Morphine-neural interactions on canine intestinal absorption and blood flow

David Mailman

Program in Physiology, Biology Department, University of Houston, Houston, Texas 77004, U.S.A.

1 Intestinal Na and H_2O fluxes and blood flow were determined in extrinsically denervated or innervated ileum of fed dogs during intra-arterial (0.2, 2, 20 μg min^{-1}) or intraluminal (4, 40, 400 μg ml^{-1}) morphine sulphate infusion.

2 ^3H_2O and ^{22}Na were used to determine unidirectional fluxes and ^3H_2O clearances were used to determine total segmental and absorptive site blood flow.

3 Net Na and H_2O absorption decreased with time in innervated gut segments but were unchanged in denervated segments.

4 Intra-arterial morphine prevented the decrease in net Na and H_2O absorption in innervated segments due to increases in unidirectional absorptive fluxes. Intra-arterial morphine did not affect absorption in denervated segments.

5 Intraluminal morphine increased net Na and H_2O absorption from both innervated and denervated ileal segments due to increases in the unidirectional absorptive fluxes.

6 Absorptive site blood flow was linearly related to unidirectional absorptive Na fluxes in each group although not with the same slopes. The increment in absorptive site blood flow *vs.* absorptive Na flux was greatest with luminal morphine, intermediate with intra-arterial morphine and in denervated segments without morphine and least in innervated segments.

7 It was concluded that intra-arterial morphine inhibits an antiabsorptive effect of extrinsic nerves and that intraluminal morphine promotes an absorptive effect which could be direct or mediated through intrinsic nerves.

Introduction

Opiates reduce induced intestinal secretion and occasionally increase basal absorption (McKay *et al.*, 1982). In some *in vitro* experiments naloxone increased short circuit current (Dobbins *et al.* 1980) suggesting basal release of endogenous opiates. Intravenous morphine increases intestinal absorption in fed dogs but not fasted dogs and this effect is blocked by naloxone (Mailman, 1980). Thus, morphine may not have a direct effect on absorption but could modulate an effect of feeding. Morphine could modify postprandial hormonal secretion as shown by Champion, *et al.*, (1981) or it could also modify neural responses. The latter possibility was the object of this study.

Opiates are thought to modulate neurotransmitter release by nerves and it is known that neural activity alters intestinal absorption. Opiates reduce acetylcholine release (Paton, 1957) and cholinergic stimuli

reduce intestinal absorption whereas adrenergic stimuli increase absorption (Morris & Turnberg, 1980; 1981). Opiates increase gut absorption *in vitro* by acting through delta receptors and this effect is blocked *in vivo* and *in vitro* by tetrodotoxin (Kachur *et al.*, 1980). The above findings suggested that the action of extrinsic nerves on gut absorption might be modified by opiates.

Previous work has shown that effective mucosal blood flow (the virtual blood flow that functionally interacts with the absorbing cells of the epithelium, e.g. through oxygen delivery or washout of absorbed solutes) and pressure can influence absorption. The increase in gastric acid secretion produced by opiates has been attributed to an increase in mucosal blood flow (Konturek *et al.*, 1978). Hence, effective intestinal mucosal blood flow was also measured.

Methods

Experimental protocol

The basic techniques have been described in previous publications (Mailman, 1980; 1981). Briefly, dogs fed *ad libitum* were anaesthetized with sodium pentobarbitone. A segment of terminal ileum was isolated and an electro-magnetic flow probe was placed on the mesenteric artery perfusing the segment after careful blunt dissection of the peri-arterial nerves. In certain experiments, the gut segment was denervated by surgically cutting all visible peri- and para-arterial nerves. A branch of the mesenteric vein draining the segment was cannulated for collecting blood samples and measuring mesenteric venous pressure. A branch of the mesenteric artery perfusing the gut segment was cannulated for infusions. A femoral artery was cannulated for measuring arterial pressure and obtaining blood samples, and a femoral vein for supplemental anaesthetic. The segment was then perfused (5 ml min^{-1}) through the lumen with saline (38 °C) containing $^{3}H_2O$, ^{14}C inulin (as a volume marker) and ^{22}Na. The effluent was collected over 20 min intervals from a cannula arranged to maintain a luminal pressure of 15 cm saline. After an equilibration period of 60 min, samples were collected for an initial period of 60 min and then either saline (0.13 ml min^{-1}) or morphine sulphate in saline administered either intraarterially (0.2, 2, 20 µg min^{-1}, i.a.) or in the luminal perfusion (4, 40, 400 µg ml^{-1} i.l.) for the next 3 h, with the morphine concentration increased each hour.

At the end of the experiment the gut segment was removed and weighed. All appropriate data are expressed as per g of wet gut weight.

Measurements

Isotope concentrations were measured in a Triton X 100/toluene mixture in a three channel liquid scintillation counter with automatic quench correction (Beckman Instruments). Sodium was measured by flame photometry (Eppendorf). Femoral artery pressure was measured with a pressure transducer (Statham) and recorded with a chart recorder (Sanborn). Mesenteric venous pressure was recorded with a saline manometer. Mesenteric artery blood flow was measured with an electromagnetic flow probe and flow meter (Carolina Medical Electronics).

Calculations

Unidirectional and net sodium and water fluxes were calculated by the method of Berger & Steel (1958). Total blood flow (TBF) and absorptive site blood flow (ASBF) were calculated as the clearance of $^{3}H_2O$ (Mailman, 1981) as

(1) $TBF = {}^{3}H_2O ABS/({}^{3}H_2O)V - ({}^{3}H_2O)A$

(2) $ASBF = {}^{3}H_2O ABS/({}^{3}H_2O)L - ({}^{3}H_2O)A$

where $^{3}H_2O ABS$ represents the total amount of $^{3}H_2O$ absorbed, $({}^{3}H_2O)$ represents a concentration and V, A, L represent mesenteric vein, artery and lumen effluent, respectively. Previous work (Mailman, 1981) has suggested that TBF provides an accurate estimate of total anatomical blood flow to the gut segment and the calculation of ASBF is a measure of the functional blood flow involved in absorption and, in addition, is a measure of a real blood flow rather than a virtual measure.

Capillary pressure was calculated by the method of Pappenheimer & Soto-Rivera (1948). The control pre/post-capillary resistance was taken as 5:1 and changes in total resistance were assumed due to precapillary resistance changes (Folkow, 1967). It should be emphasized that this is a relative estimate and only an approximation of capillary pressure.

Statistical analysis was by comparison of each 20 min experimental period to the average of the three 20 min initial periods in each animal and statistical significance determined by paired *t* test. The relationship between variables was determined by linear regression analysis. All values are given as mean ± s.e. mean. *n* = 7–10 animals for each of the six groups.

Initial periods in innervated vs denervated gut segments

There were no significant differences in the initial period between innervated and denervated control animals (Table 1), nor in the combined initial periods of animals with innervated or denervated gut segments prior to morphine administration when these were considered together (not shown).

Sodium fluxes

Net sodium (Na) absorption decreased with time in innervated controls and became stable after about 2 h at a level significantly below initial period levels (Figure 1). Intra-arterial (i.a.) morphine prevented this decrease but did not significantly increase net Na absorption. Net Na absorption from denervated gut segments was not significantly changed, nor did i.a. morphine significantly affect Na absorption in these segments. Intraluminal (i.l.) morphine significantly increased net Na absorption to the same extent in both innervated and denervated gut segments.

The secretory Na fluxes were not significantly different from initial period levels among the six groups and there was little effect of intra-arterial or intraluminal morphine on the secretory Na fluxes (Fig-

Table 1   Control period values in fed control dogs with innervated or extrinsically denervated ileal segments.

	Innervated	Denervated
Na fluxes	(μEq g^{-1} min^{-1})	
Net absorption	1.55 ± .35	1.32 ± 0.48
Secretory	0.79 ± 0.10	1.03 ± 0.11
Absorptive	2.34 ± 0.31	2.35 ± 0.51
H$_2$O fluxes	(μl g^{-1} min^{-1})	
Net absorption	8.5 ± 2.4	8.2 ± 0.33
Secretory	22.6 ± 2.6	21.4 ± 3.5
Absorptive	31.1 ± 3.8	29.6 ± 5.9
Blood pressure	(mmHg)	
Arterial	133 ± 4	135 ± 3
Mesenteric vein	15.8 ± 1.7	18.4 ± 3.0
Blood flow	(ml g^{-1} min^{-1})	
Total	0.86 ± 0.24	0.71 ± 0.12
Absorptive site	0.045 ± 0.06	0.050 ± 0.013
Resistance	(mmHg.g.min ml^{-1})	
Total	209 ± 34	209 ± 30
Absorptive site	3386 ± 427	4031 ± 1012
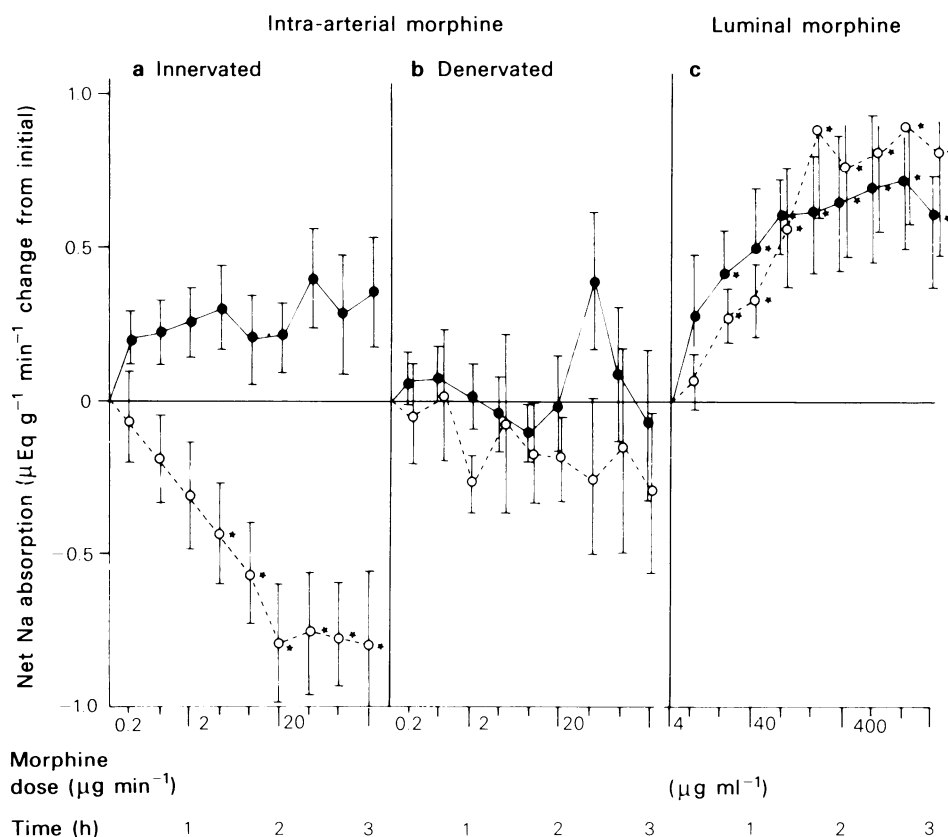
Figure 1   Net Na absorption from extrinsically innervated or denervated canine ileum during intra-arterial (a and b) (0.2, 2, 20 μg min^{-1}) or intraluminal (c) (4, 40, 400 μg ml^{-1}) morphine. In (a) and (b), (○) control; (●) morphine; in (c) (○) innervated; (●) denervated. *Represents a difference from initial periods significant to at least the 5% level.
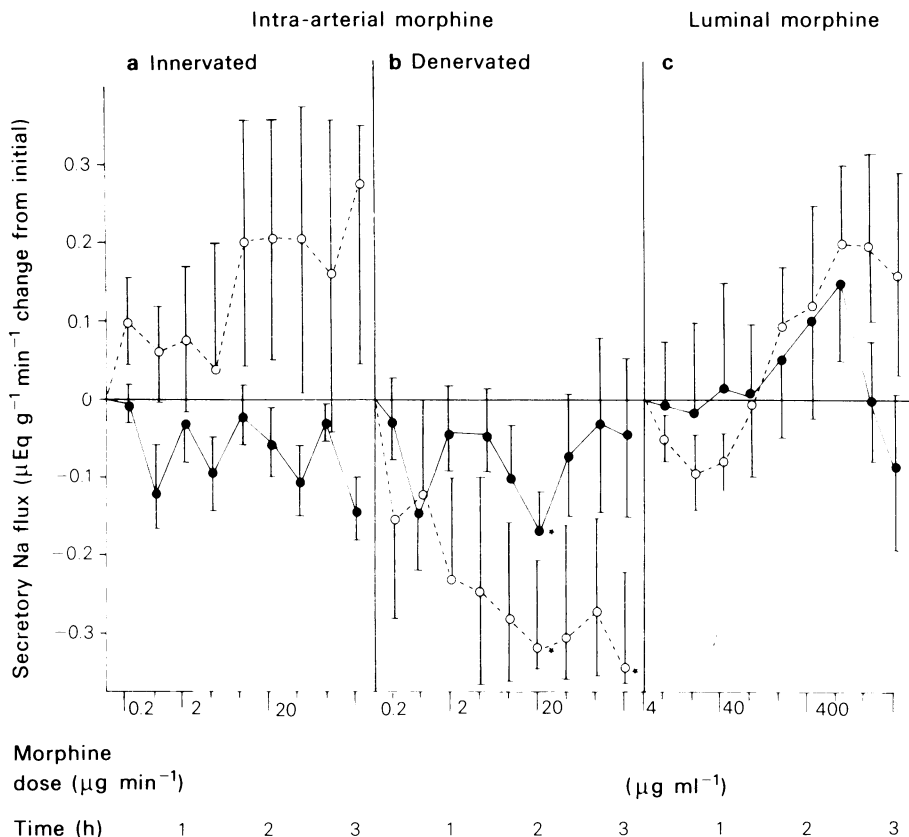
Figure 2   Unidirectional secretory Na flux into extrinsically innervated or denervated canine ileum during intra-arterial (a and b) (0.2, 2, 20 μg min^{-1}) or intraluminal (c) (4, 40, 400 μg ml^{-1}) morphine. In (a) and (b), (O) control; (●) morphine; in (c), (O) innervated; (●) denervated. *Represents a difference from initial periods significant to at least the 5% level.

ure 2.). The significant differences in net Na absorption between innervated and denervated control gut segments was quantitatively accounted for by their secretory fluxes. Although the secretory Na fluxes were themselves not significantly different from the initial periods, there was a trend for an increase in innervated segments and a decrease in denervated segments resulting in an overall significant change.

Changes in the unidirectional absorptive Na fluxes accounted for the effects of morphine on net Na absorption (Figure 3). Intra-arterial morphine prevented the significant decrease in absorptive Na flux which occurred from innervated control segments but had no significant effect on the absorptive Na flux from denervated gut segments. Intraluminal morphine significantly increased the absorptive Na flux from both denervated and innervated gut segments. Net and unidirectional H$_2$O fluxes paralleled the Na fluxes (not shown).

*Cardiovascular changes*

Absorptive site blood flow   Absorptive site blood flow paralleled the absorptive Na fluxes, although the effects of i.a. morphine infusion were not as pronounced as the effects of i.l. infusion (Figure 4). Absorptive site blood flow was not significantly changed in innervated control segments while i.a. morphine at the two lower doses significantly increased blood flow by a small amount. Blood flow did not significantly change in denervated control segments, nor was it affected by morphine. Intraluminal morphine significantly increased blood flow in denervated gut segments; however, although the increase in flow rate was of comparable magnitude in innervated gut segments, it was more variable and i.l. morphine did not significantly increase flow in innervated segments.
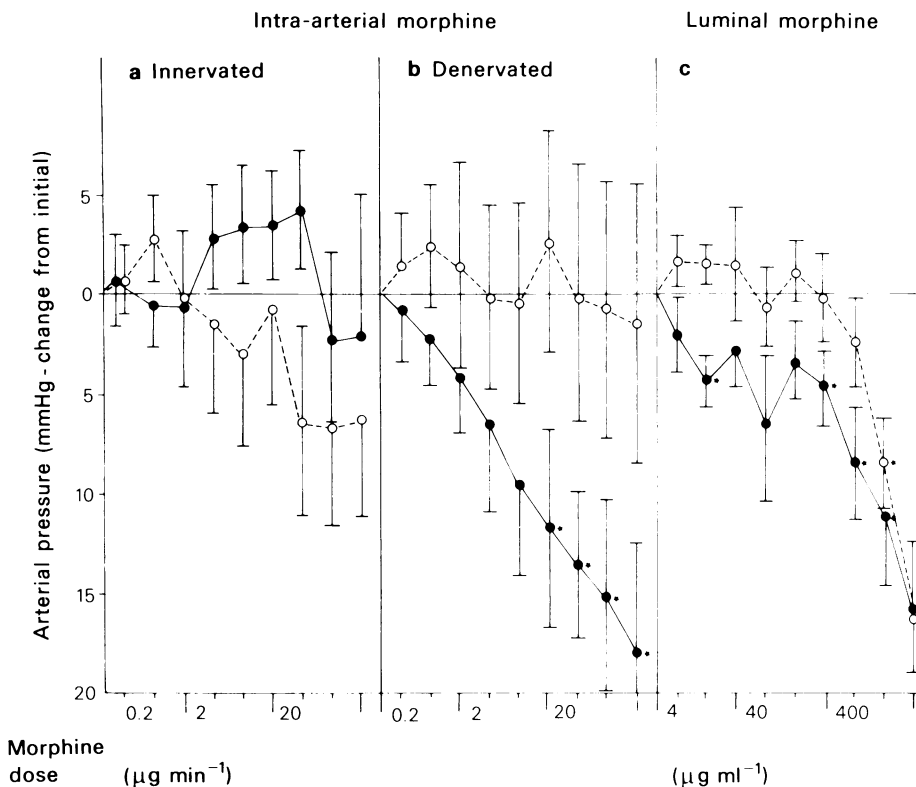
Total blood flow   Total blood flow showed few
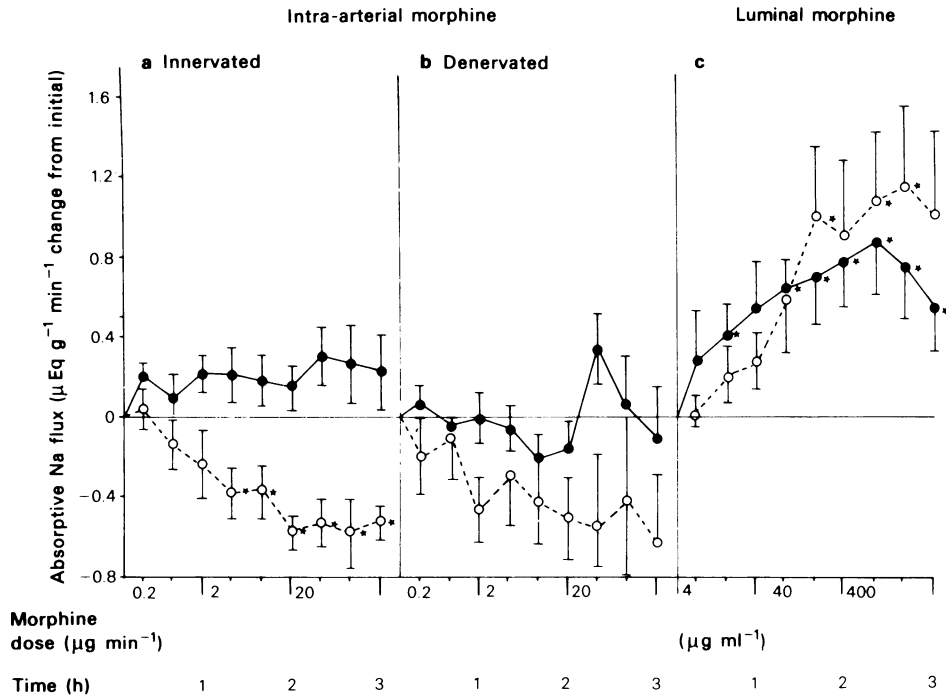
Figure 3   Unidirectional absorptive Na flux out of extrinsically innervated or denervated canine ileum during intra-arterial (a and b) (0.2, 2, 20 μg min^{-1}) or intraluminal (c) (4, 40, 400 μg ml^{-1}) morphine. In (a) and (b), (O) control; ($\bullet$) morphine; in (c), (O) innervated; ($\bullet$) denervated. *Represents a difference from initial periods significant to at last the 5% level.
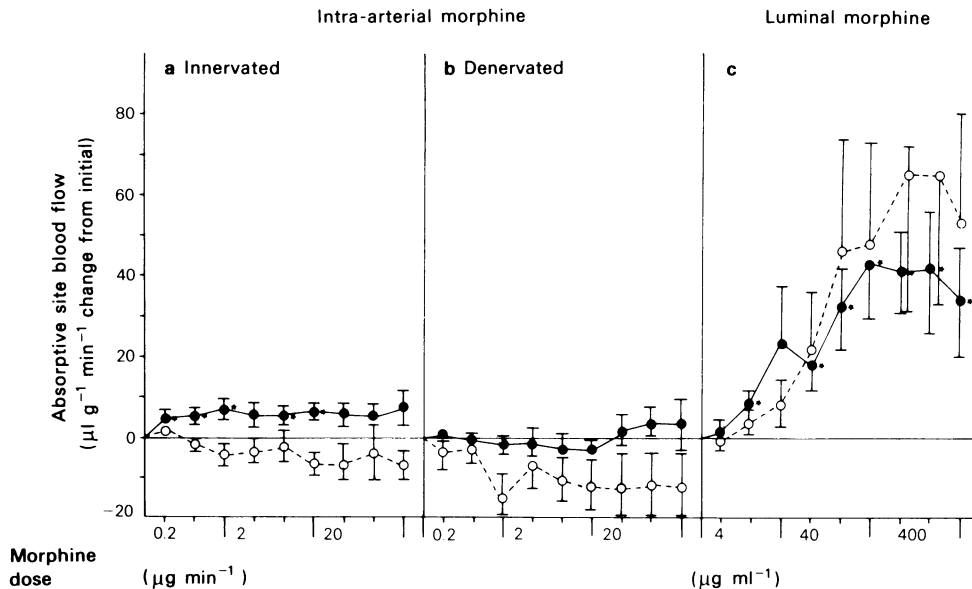
Figure 4   Absorptive site blood flow in extrinsically innervated or denervated canine ileum during intra-arterial (a and b) (0.2, 2, 20 μg min^{-1}) or intraluminal (c) (4, 40, 400 μg ml^{-1}) morphine. In (a) and (b), (O) control; ($\bullet$) morphine; in (c), ($\bullet$) innervated; ($\bullet$) denervated. *Represents a difference from initial periods significant to at last the 5% level.

Intra-arterial morphine   Luminal morphine

a Innervated   b Denervated   c

Arterial pressure (mmHg · change from initial)

Morphine
dose   (μg min^{-1})   (μg ml^{-1})

Figure 5 Arterial pressure in dogs with extrinsically innervated or denervated ileum during intra-arterial (a and b) (0.2, 2, 20 μg min^{-1}) or intraluminal (c) (4, 40, 400 μg ml^{-1}) morphine. In (a) and (b), (○) controls; (●) morphine; in (c), (○) innervated; (●) denervated. *Represents a difference from initial periods significant to at least the 5% level.

significant changes in any group. Total blood flow decreased in innervated controls with about half the periods being significantly decreased. Denervated gut segments both with and without i.a. morphine infusion had slightly decreased blood flow with no significant differences. Intra-arterial morphine infused into innervated segments and intraluminal morphine in denervated segments caused only slight increases in total blood flow with no significant differences. Total blood flow significantly increased in only one-third of the periods during intraluminal morphine perfusion of innervated gut segments.

Blood pressures Arterial pressure was not significantly changed over time in the innervated or denervated control animals, nor by i.a. morphine infused into innervated segments (Figure 5). Arterial pressure declined steadily and was significantly decreased to between 117–124 mmHg during the largest dose of i.a. morphine infused into denervated gut segments and by i.l. morphine in both denervated and innervated segments. There was only a slight tendency for the pre-/post-capillary resistance ratios and

estimated capillary pressure to decrease over time in both denervated and innervated control animals and a slight tendency to increase during i.a. morphine infusion. Intraluminal infusion caused marked decreases in the pre-/post-capillary resistance ratios and increases in estimated capillary pressures. Estimated capillary pressure (based on average values) ranged from 31.9–37.8 mmHg during the last 20 min of the initial periods and was little changed in control segments or during i.a. infusion for either innervated or denervated segments in the last 20 min (31.4–38.0 mmHg); however, it increased during i.l. morphine infusion reaching values of 54.6–67.0 mmHg. There were no significant changes in mesenteric venous pressure in any group (not shown).

Absorptive flow and Na fluxes The increments in the unidirectional absorptive Na flux, relative to the increments in absorptive site blood flow, were linearly correlated although with different slopes in each group (Figure 6). The increment in absorptive site blood flow, relative to the absorptive Na fluxes, was
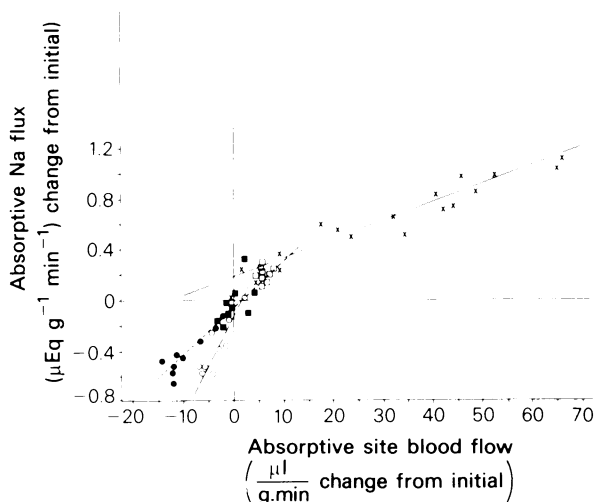
Figure 6 The relationships between absorptive site blood flow and the unidirectional absorptive Na flux in extrinsically innervated or denervated canine illeum. (●) Denervated controls; (○) innervated controls; (x) intraluminal morphine; (□) innervated morphine; (■) denervated morphine. Lines represent least squares multiple regression equations significant to at least the 5% level for the three groups indicated. There was no significant regression within innervated or denervated gut segments groups infused intra-arterially with morphine.

greatest with intraluminal morphine, intermediate with denervated controls and intra-arterial morphine infusion, and least with innervated controls.

Discussion

The present findings suggest that intra-arterial morphine does exert at least some of its effects on gut absorption through modulation of nerve activity. Na and H_2O fluxes decreased over time in innervated gut segments but remained constant in denervated segments, suggesting that the presence of extrinsic nerves had an antiabsorptive effect under these experimental conditions. Intra-arterial morphine reversed the antiabsorptive effect but, by itself, did not increase absorption because no increase in net or unidirectional fluxes occurred during i.a. morphine infusion into denervated segments. In contrast to the effects of i.a. morphine on gut absorption and blood flow, the effects of i.l. morphine are independent of the presence or absence of extrinsic nerves. It is not likely that the difference between effects of i.a. and i.l. infusions of morphine are due solely to a difference in effective concentration delivered to a basolateral or submucosal site of action, because the re-

sponses at the highest concentrations of either i.a. or i.l. administration seem to represent a maximum effect. Also, Burks & Grubb (1974) found that i.l. morphine reduced the effect of i.a. morphine on contractility. One explanation is that morphine may be exerting several actions in the gut.

Morphine probably affects gut absorption and blood flow through an indirect neural action because tetrodotoxin blocks opiate effects both *in vivo* and *in vitro* (Kachur *et al.*, 1980) and there are no opiate receptors on mucosal cells (Gaginella, *et al.*, 1983). A mechanism of action of morphine may be found in the suggestion of Tapper (1983) that the balance between acetylcholine and noradrenaline release in the mucosa may be the final common regulator of gut absorption.

Morphine is known to inhibit acetycholine release (Paton, 1957). Acetylcholine reduces gut absorption (Morris & Turnberg, 1980) and i.a. morphine could reduce acetycholine secretion under the control of extrinsic nerves, thus preventing decreased absorption. The observations that atropine mimics most, but not all, of the effects of morphine *in vivo* (Mailman, 1980, 1981) partially supports this possibility. Atropine does not mimic the effects of morphine *in vitro* (McKay *et al.*, 1982), where extrinsic nerves would not be involved.

Intraluminal morphine could stimulate the release of noradrenaline and/or inhibit basal release of acetylcholine at the mucosal level. Enkephalins inhibit acetylcholine release *in vitro* from both smooth muscle-nerve and mucosal preparations (Tapper, 1983). The α-adrenoceptor antagonist phenoxybenzamine inhibits the short circuit current response to serosal enkephalins but neither phentolamine nor catecholamine depletion with 6-hydroxydopamine shows such inhibition, making adrenergic mediation less likely (Dobbins *et al.*, 1980). Intraluminal morphine may work through stimulation of mucosal receptors which in turn act through a reflex arc similarly to the action of other mucosal stimuli (Caren, Meyer & Grossman, 1974; Cassuto, Jodal, Tuttle & Lundgren, 1981; Karlstrom, Cassuto, Jodal & Lundgren, 1981).

The linear relationship between absorptive site blood flow and the unidirectional absorptive fluxes of Na and H_2O in different groups of experiments was similar to that seen in previous experiments (Mailman, 1980). The absorptive and net Na fluxes are influenced primarily by oxygen delivery to support transport, while the absorptive H_2O flux is influenced mainly by physical washout from the absorptive site (Mailman *et al.*, 1983). Also, as in the previous study on morphine (Mailman, 1980), estimated capillary pressure had little relationship to the secretory Na fluxes. Luminal morphine caused marked increases in estimated capillary pressure but did not signific-

antly change the secretory fluxes, suggesting that the tendency for secretory fluxes to increase with capillary pressure was overrriden by other factors such as reduced active secretion or reduced mucosal permeability (Mailman & Jordon, 1975; Yablonski & Lifson, 1976). It could not be determined whether the effects on absorptive site blood flow or Na absorption were primary. A primary increase in absorptive site blood flow could supply more O_2 to support active Na

transport or an increase in active Na absorption could stimulate an increased blood flow. The different slopes of the lines relating absorptive site blood flow to the absorptive Na fluxes among the experimental groups suggests that blood flow and Na absorption are regulated partially independently.

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