Nufenoxole, a new antidiarrhoeal agent, inhibits fluid secretion in the human jejunum

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SUMMARY Nufenoxole is an orally active antidiarrhoeal agent which binds to opioid receptors in the brain and myenteric plexus of the intestine. A perfusion technique has been used to investigate the effect of nufenoxole (1 mg/kg intrajejunally) on water and solute transport stimulated by the secretagogue, dioctyl sodium sulphosuccinate, in the human jejunum *in vivo*. Nufenoxole reversed the direction of jejunal transport of salt and water from net secretion to net absorption. These changes in water and electrolyte transport were inhibited by intravenous naloxone, the opioid antagonist. Nufenoxole possesses potent antisecretory properties, which are mediated *via* opioid receptors and may contribute to its antidiarrhoeal action in man.

Nufenoxole, a new orally active agent (Fig. 1), possesses potent antidiarrhoeal properties in both animals and man, with activity comparable to that of diphenoxylate and loperamide.^{1,2} Nufenoxole, however, has a longer biological half-life and shows a wider separation of gastrointestinal and central nervous system effects.^{1,3} Animal and human studies have revealed no evidence of dependence liability.^{2,3}

Nufenoxole binds to opioid receptors in the brain and myenteric plexus of the intestine.³ Endogenous and exogenous opioids enhance water and electrolyte absorption and inhibit fluid secretion stimulated by a variety of secretagogues in mammalian intestine.⁴⁻¹³ Although opioids are thought to exert their antidiarrhoeal effects by inhibiting intestinal motility,¹⁴⁻¹⁶ such changes in transmucosal epithelial fluid transport may be at least equally as important in mediating their action.¹⁷

The present study was performed to determine whether nufenoxole inhibits fluid secretion stimulated by the secretagogue, dioctyl sodium sulphosuccinate, DSS,^{18,19} in the human jejunum *in vivo* and, if so, to establish whether it acts *via* opioid receptors.

Methods and experimental design

SUBJECTS

Twelve healthy volunteers (four men, eight women), aged 22-54 years, gave written informed consent for the study which was approved by the Ethical Committee of St. Bartholomew's Hospital, London.

INTESTINAL PERFUSION

After an eight hour fast, each subject swallowed a double lumen intestinal perfusion tube, incorporating a proximal occluding balloon, infusion and collection orifices placed 30 cm apart and a mercury bag.²⁰ The tube was positioned under fluoroscopic control such that the balloon was situated at the ligament of Treitz with the infusion orifice located in the first 5 cm of jejunum. Using a peristaltic pump, a glucose electrolyte solution containing the secretagogue, DSS, at 37°C was perfused through the infusion orifice at a rate of 15 ml/min. The perfusate contained (mM): DSS, 0.5; glucose, 10; Na 149; Cl, 124; HCO₃, 25; polyethylene glycol (PEG), 2.5 g/L and 1 μ Ci/L of [¹⁴C] PEG as a non-absorbable marker. The solution was continuously gassed throughout each experiment with 95% O₂-5% CO₂. The procedure during perfusion of each DSS-containing solution was identical. After a 30 minute equilibration period, three successive 10 minute aspirates were collected by siphonage. Aliquots

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Fig. 1 Structures of antidiarrhoeal drugs.

were taken for immediate bicarbonate estimation and samples for determination of other electrolyte concentrations were stored at -20°C before analysis.

EXPERIMENT 1

Six subjects were studied using the protocol shown (Fig. 2a). The same perfusate was infused both before and after bolus administration of buffer and

nufenoxole for four 60 minute periods in all. A rest period of one hour was allowed after each intrajeunal bolus of buffer and buffer plus nufenoxole, and between 180–240 minutes. At 180 minutes the balloon was deflated, the subjects then being allowed to drink water as desired for the next 60 minutes and the position of the tube was rechecked before recommencing the experiment at 240 minutes. At 390 minutes, venous blood for deter-

Fig. 2 Experimental design: Experiment 1 – four DSS perfusions, each separated by an interval of 60 min ($n=6$). Buffer was administered by intrajeunal bolus at 60 min and nufenoxole (1 mg/kg) at 300 min (2a). Experiment 2 – Two DSS perfusions with intrajeunal bolus administration of both buffer and nufenoxole on each study day ($n=6$). Saline or naloxone (0.114 mg/kg bolus and 0.08 mg/kg/h) infused from 120–240 min in a cross-over design (2b).

mination of plasma nufenoxole was collected into lithium heparinised tubes, centrifuged immediately, and the plasma stored at -20°C before analysis in one batch.

EXPERIMENT 2

Six different subjects were studied on two occasions (Fig. 2b), each separated by an interval of at least one week, so as to ensure that there was no carry-over effect of nufenoxole. Saline or naloxone (0.114 mg/kg bolus over five minutes and 0.08 mg/kg/h) was infused intravenously from 120–240 minutes in a randomised crossover design.

CHEMICALS

Nufenoxole was supplied as a powder and dissolved in aqueous citric acid/sodium hydroxide buffer (pH 5.2). The composition of the buffer was citric acid, hydrous (4.2 g), sodium hydroxide (1.6 g) and 180 ml sterile water, made up to a final volume of 200 ml . Nufenoxole was dissolved in the buffer to a concentration of 2 mg/ml immediately before administration. The drug was administered intrajejunally through the infusion orifice as a single bolus dose (1 mg/kg). An equal volume of buffer was administered in an identical manner. Both the nufenoxole and buffer were provided by Searle Research and Development, High Wycombe, Bucks, UK. Naloxone hydrochloride was obtained from Du Pont (UK) Limited, Stevenage, Herts, and DSS from Sigma Chemical Company, St. Louis, Missouri, USA.

ANALYSIS OF SAMPLES AND CALCULATIONS

The concentrations of sodium, potassium, chloride, bicarbonate, and $[^{14}\text{C}]\text{PEG}$ were determined in each aspirate. $[^{14}\text{C}]\text{PEG}$ was measured in an LKB 1210 Ultrabeta liquid scintillation counter.²¹ Glucose was estimated by the glucose oxidase method.²² Sodium and potassium concentrations were measured using an EEL 227 flame photometer (Evans Electro-selenium Ltd, Halstead, Essex, UK) and chloride by an EEL chloridometer. Bicarbonate concentrations were derived from measurements of CO_2 using an automated Corning 965 CO_2 analyser (Corning Ltd, Halstead, Essex, UK). Absorption rates of water and solutes from the test segment were calculated using standard formulae.²³ Net absorption (+) indicates a net transfer of water or solute from the lumen; net secretion (–) indicates net transfer of water or solute into the lumen.

PLASMA NUFENOXOLE ASSAY

Plasma nufenoxole was determined by a gas liquid chromatography-mass spectrometry (GCMS) assay using a cold label isotope dilution procedure. The

method involves buffered pH-extraction of both nufenoxole and its nonadeuterated internal standard from 1 ml plasma, followed by a quantitative analysis using a GCMS procedure, which is a modification of a previous method.²⁴

STATISTICAL METHODS

Statistical comparisons were performed using an analysis of variance.²⁵

Results

EXPERIMENT 1

Effect of nufenoxole

The effect of nufenoxole on water and solute transport from the perfusate is shown in Table 1. A comparison of the results of the second (DSS post-buffer) and fourth (DSS post-nufenoxole) perfusions shows that nufenoxole reversed the direction of DSS-induced net jejunal secretion of water ($p<0.005$), sodium ($p<0.005$) and chloride ($p<0.005$) to net absorption, inhibited net secretion of potassium ($p<0.001$), but had no significant effect on net movement of glucose or bicarbonate.

Effect of buffer

The results of DSS post-buffer and the pooled DSS controls (mean of DSS Control 1 and DSS Control 2) are compared in Table 1. Post-buffer, there was a significant inhibition of net jejunal secretion of water ($p<0.01$), sodium ($p<0.01$) and chloride ($p<0.005$), enhanced net glucose absorption ($p<0.05$), while net transport of bicarbonate and potassium were unchanged.

Effect of perfusion time

In order to determine whether transport values changed with time on repeated perfusion, the net jejunal transport of water and solutes induced by DSS Control 1 and DSS Control 2 were compared (Table 1). There were no significant differences between the two, with the exception that net potassium secretion was significantly increased from DSS Control 1 ($p<0.05$).

EXPERIMENT 2

Nufenoxole-effect of naloxone

The effect of intravenous naloxone on the antisecretory action of nufenoxole is represented in Table 2. Naloxone attenuated the inhibitory effect of nufenoxole on secretagogue-induced net jejunal secretion of water ($p=0.004$), sodium ($p=0.003$) and chloride ($p=0.009$). Neither nufenoxole nor naloxone had any significant effect on net transport of potassium, glucose or bicarbonate.

Table 1 Experiment 1

Net jejunal transport	DSS control 1	DSS post-buffer	DSS control 2	DSS post-nufenoxole
Water	-99.5±24.6	-4.20±14.0§	-62.7±26.5	+101.8±12.9*
Sodium	-14.4±4.48	-0.36±2.12§	-8.88±4.18	+16.01±1.66*
Chloride	-16.43±3.34	-4.91±1.51	-13.11±3.02	+7.23±0.81*
Potassium	-2.24±0.08	-1.94±0.08	-1.96±0.16¶	-1.38±0.09†
Bicarbonate	+2.23±1.56	+4.98±0.94	+3.88±0.87	+6.76±0.75
Glucose	+6.53±0.31	+7.57±0.30‡	+7.27±0.42	+7.80±0.32

Net transport of water is expressed in ml/30 cm/h and of solutes in mM/30 cm/h. + = absorption, - = secretion. Results are the mean±SEM of six observations. The level of significance of the differences between DSS post-nufenoxole and DSS post-buffer are shown by **p*<0.005 and †*p*<0.001, between DSS post-buffer and the pooled DSS controls (mean of DSS control 1 and DSS control 2) by ‡*p*<0.05, §*p*<0.01 and ||*p*<0.005, and between DSS control 1 and DSS control 2 by ¶*p*<0.05.

Plasma nufenoxole

Plasma nufenoxole concentrations 90 minutes after its intrajejunal administration were 1409±196 (mean±SEM) ng/ml, which is within the therapeutic range.

SIDE EFFECTS

No subject showed any signs of dehydration during the studies. All subjects passed loose stools during the rest period in Experiment 1 and at the end of Experiments 1 and 2. No side effect was experienced after administration of either nufenoxole or naloxone.

Discussion

Nufenoxole significantly inhibited DSS-induced net jejunal secretion of water, sodium and chloride, while net transport of glucose and bicarbonate were unchanged. These effects of nufenoxole on salt and water transport were strikingly similar in both experiment 1, in which the drug was administered at

300 minutes, and in experiment 2, in which nufenoxole was given at 120 minutes. The influence of nufenoxole on jejunal water and solute transport cannot therefore be attributed to a change in the transport characteristics of the jejunum with time. Neither the inhibition of potassium secretion by nufenoxole nor the apparent antisecretory effect of the buffer, which were observed in experiment 1, however, was reproduced on either study day in experiment 2. While there is no satisfactory explanation for these differences, they cannot be explained by desensitisation to the secretagogue, as the fluid secretion induced by DSS Control 1 and DSS Control 2 was comparable. This observation confirmed that comparisons between the buffer and nufenoxole were statistically valid.

A fixed sequence protocol, with administration of buffer always preceding nufenoxole, was necessary because the half-life of nufenoxole is 27 hours (Searle, unpublished). Free access to water was encouraged during the rest period from 180–240 minutes in order to prevent dehydration, which

Table 2 Experiment 2

Net jejunal transport	Saline			Naloxone			Statistical significance Δ Saline vs Δ Naloxone
	DSS post-buffer (a)	DSS post-nufenoxole (b)	Δ Saline (b-a)	DSS post-buffer (c)	DSS post-nufenoxole (d)	Δ Naloxone (d-c)	
Water	-118±36	+71±34	+189.0±14.3	-55.0±23.1	+0.3±29.2	+55.3±21.8	<i>p</i> =0.004
Sodium	-16.7±5.7	+12.3±5.7	+29.0±2.4	-4.5±3.1	+2.5±4.0	+7.0±3.3	<i>p</i> =0.003
Chloride	-18.1±4.7	+3.0±3.5	+21.1±2.69	-10.1±2.9	-4.2±3.5	+5.9±2.6	<i>p</i> =0.009
Potassium	-1.84±0.28	-1.62±0.1	+0.22±0.36	-1.71±0.18	-1.69±0.12	+0.02±0.15	NS
Bicarbonate	+1.9±1.0	+7.1±1.8	+5.2±1.5	+3.3±0.6	+5.5±1.2	+2.2±1.2	NS
Glucose	+5.7±0.6	+7.5±0.4	+1.8±0.8	+5.9±0.3	+6.9±0.4	+1.0±0.6	NS

The DSS perfusate was infused after administration of both buffer and nufenoxole on both study days. Δ values refer to the Δ (DSS post-nufenoxole - DSS post-buffer) on the iv saline and iv naloxone study days respectively. Net transport of water is expressed in ml/30 cm/h. and of solutes in mM/30 cm/h. + = enhanced net absorption. Results are the mean±SEM of six observations. *p* values refer to the level of significance of the difference between Δ saline and Δ naloxone. Naloxone significantly inhibited the anti-secretory effect of nufenoxole on net transport of water, sodium and chloride.

might have influenced transport results. The secretory effect of DSS in the human jejunum completely reverses within 60 minutes of exposure to the secretagogue;¹⁸ hence the rationale for the protocol adopted, with 60-minute intervals between successive perfusions. This design also ensured that intrajejunal administration produced therapeutically effective plasma concentrations of nufenoxole 90 minutes later, at which time its effects on water and solute transport were evaluated. Plasma concentrations of 1400 ng/ml are within the range of 200–3000 ng/ml, at which an antidiarrhoeal effect has been shown (Searle, unpublished).

Intravenous infusion of naloxone, the specific opioid antagonist,²⁶ markedly inhibited the anti-secretory effect of nufenoxole, showing that nufenoxole acts *via* opioid receptors. Naloxone has a plasma half-life of 60–90 minutes,²⁷ and when infused in the dose used in this study, rapidly produces plateau plasma concentrations without systemic side effects.²⁸ Doses of this magnitude are required to block opioid receptors of the delta and kappa type, as they are relatively naloxone-resistant.²⁹ The effect of nufenoxole is likely to be mediated *via* peripheral rather than central opioid receptors, because opioids retain their antiseecretory properties in pithed animals.³⁰ Although not found on intestinal epithelial cells,³¹ opioid receptors of the delta, kappa and mu types are present in the submucosa and myenteric plexus,^{32–36} the delta receptors being thought to play a role in the regulation of intestinal fluid transport. The relative affinity of nufenoxole for the three classes of opioid receptor is unknown.

Intestinal secretion stimulated by DSS appears to be mediated by endogenous prostaglandins of the E series.³⁷ Prostaglandin-induced intestinal fluid secretion is also inhibited by morphine,^{5 38} enkephalins³⁰ and loperamide.^{5 8 11–13} Loperamide increases the permeability of the serosal border of the enterocyte to chloride, which facilitates the absorption of sodium chloride and water into the submucosal space and reduces the net effect of the secretagogue on luminal fluid secretion.³⁹ Nufenoxole might act either by inhibiting directly the action of prostaglandins, released by DSS, on mucosal fluid transport, or by reducing their net secretory effect by stimulating an independent absorptive process.

Classically, the antidiarrhoeal activity of opioids has been attributed to their effects on motility, the slowing of intestinal transit increasing the contact time of the luminal contents with the intestinal mucosa, which promotes absorption of water and electrolytes.^{14 15} In a previous study in man, it was concluded that the antidiarrhoeal action of codeine

could be explained entirely by its inhibition of intestinal motility,¹⁶ and the antiseecretory properties of loperamide have been well documented.^{5 8–13} Nufenoxole, in common with other opioid antidiarrhoeals, increases circular muscle contractile activity in the intestine,¹ which may partly explain its antidiarrhoeal activity.

In the present study, nufenoxole markedly inhibited the effects of the secretagogue, DSS, on water, sodium, and chloride movement in the human jejunum. These changes in intestinal salt and water transport are mediated *via* opioid receptors and may contribute to the antidiarrhoeal action of nufenoxole.

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