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Auranofin inhibits the active uptake of bile acid by rat ileum

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Auranofin in the mucosal fluid inhibited the active Na^+-dependent absorption of taurocholic acid by everted sacs of rat ileum. It did not however, affect the passive uptake of taurocholic acid by sacs of mid-intestine. The inhibition by auranofin of active bile acid absorption could result from its previously demonstrated ability to inhibit Na^+, K^+-ATPase activity. A reduction in the reabsorption of bile acids by the ileum may contribute to the diarrhoea experienced by some patients taking auranofin.

Auranofin (2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*(triethylphosphine) gold) is an orally effective gold preparation which is used in the treatment of rheumatoid arthritis. It does however, cause some side-effects, the most common of which is a change in bowel habit, usually diarrhoea (Heuer & Morris 1983). A factor which may contribute to this is the drug's inhibition of the active absorption of sugars and amino acids by the small intestine (Hardcastle et al 1984, 1986). Both these groups of nutrients utilize the Na^+ gradient in their absorption, and the ability of auranofin to reduce Na^+ pump activity by inhibiting Na^+,K^+-ATPase could explain its effects on nutrient uptake (Hardcastle et al 1986). Another group of compounds that depends on the Na^+ gradient for absorption is the bile acids which are actively reabsorbed by a Na^+-dependent mechanism in the terminal ileum (Holt 1964). This process could be inhibited by auranofin with important consequences on net fluid movement across the intestinal tract and the present investigation was designed to assess this possibility.

Methods

Experiments were carried out on male albino rats (230–260 g) obtained from the Sheffield Field Laboratories. 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.).

The transport of fluid and taurocholic acid was measured in everted sacs (Wilson & Wiseman 1954) taken from two regions of the intestine: the mid-intestine and the last 10 cm of the ileum. Each 10 cm sac was filled with 0·5 mL fluid (serosal fluid) and incubated at 37 °C for 30 min in 25 mL mucosal fluid. The incubation medium was Krebs bicarbonate saline (Krebs & Henseleit 1932), gassed with 95% O_2/5% CO_2. When low Na^+ conditions were required, the Na^+ concentration was reduced to 25 mM by replacing all the NaCl in the Krebs with isotonic Tris Cl. Fluid transport was measured gravimetrically and expressed as mL g^{-1} initial wet weight/30 min. The absorption of taurocholic acid was assessed using ^{14}C-labelled taurocholic acid (0·5 μCi/100 mL) which was added to 2 mM taurocholic acid in the mucosal fluid. At the end of the incubation period the serosal fluid was collected and the sac was deproteinized with 5 mL 10% sodium tungstate and 5 mL 0·33 M H_2SO_4, homogenized and then filtered. Samples of mucosal fluid, serosal fluid and gut homogenate were then added to scintillation fluid (Bray 1960) and counted in a liquid scintillation counter (LKB, 1215 Rackbeta). Taurocholic acid absorption was expressed in two ways: firstly as the amount taken up into the gut and serosal fluid, expressed in μmol g^{-1} initial wet weight/30 min, and secondly as the T/M ratio, i.e. the ratio of the taurocholic acid concentration in the tissue water compared with its concentration in the mucosal fluid at the end of the incubation. The tissue water content was 80% initial wet weight plus the volume of fluid taken up by the gut during the incubation. Auranofin was added to the mucosal fluid to give a concentration of $1·47 \times 10^{-4}$ M and control sacs received an equivalent volume (1 mL%) of the ethanol vehicle.

Results were expressed as mean values ±1 s.e.m. of the number of observations (n) indicated. Significance was assessed using an unpaired *t*-test.

Results and discussion

The uptake of taurocholic acid by the mid-intestine was not active, as indicated by a T/M ratio of less than 1 (Table 1). The presence of auranofin in the mucosal fluid reduced fluid uptake, consistent with its inhibition of Na^+,K^+-ATPase activity, but did not affect the passive movement of taurocholic acid. This suggests that auranofin was not altering intestinal permeability, although other studies have reported that patients on treatment with auranofin who develop overt diarrhoea demonstrated an increase in intestinal permeability (Behrens et al 1986). The taurocholic acid itself had no significant effect on fluid absorption ($P > 0·05$).

In the ileum, taurocholic acid was actively absorbed, with a T/M ratio significantly ($P < 0·001$) greater than 1 (Table 1). This process was Na^+-dependent since reducing the Na^+ concentration of the medium from 143 to 25 mM caused a significant inhibition (Table 1). These observations that taurocholic acid was absorbed actively

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Table 1. Effect of auranofin ($1{\cdot}47 \times 10^{-4}$ M) on the absorption of fluid and taurocholic acid (initial concentration 2 mM) by everted sacs of rat ileum and mid-intestine. Additions were made to the mucosal fluid as shown. The effect of Na^+ on taurocholic acid absorption in the ileum was assessed by reducing the Na^+ concentration of the medium to 25 mM (low Na^+). Each value represents the mean ± 1 s.e.m. of the number of observations (n) indicated. The action of auranofin was compared with the ethanol controls (1 mL % ethanol in mucosal fluid) and that of low Na^+ was compared with taurocholic acid alone. Significance was assessed with an unpaired *t*-test.

Additions to mucosal fluid	n	Mucosal fluid transport (mL g^{-1} iww/30 min)	Taurocholic acid uptake (μmol g^{-1} iww/30 min)	T/M ratio
Mid-intestine				
None	5	0·37 ± 0·05	—	—
Taurocholic acid	10	0·40 ± 0·02	0·89 ± 0·07	0·31 ± 0·03
Taurocholic acid + ethanol	11	0·35 ± 0·02	0·89 ± 0·05	0·33 ± 0·02
Taurocholic acid + auranofin	13	0·16 ± 0·02*	0·81 ± 0·10^{NS}	0·37 ± 0·04^{NS}
Ileum				
None	4	0·51 ± 0·06	—	—
Taurocholic acid	9	0·17 ± 0·01	3·78 ± 0·19	1·56 ± 0·07
Taurocholic acid, low Na^+	6	0·13 ± 0·01^{NS}	2·72 ± 0·19*	1·13 ± 0·05**
Taurocholic acid + ethanol	15	0·19 ± 0·01	4·38 ± 0·16	1·80 ± 0·07
Taurocholic acid + auranofin	15	0·17 ± 0·02^{NS}	3·24 ± 0·13**	1·35 ± 0·03**

*$P < 0{\cdot}01$, **$P < 0{\cdot}001$, ^{NS} Not significant.

from the ileum but not from more proximal regions are in agreement with earlier studies (Holt 1964). In the ileum, fluid uptake was significantly smaller ($P < 0{\cdot}001$) in the presence of taurocholic acid than in its absence, consistent with the view that bile acids can stimulate secretion (Binder 1980). Auranofin failed to affect fluid uptake further, although it did reduce the absorption of taurocholic acid (Table 1) when compared with the ethanol control. This action may be a consequence of the inhibition of Na^+,K^+-ATPase activity caused by the drug (Hardcastle et al 1986) which will reduce Na^+ pump activity and so decrease the Na^+gradient between intra- and extracellular fluids which provides the driving force for active absorption.

Any decreased absorption of bile acids could have several consequences, many resulting in an accumulation of fluid in the lumen (Binder 1980). Thus the ability of auranofin to inhibit bile acid reabsorption may have more profound effects on net fluid movement in the gut than its inhibition of hexose and amino acid absorption, and may make a significant contribution to the diarrhoea experienced by patients receiving this drug.

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