(+)-isomer preferentially affects 5-HT (Crunelli et al 1980). It is conceivable that prolactin could be elevated through more than one mechanism by these compounds, either enhancement of 5-hydroxytryptaminergic function or inhibition of dopaminergic function. However, the evidence favours the idea that enhancement of 5-hydroxytryptaminergic function is the mechanism by which fenfluramine increases serum prolactin concentration in rats (Quattrone et al 1978). The current findings are consistent with that interpretation, but a differing involvement of 5-HT and dopamine in the actions of the individual stereoisomers of the compounds studied cannot be ruled out.

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Evidence implicating a prostaglandin as the mediator of intestinal fluid secretion induced by pithing: inhibition by indomethacin, morphine and pentobarbitone

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There is evidence that the fluid transport mechanism of the small intestine is influenced by the autonomic nervous system. For example, noradrenaline stimulates the absorption of sodium and chloride by activating α-adrenoceptors (McColl et al 1968), while cholinergic stimulation results in the net secretion of fluid (Hubel 1976). However, Beubler et al (1978) and Lembeck & Beubler (1979) have shown, using the 'enteropooling' assay, that destruction of the extrinsic nerve supply of the rat small intestine by pithing does not alter the fluid volume within the lumen.

The aim of the present study was to re-examine these findings using a more accurate recirculation method, so that further experiments could be undertaken to investigate the effects of drugs on fluid transport in vivo without the complications of extrinsic nerve activity and the use of an anaesthetic. This latter point was prompted by the suggestion of Tothill (1976) that the anaesthetics pentobarbitone and urethane may interfere with the release of PGE_2 from the mucosa of the rat small intestine. This may be of some importance since prostaglandins have been implicated as physiological modulators of intestinal fluid transport (Beubler & Juan 1977).

Materials and methods

Male and female hooded rats (190–290 g) were kept in wire-bottomed cages and were deprived of food for 24 h but were given free access to drinking water. Anaesthesia was produced using halothane inhalation, the trachea cannulated and the brain and spinal cord destroyed by a 2 mm diameter needle introduced through the eye orbit. The method of pithing used was the same as the initial procedure described by Shipley & Tilden (1947) except that the vagi were not cut, the carotid arteries and jugular veins were not tied off and the animals were not pre-treated with atropine as in the preparation described by Gillespie & Muir (1967). The animal were then placed on an electrically heated pad (approximately 35 °C) and artificially respired using an air pump at the rate of 53 strokes min^{-1} and a volume of 1 ml/100 g of body weight.

A recirculation technique was used to measure the net fluid transported by the jejunum over 20 min, as has been described previously (Coupar 1978). This involved using an isosmotic solution containing (g $litre^{-1}$): NaCl 8·57, KCl 0·37, dextrose 1·0 and phenolsulphonphthalein (PSP) 0·02, to act as a non-absorbable marker for water transport. Recirculation of this solution through the lumen of the jejunum by gas lift was initiated 10 min after completing the pithing procedure. Results are expressed as the net amount of water absorbed (+) or secreted (−) per g wet weight of intestinal tissue during the 20 min perfusion.

Samples of recirculation fluid were also extracted for stable prostaglandins by an extraction procedure similar to that described by Unger et al (1971). The samples were acidified to pH 3·5 with 1% v/v formic acid and extracted with two equal volumes of chloroform, which were evaporated at 35 °C by the use of a rotary evaporator. The dry residue was blown in a stream of N_2 until all traces of formic acid were removed.

The dry extract was dissolved in 5 ml of Krebs-Henseleit solution and bioassayed against authentic PGE_2 using superfused rat fundus strips by the method similar to that of Ferreira & De Souza Costa (1976). Fundus strips were

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Table 1. The effect of various drug treatments on net fluid secretion induced by pithing. Values are of net water transport (+ absorption, − secretion) ± s.e. mean from 5 rats in each group.

Treatment	Net water transport µl g^{-1} in 20 min	*P* value compared to control (Dunnett's *t*-test)
Pithed-only control	− 140 ± 75	
+ Indomethacin	+ 102 ± 50	<0·025
+ Pentobarbitone	+ 114 ± 58	<0·005
+ Morphine	+ 105 ± 40	<0·025
+ Morphine + naloxone	− 201 ± 47	=0·28

suspended in an organ bath containing liquid paraffin maintained at 37 °C and were superfused at 0·2 ml min^{-1} down their serosal surface from a fine polyethylene tube (PE 10) acting as a connecting thread. The superfusing fluid was Krebs-Henseleit solution containing a mixture of antagonists (atropine, mepyramine, phenoxybenzamine 0·1 µg ml^{-1}; methysergide, propranolol 0·2 µg ml^{-1}; indomethacin 2 µg ml^{-1}).

Indomethacin (Merck, Sharp & Dohme) was suspended in tragacanth mucilage, so that 28·8 mg kg^{-1} was given orally in a volume of 1 ml kg^{-1}, 24 h before experiments. A slow-release preparation of morphine was prepared by incorporating morphine base into an emulsion as described by Collier et al (1972). The dose of morphine administered was 75 mg kg^{-1} subcutaneously (s.c.) in a volume of 10 ml kg^{-1}; experiments were performed 24 h later. This procedure has been shown to induce physical dependence within 24 h (Laska & Fennessy 1976). Naloxone HCl (Endo) was dissolved in 0·9% w/v NaCl and injected in a volume of 1 ml kg^{-1}, 5 min before recirculating the perfusion solution of the jejunum. The source of the other drugs used were: atropine sulphate (Sigma), halothane (ICI), methysergide hydrogen maleate (Sandoz), pentobarbitone sodium ('Nembutal', Abbott), phenoxybenzamine hydrochloride (Smith, Kline & French) and prostaglandin E_2 (PGE_2) (Upjohn).

The values in the text are of means ± standard error means. Pairs of means derived from single experiments were analysed by Student's unpaired *t*-test. The effect of different treatments on means was determined by analysis of variance and the significance of individual means against a common control was determined by Dunnett's *t*-test. Two-tailed tests of significance were used unless specifically indicated. Differences were considered statistically significant when the value of *P* was smaller than 0·05.

Results

In non-pithed rats anaesthetized with pentobarbitone (80 mg kg^{-1} s.c.) there was net fluid absorption of 257 ± 67 µl g^{-1} from the jejunum in the 20 min perfusion period (n = 5). By contrast, pithing induced a net secretion of 140 ± 75 µl g^{-1} in 20 min (n = 5, $P < 0·001$). Three drug treatments prevented net fluid secretion in pithed animals (Table 1). These were indomethacin (28·8 mg kg^{-1}, orally 24 h before experiments), an anaesthetic dose of pentobarbitone (80 mg kg^{-1} s.c. 1 h before perfusing the jejunum) and a slow release preparation of morphine (75 mg kg^{-1} s.c.) given 24 h before experiments. This antisecretory effect of morphine was blocked by naloxone (2 mg kg^{-1} s.c. 5 min before perfusing the jejunum). The potential effect of naloxone alone was not determined. However, previous results have shown that the above dose of naloxone does not alter the values of normal water absorption or secretion induced by PGE_1 in the rat jejunum (Lee & Coupar 1980).

The amount of PG-like material secreted into the jejunal lumen was higher in pithed-only rats compared with non-pithed pentobarbitone-anaesthetized rats. The values were 1·80 ± 0·43 (n = 6) and 0·09 ± 0·02 (n = 6) ng PGE_2 equivalents g^{-1} in the 20 min perfusion respectively ($P < 0·01$).

Table 2. Prostaglandin E_2-like activity released into the lumen of the jejunum. In the first set of results, the treatments (control, indomethacin or pentobarbitone) had no significant effect on the means ($P = 0·14$, analysis of variance). * Indicates the difference between control and indomethacin was significant on the basis of a 1-tailed Student's *t*-test ($P < 0·05$). In the second series of results, the means were not affected significantly by the treatments ($P = 0·83$, analysis of variance).

Treatment	ng PGE_2 equivalents g^{-1} in 20 min	Number of determinations
Pithed-only control	1·80 ± 0·43	6
Indomethacin	0·78 ± 0·12*	5
Pentobarbitone	2·30 ± 0·69	6
Pithed-only control	1·54 ± 0 88	5
Morphine	1·94 ± 1·00	5
Morphine + naloxone	1·17 ± 0·66	5

Further assays of PGE-like material were performed in two blocks each with their own controls in order to reduce systematic error. The results are shown in Table 2. Compared to the pithed-only group, the first assay indicated slightly more PGE_2-like material in the pentobarbitone-treated group ($P = 0·55$, Student's *t*-test) and less than half in the indomethacin-treated group ($P < 0·05$, 1-tailed Student's *t*-test). The results of the second assay showed that the means of PGE_2-like activity were not significantly different in control, morphine-treated and morphine plus naloxone groups ($P = 0·83$, analysis of variance).

Discussion

The present study demonstrates that fluid transport by the jejunum is reversed to net secretion in pithed rats, compared with non-pithed pentobarbitone-anaesthetized animals. This difference may be caused by increased synthesis of prostaglandin E_2 (PGE_2)-like material in pithed rats on the basis of the following evidence.

An E-type prostaglandin was a candidate for mediating the fluid secretion since PGE compounds cause dose-related inhibition of absorption, followed by stimulation of water secretion (Greenough et al 1969; Matuchansky & Bernier 1971; Coupar & McColl 1975). The main products of cyclo-oxygenase in extracts of rat jejunum were reported to be PGE_2, $PGF_{2\alpha}$ and PGD_2 (Knapp et al 1978). PGE_2 stimulates rat intestinal fluid secretion when present in the blood (Coupar & McColl 1975), when instilled into the lumen (Tothill 1976) or perfused through the lumen of the

small intestine (Beubler & Juan 1977). PGE_2 is a more potent secretagogue than $PGF_{2\alpha}$ (Robert 1976; Beubler & Juan 1977), and PGD_2 is only a weak partial agonist (Robert et al 1979).

The extraction method used in this study is one that isolates stable prostaglandins, free from interfering bases. The bioassay using rat fundus strips is 17·5 times more sensitive to PGE_2 than to $F_{2\alpha}$ (Bergström et al 1968) and several orders less sensitive to prostaglandins of the A, B and D-series (Salmon & Karim 1976; Whittle et al 1979). Using these methods, the present results demonstrate that a small amount of PGE_2-like material is secreted into the perfusion solution of the jejunum of pentobarbitone-anaesthetized rats. However, approximately 20 times more was recovered from the jejunum of non-pentobarbitone-anaesthetized pithed rats. Indomethacin prevented fluid secretion induced by pithing and reduced the output of PGE_2-like material into the perfusion fluid. The dose of indomethacin was selected on the basis that it inhibits cyclo-oxygenase in rat small intestine (Fitzpatrick & Wynalda 1976) but the reduction in jejunal PGE_2-like secretion was modest (about 50%) and only of borderline statistical significance. This was probably a result of the variable amounts of PGE_2-like material recovered from the luminal fluid. It is also possible that since indomethacin was given 24 h before experiments, that only a small inhibition of cyclo-oxygenase remained at the time of measurements.

The effect of morphine was expected since it non-selectively inhibits intestinal fluid secretion caused by PGE_1 (Coupar 1978), carbachol (Beubler & Lembeck 1979) or vasoactive intestinal peptide (Beubler & Lembeck 1979; Lee & Coupar 1980). The antisecretory effect of morphine involves interaction with an opiate receptor (Coupar 1978; Beubler & Lembeck 1979; Lee & Coupar 1980), and the present results show no inhibition by morphine on the output of PEG_2-like material. Since the central nervous system was destroyed by pithing, the present results also demonstrate that morphine acts peripherally to block secretion.

Tothill (1976) suggested that anaesthetics interfere with the release of PGE_2 from the intestine: crude cholera enterotoxin stimulted the intraintestinal secretion of both fluid and PGE_2-like material in conscious rats, but not in animals anaesthetized with pentobarbitone or urethane. However, the present results indicate that an anaesthetic dose of pentobarbitone does not inhibit pithing-induced release of PGE_2-like material in rat intestine, so that pentobarbitone may inhibit the effect of PGE_2-like material. Another possibility is that pentobarbitone inhibits the activity of nerves or mediators which co-operate with secretory stimuli. However, Beubler et al (1978) showed that pentobarbitone (or urethane) did not affect 'entero-pooling' in response to PGE_2. This 'enteropooling' method (Robert et al 1979) measures the intraluminal fluid volume of the entire small intestine, including contributions of fluid from gastric, biliary and pancreatic secretions. Furthermore, the results may be influenced by changes in gastrointestinal motility.

In conclusion, the present results, obtained by a recirculation method, provide evidence that pithing induces intestinal fluid secretion stimulated by the release of an E-type prostaglandin. The site and the mechanism of the prostaglandin release has not yet been determined. Additionally, the results indicate that the secretory effect of PGE_2 may be inhibited by pentobarbitone or that pentobarbitone might inhibit the activity of nerves which co-operate with secretory stimuli.

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Evidence for an A_2-like adenosine receptor on cerebral cortical neurons

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The concept of extracellular purine receptors has been generally accepted for both peripheral and central tissues where adenosine and the adenine nucleotides exert dose dependent depressant or excitant effects on activity (Phillis & Wu 1981). Burnstock (1978) has proposed the existence of two types of extracellular receptor in peripheral tissues. The P_1 purinoceptor is more responsive to adenosine and adenosine 5′-monophosphate (AMP) than to adenosine 5′-triphosphate (ATP) and is antagonized by the methylxanthines while the P_2 purinoceptor is activated by ATP and adenosine 5′-diphosphate (ADP), and is antagonized by quinidine and 2,2′-pyridilisatogen tosylate. Studies on the effects of adenosine on adenylate cyclase from various tissues have led to a further classification of the adenosine receptors into a high affinity A_1 type which inhibits, and a lower affinity A_2 type which activates, the enzyme (Van Calker et al 1979; Londos et al 1980). The order of potency of certain adenosine analogues is different for the two receptor subtypes. However both are antagonized by methylxanthines. The purpose of the present study was to determine whether the P_1 receptor on cerebral cortical neurons can be equated with either the A_1 or A_2 subtypes of adenosine receptor associated with adenylate cyclase.

Experiments were performed on 18 male Sprague-Dawley rats (350–500 g). Following induction of anaesthesia with halothane, and tracheal intubation, the animals were placed in a stereotaxic frame and maintained on methoxyflurane in a mixture of nitrous oxide and oxygen (80:20). An electric heating pad controlled by a rectal probe maintained body temperature at 37°C. A small hole was drilled through the parietal bone 2 mm lateral to the sagittal suture and 1·5 mm posterior to the coronal suture line. A small slit was made in the exposed dura to expose the sensorimotor cortex. The exposed subcutaneous areas, muscle and bone were covered with a thin layer of 4% agar in Ringer solution to prevent drying.

Eleven of these rats had a cannula placed in the right femoral vein for intravenous drug injections and an arterial cannula inserted into the right femoral artery for recording arterial blood pressure on a Grass polygraph.

The recording of neuronal activity and iontophoresis of drugs was accomplished with seven barrelled micropipettes with overall tip diameters of 6–10 μm. The central recording barrel contained 2 M NaCl and the remaining barrels were filled by centrifugation with various combinations of the following solutions: adenosine hemisulphate (0·1 M pH 4, Sigma), AMP (0·1 M, pH 6·0, Sigma), 2-chloroadenosine (0·01 M, pH 5·5, Sigma), adenosine 5′-*N*-ethylcarboxamide (NECA, 0·01 M, pH 6·2, Byk Gulden Pharmazeutika), adenosine 5′-*N*-methylcarboxamide (NMCA, 0·01 M, pH 5·8, Abbott), adenosine 5′-*N*-cyclopropylcarboxamide (NCPCA, 0·005 M, Abbott), L-N^6-phenylisopropyladenosine (L-PIA, 0·001 M, pH 5·8 Boehringer Mannheim, GmbH), N^6-cyclohexyladenosine (CHA, 0·001 M, pH 5·5, Calbiochem). Substances were applied from at least three different electrode barrels onto neurons in the sensorimotor cortices of a minimum of two animals. Drugs were tested on deep (800–1200 μm) 'spontaneously firing' cortical neurons. Drug effects were evaluated by observing the alterations in the rate of neuronal firing. The relative depressant potencies of individual compounds were evaluated either on the basis of the magnitude of the currents which had to be passed through the various electrode barrels for a standard time interval to elicit comparable reductions in the firing rate or by observing the magnitude of the depressions elicited when currents of similar magnitude were passed through different barrels.

The adenosine derivatives were also tested as hypotensive agents to ascertain the structure-activity relationships of these compounds in another test system (Phillis & Kostopoulos 1975). The amount of adenosine, administered intravenously, required to produce a discernible fall in blood pressure was determined and then the amounts of the other compounds which produced a comparable fall were ascertained.

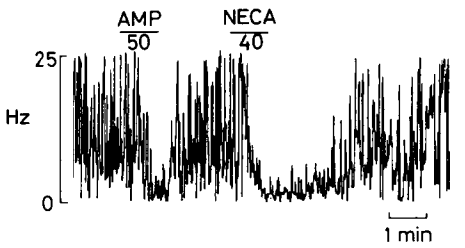

FIG. 1. Firing frequency record from a cerebral cortical neuron. This is a ratemeter record of spontaneous firing with the number of action potentials per second on the ordinate. Horizontal bars above the record indicate periods of drug application. AMP (50 nA) and NECA (40 nA) depressed the firing of the neuron. The NECA effect was more pronounced and of longer duration.