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Effects of neomycin on galactose absorption across rat jejunum

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1 The effects of neomycin sulphate on galactose absorption have been studied using *in vivo* and *in vitro* preparations of rat small intestine.

2 Neomycin (10^{-3} M) produced an increase in the maximum transport capacity (J_{max}) for the active component of absorption *in vivo*. The apparent K_t for absorption was unaffected.

3 The antibiotic caused a dose-dependent increase in the potential difference across the mucosal membrane (V_m) measured *in vitro*, a maximal effect being seen at a concentration of 10^{-4} M. Furthermore, the magnitude of the depolarization induced by the addition of galactose (4 mM) to the mucosal fluid was enhanced by neomycin (10^{-4} M). Phlorhizin (10^{-4}M) abolished the galactose-induced depolarization in both the absence and presence of the antibiotic.

4 It is concluded that neomycin increases the electrical driving force for Na^+ during Na^+-coupled galactose entry into the enterocyte.

Introduction

Previous studies have shown that the feeding of neomycin sulphate increases the absorption of sugars and amino acid across the small intestine of the rat measured *in vitro* (Robinson *et al.*, 1966; Rogers *et al.*, 1968; Kessler *et al.*, 1978). This response appears to be unrelated to the antibiotic properties of neomycin since the addition of the compound to the mucosal fluid enhances the intestinal uptake of glucose both *in vitro* (Rogers *et al.*, 1968) and *in vivo* (Small *et al.*, 1966; Broitman *et al.*, 1967). One important factor which has not been considered in previous studies of the effect of neomycin on sugar uptake *in vivo* is the existence of at least two components of absorption, an active electrogenic Na^+-dependent mechanism upon which is superimposed a diffusive process (Debnam & Levin, 1975; Murakami *et al.*, 1977; Debnam, 1982). The present investigation was undertaken primarily to study the effects of neomycin on the active pathway of sugar absorption. Accordingly, the uptake by the jejunum of galactose was measured using a technique which allowed the kinetic parameters of 'apparent' K_t and the maximum absorptive capacity (J_{max}) of the active transfer mechanism to be obtained *in vivo* (Debnam & Levin, 1975).

The potential difference across the brush border membrane of the enterocyte is known to be an important driving force for Na^+-sugar cotransport into the cell (Schultz, 1977). Thus, in order to gain further insight into the effects of neomycin on active sugar absorption, experiments have been carried out to examine the effects of the antibiotic on electrophysiological changes associated with galactose absorption across the mucosal membrane. A preliminary account of this work has been presented to the Pharmacological Society (Debnam & Thompson, 1983).

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Methods

All experiments were carried out on male Sprague-Dawley rats weighing 230–260 g maintained on Diet 86 (Oxoid, London).

Measurement of sugar absorption in vivo

Rats were anaesthetized with pentobarbitone (90 mg kg^{-1} i.p. Sagatal, May & Baker Limited). A mid-line incision into the abdominal cavity was made and a section of the mid-jejunum approximately 25 cm long was selected. The segment was washed through with warm NaCl (154 mM) and cannulated at each end. The cannulae were connected to a fluid circuit through which bicarbonate saline (Krebs & Henseleit, 1932) maintained at 37°C and gassed with 95% O_2 : 5% CO_2 (v/v) was pumped at a flow rate of 2 ml min^{-1} by means of a peristaltic pump (Watson-

Marlow, England). The solution was recirculated for a period of 15 min duration from a reservoir containing 12.5 ml of bicarbonate saline. The diameter of the distal cannula relative to that of the proximal cannula ensured that distension of the intestine did not occur. Rectal temperature was maintained at 37°C throughout the experiment by a blanket control system (BioScience, England).

The kinetics of galactose absorption were determined by circulating increasing concentrations of the sugar (up to 64 mM) dissolved in bicarbonate saline through the intestinal segment. All solutions were made to the same tonicity as described by Debnam & Levin (1975). After the circulation period, the sugar solution in the intestine and circulation system was washed out, diluted to a known volume and deproteinised using 0.3N barium hydroxide/5% zinc sulphate. The resulting solution was filtered and an aliquot of the filtrate was estimated for galactose by the method of Nelson (1944) as modified by Somogyi (1945). The segment of the intestine was removed and its length measured. Absorption of galactose at each concentration was calculated as luminal loss in the amount initially present minus that recovered after 15 min and expressed as μmol 10 cm^{-1} 15 min^{-1}.

In separate experiments, the rate of galactose absorption at each initial concentration was corrected for the non-saturable component of absorption using phlorhizin (2×10^{-3} M). Preliminary experiments showed that this concentration was the minimum necessary to abolish the galactose-induced transmural potential difference measured *in vivo*. Furthermore, the inhibitory effect on the potential difference was reversible. Subtraction of the amount of sugar absorbed in the presence of phlorhizin from that absorbed in its absence at each concentration of galactose used allowed an estimate of uptake via the active, saturable transport mechanism (Debnam & Levin, 1975). The kinetic parameters of 'apparent' K_t (an index of the affinity of the intestinal mechanisms for the transferred sugar) and J_{max} (maximum absorptive capacity) for the active component were determined by Lineweaver-Burk analysis of the corrected absorption data. Lines were determined by least-square analysis of the data.

Measurement of potential difference across the mucosal membrane

A 20 cm section of jejunum of anaesthetized rats was selected, washed through with warm NaCl (154 mM) and a 1 cm portion rapidly removed. The tissue was cut longitudinally along the mesenteric border and mounted as a flat sheet on a Perspex disc using cyanoacrylate adhesive (RS Components Limited) applied to the muscle side. The disc formed the base of a tissue bath, a rubber gasket being used to prevent fluid leakage. The preparation was superfused at 35 ± 1°C with bicarbonate saline gassed with 95% O_2 : 5% CO_2 (v/v) and fed by gravity to achieve a flow rate of 2 ml min^{-1}.

In some experiments, galactose (4 mM) was present in the bicarbonate saline. The time period between the removal of intestine from the animal and exposure to buffer never exceeded 1 min. A maximum of four 1 cm sections of intestine were used from the same initial 20 cm length. The time period during which electrical measurements were made on any one section of tissue never exceeded 30 min in duration. During this time, the animal's body temperature was maintained at 37°C with a heating blanket (Biosciences, England).

Microelectrodes were prepared from 1.5 mm o.d. borosilicate glass tubing (Kwik-fil, Clark Electromedical Instruments, England) and were filled with filtered 3M KCl. Electrodes were selected for a resistance of 15–25 MΩ and a tip potential of less than 5 mV. The size of the electrode tip was in the range 0.5–1.0 μm. A silver/silver chloride miniature half cell (Clark Electromedical Instruments, England) connected the microelectrode to the input of a high impedance preamplifier (Model KS700, W.P. Instruments, U.S.A.). A 3M KCl-agar bridge in contact with the bathing solution was used as the reference electrode and, therefore, in all further discussion the electrical potential of the bathing solution is taken as zero. The microelectrode was supported vertically and lowered gradually using a hydraulic drive micromanipulator (Trent Wells Inc., U.S.A.). Although impalements were not under direct visual control, the electrophysiological study by Cremaschi *et al.*, (1982) would suggest that cells studied by random impalements are sited on the top part of the villus. The criteria for an acceptable impalement were (a) an immediate negative deflection; (b) maintenance of a stable potential difference for at least 15 s; (c) an abrupt return to baseline upon withdrawal of the microelectrode and (d) a similar electrode resistance (± 10%) and tip potential(± 2 mV) before and after impalement. A strip chart recorder (Model SR 201A, Heath Electronics) was used to obtain permanent traces of the results and, in addition, the output of the microprobe was continuously monitored on an oscilloscope (Telequipment Model D1011).

Preliminary experiments indicated that the technique used to change the bathing fluid during impalement resulted in instability of the V_m. It was therefore not possible to monitor continuously changes in V_m. in individual cells during exposure of the tissue to buffer containing galactose. All results presented are, therefore, values obtained in different, successively punctured cells.

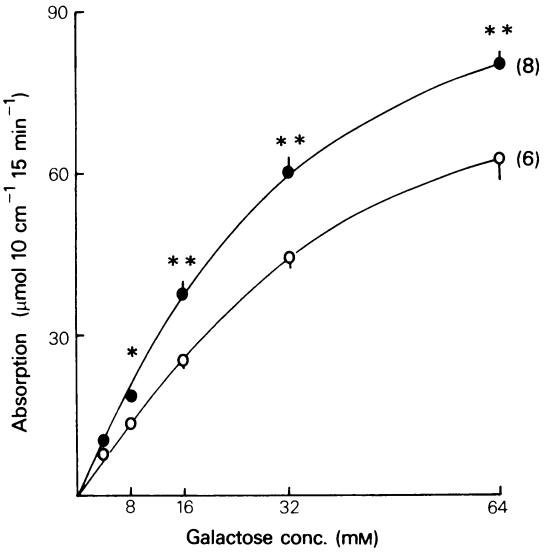

Figure 1 The kinetics of galactose absorption measured *in vivo* with (●) or without (○) neomycin sulphate (10^{-3} M). Values have been corrected for the diffusive component (see text). The data are given as mean (s.e. indicated by vertical lines) with number of animals used in parentheses. * $P<0.05$; ** $P<0.005$.

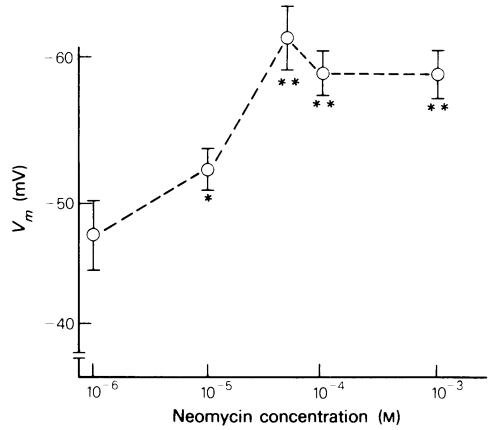

Figure 2 Dose-response curve showing the effect of neomycin sulphate on the membrane potential across the brush border membrane of rat jejunum. Results are given as mean with s.e.mean indicated by vertical lines. * $P<0.02$; ** $P<0.001$.

Statistical analysis

All values are expressed as mean ± s.e. Differences between means were evaluated by a Student's *t* test for unpaired data and considered not significant at $P>0.05$.

Chemicals

D-Galactose (glucose-free), phlorhizin and neomycin sulphate were obtained from Sigma U.K. Limited. All other chemicals were of Analar Grade from B.D.H. Limited.

Results

Effect of neomycin on the kinetics of active galactose absorption in vivo

By using phlorhizin to inhibit the active, electrogenic component of absorption, the relative contributions of the 'active' and 'linear' processes can be identified and quantified. The influence of neomycin sulphate on the corrected data for galactose absorption is shown in Figure 1. A stimulatory effect of neomycin on absorption was seen at sugar concentrations from 8–64 mM. At 4 mM the difference was not statistically significant ($P>0.05<0.1$) despite a 22.6% increase in absorption. Kinetic analysis of the corrected absorption data revealed an apparent *K*t for control and neomycin conditions of 36.8 ± 2.1 (8) and 39.4 ± 3.9 mM (6) respectively ($P>0.06$) and a J_{max} of 96.0 ± 7.1 (8) and 134.4 ± 4.9 (6) μmol 10 cm^{-1} 15 min^{-1} respectively ($P<0.001$).

Effect of neomycin on the phlorhizin-insensitive component of absorption

In contrast to the effect of neomycin on the active, phlorhizin-sensitive component of absorption, no effect of the antibiotic was observed on galactose absorption in the presence of phlorhizin (2×10^{-3} M). As an example, absorption of galactose from an initial concentration of 64 mM was found to be 67.1 ± 5.2 (8) and 67.0 ± 2.8 μmol 10 cm^{-1} 15 min^{-1} (6) respectively in the absence and presence of neomycin ($P<0.95$).

Effect of neomycin on mucosal membrane potential (V_m)

The presence of neomycin sulphate in the mucosal fluid caused a dose-dependent increase in V_m (Figure 2), with no effect observed at a concentration of 10^{-6} M and a maximum response being seen at 10^{-4} M. The addition of galactose (4 mM) to the mucosal fluid caused a significant depolarization of V_m in both the absence ($P<0.02$) and the presence ($P<0.001$) of 10^{-4} M neomycin (Table 1). However, the magnitude of the galactose-induced reduction in membrane potential difference in the presence of the antibiotic was significantly greater than that in the

Table 1 Effect of neomycin sulphate (10^{-4} M) on the galactose-induced change in the potential difference across the brush border membrane of rat small intestine

	V_m (mV)	
	Control	*+ Neomycin*
Bicarbonate buffer	-45.9 ± 1.3 (116)	-58.7 ± 1.6 (35)
+ Galactose (4 mM)	-40.3 ± 1.9 (50)	-44.8 ± 2.1 (29)
+ Galactose (4 mM) + phlorhizin (10^{-4} M)	-47.2 ± 2.1 (30)	-58.8 ± 1.6 (29)

Results are given as mean ± s.e.mean with numbers of impalements shown in parentheses.

buffer alone ($P > 0.01 < 0.02$). Phlorhizin (10^{-4} M) abolished the depolarization induced by galactose in both conditions.

Discussion

Although previous studies have claimed that neomycin sulphate causes an increase in hexose absorption across rat small intestine, the cellular mechanisms involved are unknown. This study is the first to describe the effects of the antibiotic on the kinetics of active sugar absorption and on the electrophysiology of the brush border membrane.

Broitman *et al.* (1967) using an *in vivo* preparation of rat small intestine noted that glucose uptake from initial luminal concentrations ranging from 5–50 mM was increased by neomycin. In this present study we have confirmed and extended the work of Broitman *et al.* (1967) using a technique which allows a separation and quantification of the active, saturable pathway for absorption from the linear, phlorhizin-insensistive component of uptake (Debnam & Levin, 1975). Our results show that neomycin sulphate caused a highly significant increase of 40% in the maximum transport capacity (J_{max}) of the active mechanism of galactose absorption *in vivo* whilst leaving the 'apparent' K_t unaffected. In contrast, no effect of neomycin was seen on uptake via the phlorhizin-insensitive component of absorption. This change in J_{max} for active uptake was not due to an alteration in the metabolism of the transported sugar since galactose is a hexose known to be poorly metabolised by the intestinal mucosa.

The addition of neomycin to the mucosal fluid caused a dose-dependent hyperpolarization of the brush border membrane, a maximum effect being observed at a concentration of 10^{-4} M neomycin. Furthermore, the addition of galactose to neomycin-treated tissue resulted in an enhanced depolarization of the membrane potential. The inhibition by phlorhizin of the galactose-induced depolarization in both the absence and presence of neomycin confirms our belief that the reduction in membrane potential upon addition of the sugar is a consequence of Na^+-sugar cotransport into the cell. Since the magnitude of the potential difference is recognised to be an important driving force for hexose uptake by intestinal enterocytes (Schultz, 1977), we suggest that the enhanced depolarization shown by galactose in the presence of neomycin is a result of the hyperpolarization induced by the antibiotic.

The recent work of Carter-Su & Kimmich (1980) using isolated intestinal cells showed that an increased membrane potential enhances the J_{max} but not the K_t for sugar entry. The results of both the *in vivo* and *in vitro* studies in this present work imply therefore that neomycin causes an increase in the electrical gradient for Na^+ entry during Na^+-galactose movement across the mucosal membrane.

The mechanism of action of neomycin on active absorption remains unclear. The compound is likely to have an extracellular action since it is known to be only weakly absorbed by the intestinal epithelium (Feingold, 1963). A recent study by Lemaire *et al.* (1982) using membrane vesicles prepared from rabbit intestine concluded that the polycationic nature of neomycin was responsible for the increased hexose uptake which they observed. It was suggested by Lemaire *et al.* (1982) that screening of membrane negative charges by the positively charged neomycin causes an accumulation of anions at the membrane surface resulting in an increase in the transmembrane potential difference and thus hexose uptake. However, Bieberdorf *et al.* (1975) and Elsenhans *et al.* (1983) proposed that cationic compounds rather than stimulating absorption actually inhibit the intestinal absorption of sugar by displacing Na^+ from the transport carrier or its vicinity. Clearly, a property of neomycin other than its cationic nature must be responsible for the increase in sugar transport described in this present study. It is possible that the antibiotic induces a change in Na^+ and/or K^+ conductance of the brush border membrane. Studies designed to reveal the mechanism of action of neomycin at the membrane level are at present being carried

out. A knowledge of the process by which the antibiotic achieves its effect would be of possible therapeutic benefit in conditions where it is desirable to increase the absorptive capacity for nutrients. Such treatment would, for example, reduce the severity of malabsorption resulting from a defective absorptive surface.

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