of auranofin on (Na^+ + K^+)ATPase activity did not appear to be non-specific since Mg^{2+} ATPase activity was little affected by the drug (Fig. 2).

Discussion

The possibility that colonic transport mechanisms might be affected by auranofin was first suggested by the data of Van Riel et al (1983) when they observed changes in faecal Na^+ and K^+ levels during auranofin treatment in

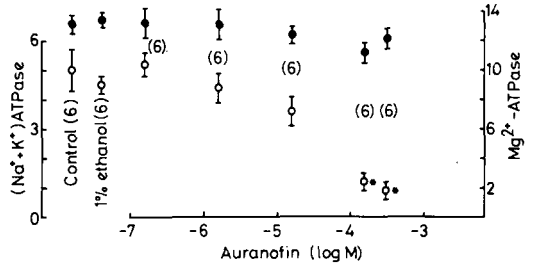

FIG. 2. Effect of varying doses of auranofin on the ATPase activity of homogenates of rat colonic mucosa. Enzyme activity was measured as the release of Pi in a timed assay. $(Na^+ + K^+)$ATPase activity (μmol Pi mg protein^{-1} h^{-1}; ○) was calculated as the inhibition of total ATPase activity caused by the presence of 10^{-3} M ouabain. Mg^{2+}ATPase activity (μmol Pi mg protein^{-1} h^{-1}; ●) was that portion of the total ATPase activity that was not affected by ouabain. Each value represents the mean ± 1 s.e.m. with the number of observations in parentheses. The values obtained with auranofin were compared with the ethanol controls and significance was assessed using a paired *t*-test. A significant difference ($P < 0.001$) is represented with an asterisk.

man. It has been suggested that the drug could act by inhibiting colonic $(Na^+ + K^+)$ATPase activity (Heuer & Morris 1982) and auranofin has been shown to act in this way in the small intestine (Fondacaro et al 1986; Henderson et al 1984). The present investigation in the rat has shown that auranofin does indeed inhibit colonic $(Na^+ + K^+)$ATPase activity (Fig. 2) and that this is associated with a reduction in the ability of the colon to absorb Na^+ and fluid (Fig. 1).

The inhibition by auranofin of $(Na^+ + K^+)$ATPase activity in the small intestine leads, via dissipation of the Na^+ gradient, to a reduction in Na^+-linked transport processes (Hardcastle et al 1984). Hence the absorption not only of Na^+, but also of sugars and amino acids, is impaired and leads to an increase in the fluid load reaching the colon. Under normal circumstances the colon has a considerable reserve capacity (Binder 1979), but as the present study has shown, colonic transport mechanisms are also inhibited by auranofin. Thus an increased fluid load is presented to a colon that is less capable of coping with it and this may be responsible for the diarrhoea that is experienced by patients receiving auranofin.

Since auranofin inhibits Na^+-linked transport processes via its inhibition of the Na^+ pump, it also reduces the reabsorption of bile acids in the terminal ileum (unpublished observations). This will lead to an accumulation of bile acids in the small intestine and their passage into the colon. In both these locations bile acids stimulate a net secretion of fluid and electrolytes (Binder 1980), so leading to further intestinal fluid loss.

Auranofin is administered orally and so comes into contact with the mucosal surface of the alimentary tract. Since only 25% of auranofin is absorbed (Gottlieb 1983), a substantial proportion of the dose administered is likely to reach the colon. Thus the inhibitory effects of auranofin observed in the in-vitro colonic preparations described in this study may also apply to patients taking the drug.

In conclusion auranofin reduces absorptive processes not only in the small intestine but also in the colon. This results from the ability of the drug to inhibit the Na^+ pump by reducing $(Na^+ + K^+)$ATPase activity. This action may contribute to the diarrhoea experienced during auranofin therapy.

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