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Effects of atropine and tetrodotoxin on neurotensin-induced ileal sodium transport in the dog

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1 Neurotensin was infused intravenously, in the presence or absence of intravenous atropine or intraarterial tetrodotoxin, into dogs anaesthetized with sodium pentobarbitone. Net and unidirectional fluxes of sodium and blood flows in the ileum were measured. Arterial and mesenteric venous blood pressures, haematocrits and plasma total solids were also determined.

2 Neurotensin caused a transient increase in net sodium absorption which was not associated with significant changes in unidirectional fluxes. This was followed by prolonged net secretion which was associated with an increase in unidirectional sodium secretion and a smaller decrease in sodium absorption. Potassium secretion was also increased when net sodium secretion increased.

3 Neurotensin increased haematocrit and total solids and decreased arterial pressure at the same time that secretion occurred.

4 Atropine blocked all the cardiovascular effects of neurotensin and reduced its early effects on both absorption and secretion but not the later effects on secretion. Tetrodotoxin only blocked the increase in absorption but not the secretion or the cardiovascular effects.

5 It was concluded that there is a cholinergic step in the cardiovascular effects of neurotensin and that the early effects of neurotensin on secretion are due to active secretion supported by fluid leakage from the plasma. The later effects of neurotensin on secretion do not have a cholinergic step and are due primarily to an active secretion. The increased absorption is mediated partly through intrinsic nerves of the gut.

Introduction

Neurotensin is a regulatory peptide found primarily in the ileum and it has a wide variety of effects on the gut and cardiovascular system. Neurotensin is known to cause gut secretion, although in the present experiments a transient stimulation of absorption was also observed. The secretion induced by neurotensin *in vitro* is associated with the neurally mediated release of substance P (Miller *et al.*, 1981). Neurotensin can be either hypotensive or hypertensive depending on the anaesthetic state of the animal and these cardiovascular effects are mediated partly through autonomic nerves (Rioux *et al.*, 1982). These involvements of nerves in the secretory and cardiovascular effects of neurotensin prompted an investigation of the role of muscarinic cholinergic sites and intrinsic gut nerves in the effects of neurotensin and also the role of Starling forces in the changes of gut transport.

There are many examples of regulatory agents that act on gut transport, blood flow, or motility through intermediary nerves (Mailman, 1978; Sakai *et al.*, 1984; Hubel, 1985; Fondacaro, 1986). In general, cholinergic stimuli decrease absorption and adrenergic stimuli increase absorption (Tapper, 1983). Nerves or regulatory agents can alter transport by acting on the enterocyte to increase or decrease active transcellular absorption or secretion. Additionally, *in vivo*, transport can be changed by altering mucosal interstitial pressure through changes in capillary permeability and/or blood pressure (Granger *et al.*, 1980; Granger, 1981; Mailman, 1984). Since neurotensin has both secretory and cardiovascular effects the role of each was considered in these experiments.

Net absorption or secretion across the gut represents the difference between the blood-to-lumen secretory flux and the lumen-to-blood absorptive flux. Both the unidirectional secretory and absorptive fluxes can result from active transcellular transport. In addition both these fluxes can be influenced by local effective mucosal blood flow and pressure (Mailman, 1984). In general, increasing blood flow

increases both fluxes by increasing the degree of diffusional exchange across the mucosa and increases the absorptive flux by increasing the supply of nutrients to support active transport. Increasing local capillary pressure increases the secretory fluxes by increasing pressure-driven ultrafiltration across the mucosa. Previous work has employed a measure of the effective mucosal blood flow which is determined by the clearance of ^3H_2O and is referred to as absorptive site blood flow (see below). A comparison of the changes in local blood pressure and absorptive site blood flow with the changes in unidirectional fluxes sometimes allows inferences to be made as to whether the changes in the fluxes are due to active or physical mechanisms.

Methods

Dogs, fasted for 18 h but allowed access to water, were anaesthetized with sodium pentobarbitone. The techniques employed have been described previously (Mailman, 1981; 1984). A segment of terminal ileum with its nerve and blood supply intact was perfused through luminal cannulae with isotonic saline containing ^3H_2O, [^{14}C]-inulin (as a volume marker) and ^{22}Na. A branch of the mesenteric vein draining the gut segment was cannulated for measuring mesenteric venous pressure with a saline manometer and for obtaining plasma for the measurement of radioisotopes, total solids and haematocrit. A branch of the mesenteric artery just proximal to the perfused gut segment was cannulated for the infusion of tetrodotoxin ($0.43\,\mu g\,min^{-1}$, Calbiochem) in $0.15\,ml\,min^{-1}$ saline to cause a relatively localized inhibition of intrinsic gut nerves. A femoral vein was cannulated for infusion of neurotensin ($0.68\,\mu g\,min^{-1}$, Vega Biochemicals) and/or atropine sulphate ($1\,mg\,kg^{-1}$ followed by $0.1\,mg\,kg^{-1}\,h^{-1}$) in saline ($0.15\,ml\,min^{-1}$) and supplemented anaesthetic. Arterial pressure was obtained from a femoral artery with a mercury manometer.

After completion of surgery and the beginning of the luminal perfusion, a 60 min equilibration period was allowed for recovery. When atropine sulphate was infused, it was started in the middle of the equilibration period. After the equilibration period, an initial hour consisting of three-20 min measuring periods was begun and used as a reference for the subsequent changes due to the neurotensin by itself or combined with the other agents. After the initial periods, the infusion of neurotensin or tetrodotoxin was started and maintained for 3 h and measurements taken over 20 min intervals. One group of dogs was infused only with neurotensin ($n = 8$), another group with atropine plus neurotensin ($n = 7$), a third group with atropine alone ($n = 4$), a fourth group with 60 min of tetrodotoxin followed by a simultaneous infusion of intravenous neurotensin while continuing the intraarterial tetrodotoxin ($n = 6$) and a group of control dogs to assess the effects of time ($n = 7$).

Radioisotope concentrations were determined by liquid scintillation counting using external standardization to correct for quenching (Beckman). Sodium and potassium concentrations were measured by flame photometry (Perkin Elmer). Haematocrits of mesenteric venous and femoral arterial blood were measured in microhaematocrit tubes with a haematocrit reader. Plasma total solids were measured by refractometry (American Optical).

Separate standard quench curves for plasma and gut effluent were used to determine quench and spillover for the isotopes. The concentrations of ^3H_2O, [^{14}C]-inulin and ^{22}Na were approximately 25 000, 5000 and 500 c.p.m. per ml in their narrow counting windows, respectively. Two ml of sample was counted to an accuracy of at least 2%. ^3H_2O was used to calculate total and absorptive site blood flows, ^{22}Na for the calculation of unidirectional Na fluxes and [^{14}C]-inulin as a volume marker for calculating net absorption or secretion as the change in the volume of perfusate after it had traversed the gut.

The secretory Na flux results in a decrease in the specific activity of Na due to the entry of unlabelled Na from the plasma relative to the net absorption determined from the change in concentration of [^{14}C]-inulin (Berger & Steele, 1958). The secretory flux (SF) was calculated as:

$$SF = -NF \times \frac{\ln(SA_{lf} - SA_{pf})/(SA_{li} - SA_{pi})}{-\ln(Na_f/{}^{14}C_f)/(Na_i/{}^{14}C_i)}$$

and the absorptive flux (AF) as:

$$AF = NF + SF$$

where NF represents net flux, SA represents specific activity, ^{14}C represents the concentration of inulin, Na represents sodium concentration, i and f represent initial (inflow) and final (outflow) values, respectively and l and p represent lumen and arterial plasma, respectively.

Total blood flow (TBF) to the perfused segment was calculated as:

$$TBF = {}^3H_2Oabs/({}^3H_2O_v - {}^3H_2O_a)CF$$

and absorptive site blood flow as:

$$ASBF = {}^3H_2Oabs/({}^3H_2O_l - {}^3H_2O_a)CF$$

where abs represents the amount absorbed, CF represents a correction for haematocrit and l, v and a represent the concentration in lumen effluent and venous and arterial plasma, respectively. Previous work has shown that TBF is a measure of the real

Table 1 Initial values for dogs with or without atropine pretreatment before neurotensin infusion

	Pretreatment	
	None	*Atropine*
Fluxes	($n = 6$)	($n = 7$)
Sodium (μEq g^{-1} min^{-1})		
Net	1.63 ± 0.28	2.02 ± 0.53
Secretory	0.82 ± 0.14	0.59 ± 0.07
Absorptive	2.46 ± 0.36	2.61 ± 0.56
Potassium (nEq g^{-1} min^{-1})		
Net	37.3 ± 6.5	25.5 ± 5.0
Blood flows (ml g^{-1} min^{-1})		
Total	1.08 ± 0.32	1.03 ± 0.27
Absorptive site	0.074 ± 0.028	0.063 ± 0.019
Pressures (mmHg)		
Arterial	133 ± 10	124 ± 8
Mesenteric venous	10.8 ± 1.0	9.9 ± 0.6
Haematocrit (%)	39.2 ± 2.9	38.4 ± 2.7
Total solids (g%)	5.76 ± 0.16	5.56 ± 0.24

blood flow to the perfused gut segment and ASBF is a functional blood flow effectively perfusing the absorbing mucosa and probably reflects a real blood flow although the exact anatomical site can not be specified (Mailman, 1981).

Statistical analysis was carried out by paired t test in which each animal served as its own control. All values, where appropriate, are expressed per g of wet gut weight which was obtained at the end of the experiment. The averages of the three measurements for each parameter, obtained in the hour before neurotensin infusion began, were used as an initial level and subtracted from the subsequent values during each 20 min measuring period during neurotensin infusion. A similar calculation was carried out over the same time periods in control animals which had no neurotensin infusion. Therefore, an increase or decrease in the values of the parameters shown in the figures represents a change due to neurotensin (or time) relative to the value before neurotensin infusion. The null hypothesis was that there was no change from initial levels. Hence, each mean ± s.e. mean was tested separately at its time interval to determine if it was significantly different from zero, i.e. initial levels. Implicit in this comparison was the lack of significant changes in control animals over the same time intervals (see below). Comparisons between groups were made only by the presence or absence of significant changes within each separate group. For example, if neurotensin caused a significant change during one period but the corresponding period was not different during the infusion of neurotensin and one of the other agents, then it was considered that the agent inhibited the effect of neurotensin. All values are given as mean ± s.e. mean.

Results

Control animals and initial values

There were no significant changes in any parameter in control animals over time. The initial values for all four groups were not significantly different including those pretreated with atropine. The initial values for the dogs infused with neurotensin and tetrodotoxin with no pretreatment and those pretreated with atropine are shown in Table 1.

Ion fluxes

Net sodium absorption was significantly increased by neurotensin within 20 min after beginning the infusion but after 20 min there was a net secretion which continued for the remainder of the infusion time at about the same level (Figure 1). Atropine, by itself, caused small but significant increases in net sodium absorption for the first 80 min and absorption did not fall below initial levels for the remainder of the atropine infusion. The increased absorption was a continuation of an increase which began in the initial periods but which had not reached significance. Atropine reduced both the initial increase in net sodium absorption and the subsequent secretion for 80 min. The later times of neurotensin-induced secretion were not affected by atropine.

The secretory sodium flux was increased by neurotensin after 20 min and remained elevated for the duration of neurotensin infusion although it tended to fall with time (Figure 2). Atropine by itself had no effect on the secretory sodium flux but it inhibited the increase due to neurotensin for 40 min and after this time it had no effect.

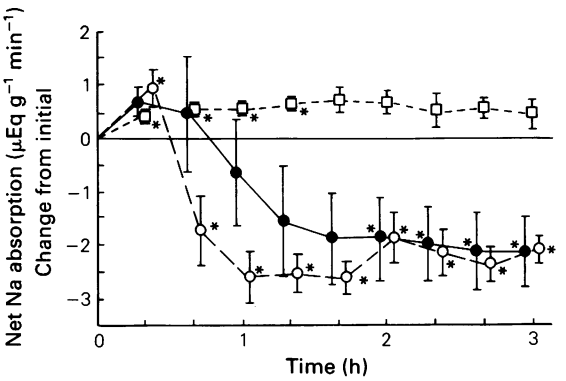

Figure 1 Net Na absorption from canine ileum during i.v. infusion of neurotensin, atropine or both. * represents a difference from initial values significant to at least the 5% level. Values are mean with s.e. mean shown by vertical lines; (○) 0.68 μg min^{-1} neurotensin; (□) 1 mg kg^{-1} + 0.1 mg kg^{-1} h^{-1} atropine; (●) atropine + neurotensin.

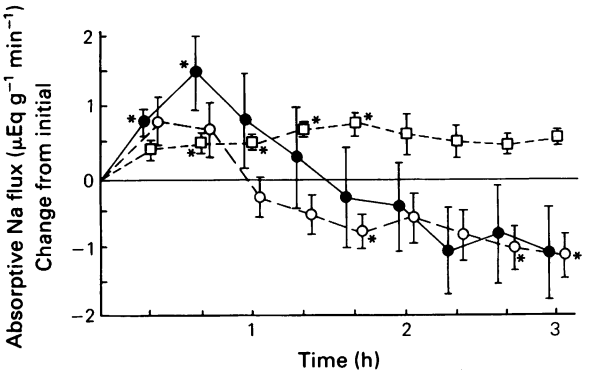

Figure 3 Absorptive Na fluxes out of canine ileum during i.v. infusion of neurotensin, atropine or both. * represents a difference from initial values significant to at least the 5% level. Values are mean with s.e. mean shown by vertical lines; (○) 0.68 μg min^{-1} neurotensin; (□) 1 mg kg^{-1} + 0.1 mg kg^{-1} h^{-1} atropine; (●) atropine + neurotensin.

Neurotensin caused significant decreases in the absorptive sodium fluxes only during the latter half of the time of neurotensin infusion (Figure 3). Atropine increased the absorptive sodium flux from 20–100 min during its infusion. During the simultaneous infusion of atropine and neurotensin, the absorptive Na flux increased significantly for the first 40 min and then declined and became nonsignificant. After 80 min the effects of neurotensin were little affected in magnitude by atropine although they were not significantly decreased in the presence of atropine as they were with neurotensin alone.

Potassium secretion was increased by neurotensin after 20 min but atropine had no effect by itself (Figure 4). Atropine reduced the early increase in potassium secretion but not the later secretion although it was consistently decreased by about 40%.

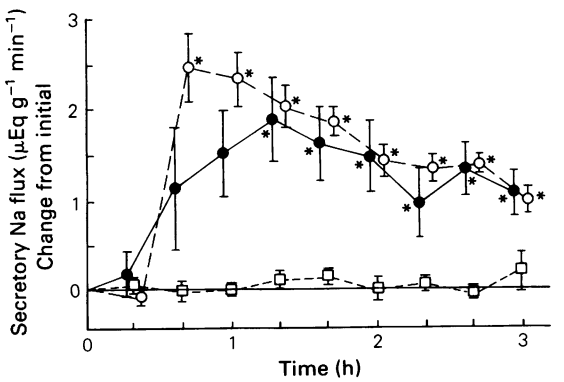

Figure 2 Secretory Na fluxes into canine ileum during i.v. infusion of neurotensin, atropine or both. * represents a difference from initial values significant to at least the 5% level. Values are mean with s.e. mean shown by vertical lines; (○) 0.68 μg min^{-1} neurotensin; (□) 1 mg kg^{-1} + 0.1 mg kg^{-1} h^{-1} atropine; (●) atropine + neurotensin.

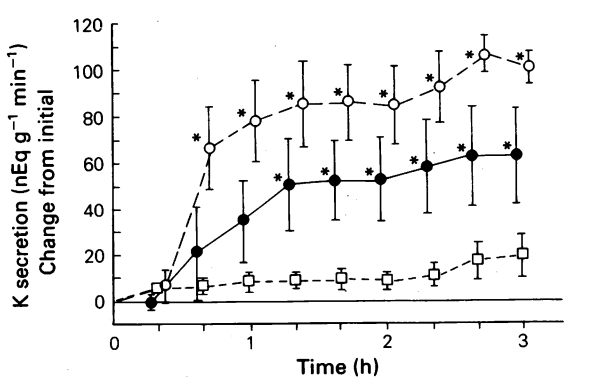

Figure 4 Net K secretion into canine ileum during i.v. infusion of neurotensin, atropine or both. * represents a difference from initial values significant to at least the 5% level. Values are mean with s.e. mean shown by vertical lines; (○) 0.68 μg min^{-1} neurotensin; (□) 1 mg kg^{-1} + 0.1 mg kg^{-1} h^{-1} atropine; (●) atropine + neurotensin.

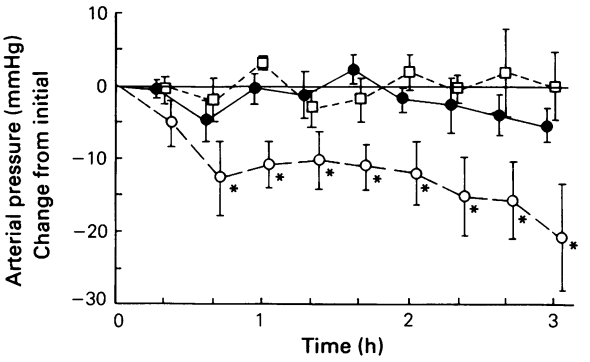

Figure 5 Arterial pressure during i.v. infusion of neurotensin, atropine or both. * represents a difference from initial values significant to at least the 5% level. Values are mean with s.e. mean shown by vertical lines; (○) 0.68 $\mu g\,min^{-1}$ neurotensin; (□) 1 $mg\,kg^{-1}$ + 0.1 mg $kg^{-1}\,h^{-1}$ atropine; (●) atropine + neurotensin.

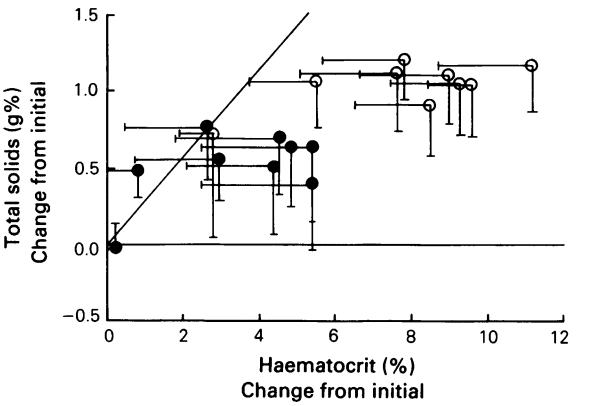

Figure 6 Changes in total plasma solids and haematocrit in sequential periods during i.v. infusion of neurotensin or neurotensin plus atropine. The solid line represents the theoretical values if the initial levels of total solids and haematocrit were increased solely by loss of colloid-free fluid from the plasma. Values are mean with s.e. mean shown by horizontal and vertical lines; (○) neurotensin; (●) neurotensin + atropine.

Cardiovascular effects

Neurotensin decreased arterial pressure after 20 min of infusion and the pressure remained at the same reduced level for the remainder of the infusion (Figure 5). Atropine by itself had no effect on arterial pressure but it completely inhibited the decrease in blood pressure caused by neurotensin. Mesenteric venous pressure was not affected by any of the infusions (not shown). There were no significant effects of any treatment on total blood flow. For example, the largest change in any period for mesenteric venous pressure was a decrease of 0.56 ± 0.59 mmHg and for total blood flow a decrease of 0.27 ± 0.21 ml $g^{-1}\,min^{-1}$, respectively, during neurotensin infusion which can be compared to their initial values given in Table 1. Absorptive site blood flow was significantly increased by $86 \pm 38\,\mu l\,g^{-1}\,min^{-1}$ only 20–40 min after beginning the infusion and there were no significant effects of atropine or atropine plus neurotensin (not shown).

The haematocrit was significantly increased by neurotensin in every period with the first increase of $2.8 \pm 0.9\%$ occurring in the first 20 min and reaching a maximum of $11.2 \pm 1.9\%$. It should be pointed out that the changes in mesenteric venous haematocrit were paralleled by changes in femoral arterial haematocrits and therefore the haemoconcentration was not restricted solely to the intestine. Plasma total solids were significantly increased after 20 min of neurotensin infusion and remained at the same level for the remainder of the infusion time. The increase in total solids was 1.08 ± 0.31 g% after 20 min and 1.06 ± 0.35 g% after 60 min of the neurotensin infusion. Atropine had no effect on either total solids or haematocrit but completely inhibited the increase in both due to neurotensin. Figure 6 expresses the change in haematocrit and total solids for each period. The solid line represents the theoretical change in total solids and haematocrit if the initial values were changed solely by loss of fluid from the plasma, i.e., the amount of total solids and the volume of red blood cells in the blood remained constant. As the haematocrit increased during neurotensin infusion, the concentration of total solids deviated further below the theoretical line. Hence, there was a relative loss of colloids from the plasma. After atropine, there were no significant differences between the observed and theoretical values during neurotensin infusion.

Tetrodotoxin effects

Intraarterial tetrodotoxin, by itself, caused small but significant increases in net Na absorption (Figure 7). Neurotensin, infused intravenously during the continuing intraarterial tetrodotoxin infusion, did not increase net Na absorption as did neurotensin by itself but the secretion still occurred and was about 20–25% larger than with neurotensin alone (compare to Figure 1). The secretory Na flux was increased to about the same degree by neurotensin in the presence of tetrodotoxin (Figure 7) as by neurotensin alone (Figure 2). The absorptive Na flux was decreased significantly in four periods during the infusion of neurotensin and tetrodotoxin (Figure 7) as compared to a decrease in only one period over

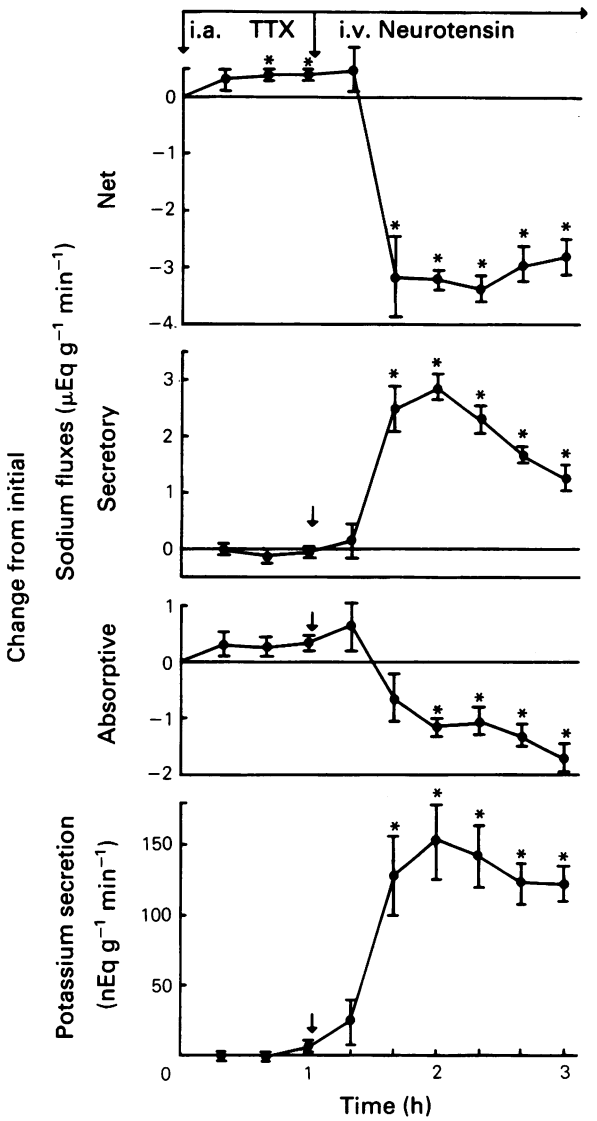

Figure 7 Changes in net, secretory and absorptive Na^+ fluxes and net K^+ secretion by canine ileum during i.a. tetrodotoxin (TTX) and TTX plus i.v. neurotensin (arrow). * represents a difference from initial values significant to at least the 5% level. Values are mean with s.e. mean shown by vertical lines.

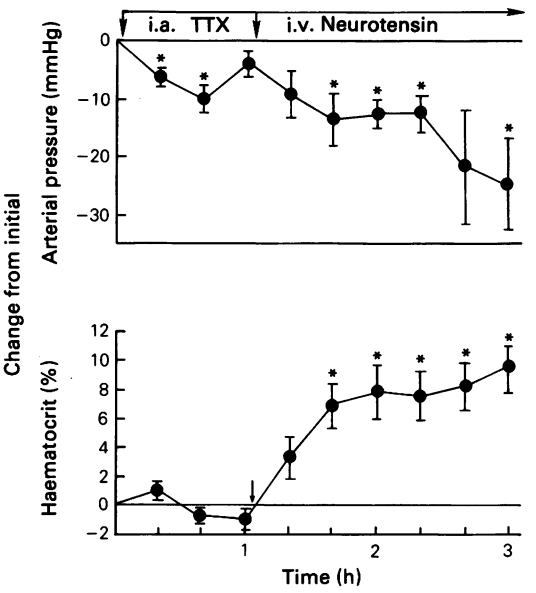

Figure 8 Changes in arterial pressure and mesenteric venous haematocrit in canine ileum during i.a. tetrodotoxin (TTX) and TTX plus i.v. neurotensin (arrow). * represents a difference from initial values significant to at least the 5% level. Values are mean with s.e. mean shown by vertical lines.

the same time interval during the infusion of neurotensin alone (Figure 2). The change (decrease) in the absorptive Na flux was about 100% larger during neurotensin infusion in the presence of tetrodotoxin than during the infusion of neurotensin alone. Potassium secretion was increased about 70% more by neurotensin during tetrodotoxin (Figure 7) than by neurotensin alone (Figure 4).

Tetrodotoxin alone caused a transient decrease in arterial pressure but did not affect the haematocrit (Figure 8). Arterial pressure was decreased and the haematocrit was increased to about the same extent by neurotensin in the presence or absence of tetrodotoxin (Figure 8 compared to Figures 5 and 6). Absorptive site blood flow was increased by neurotensin in the presence of tetrodotoxin only at 40–60 and 60–80 min of neurotensin infusion by 143 ± 65 and 109 ± 37 $\mu l\, g^{-1}\, min^{-1}$, respectively, similar to the effect of neurotensin alone (not shown). Neither total blood flow nor mesenteric venous pressure were affected by tetrodotoxin or by neurotensin in the presence of tetrodotoxin (not shown).

Discussion

There have been a number of studies which have shown that regulatory agents can alter gut transport through effects on active transcellular transport and/or through changing the interstitial pressure in

the mucosa and physically changing paracellular fluxes (MacFerran & Mailman, 1977; Granger *et al.*, 1979; 1980; Fondacaro, 1986). Neurotensin can increase intestinal secretion *in vitro*, as judged by electrical measurements, which suggests that active secretion was involved (Miller *et al.*, 1981). Neurotensin causes oedema and tissue accumulation of colloids and increases the large pore radius in the intestinal vasculature (Leeman & Carraway, 1982; Harper *et al.*, 1984) and losses of plasma colloids were also observed in the present experiments. These observations suggest that at least a part of the increased secretory fluxes, which account for the net secretion, are due to a pressure-driven secretion caused by the loss of fluid and colloids into the interstitial space and a consequent increase in interstitial pressure (Granger, 1981; Mailman, 1984). However, arguing against the possibility of neurotensin increasing net secretion through an effect on pressure driven secretion are the observations that arterial pressure decreased with little change in ASBF or mesenteric venous pressure. Thus, capillary pressure would have tended to decrease which would, in turn, tend to decrease net secretion. The lack of change of ASBF and the increase in the secretory Na flux at the same time that there was a decrease in the absorptive flux also indicates that diffusional exchange between the blood and lumen was not a major contributor to the change in unidirectional fluxes since any change in ASBF would change both fluxes in the same direction (Mailman, 1981). It is possible that an increase in tissue pressure due to fluid entry into the tissue space could occur even if capillary pressure were tending to decrease.

All of the cardiovascular changes, the decreased arterial pressure and the increased haematocrit and total plasma solids, caused by neurotensin are inhibited almost completely by atropine indicating that a cholinergic step is a link in the sequence of mechanisms between neurotensin and its cardiovascular effects. Atropine, in the rat, did not block neurotensin-induced secretion of fluid measured over a 50 min period although hexamethonium did block the secretion (Rokaeus, 1984). Perhaps, the measurement period in those experiments was long enough that the initial reduction in secretion could not be detected or there is a species difference in the effect of atropine on secretion. Since the later stages of sodium and potassium secretion induced by neurotensin are not affected by atropine then this portion of the secretion is not due to cardiovascular mechanisms. This phase of the secretion may be the active secretion which was observed *in vitro* and reflects a mechanism which does not have a cholinergic link (Miller *et al.*, 1981). The *in vitro* secretion depended on a neurally mediated release of substance P. The nerves involved in the release of substance P were inhibited by adrenaline and based on the findings in the present experiments are not associated with muscarinic cholinergic effects (Miller *et al.*, 1981).

Since the cardiovascular effects of neurotensin were reduced by atropine as were the early stages of secretion it is possible that this portion of the secretion is dependent on increased interstitial pressure. However, other studies have shown that intraarterial hyperoncotic dextran does not reverse the secretory fluxes which are increased by neurotensin and that the water content of the gut tissue is decreased by neurotensin (unpublished observations). Neither observation is consistent with pressure driven secretion. The reduced tissue water content is consistent with an effect of the reduced blood pressure altering Starling forces in a direction to promote fluid uptake from the tissues into the plasma and/or loss of mucosal fluid to active secretion (Granger, 1981). Losses of fluid into the interstitial space could support an increase in active secretion without causing a pressure driven secretory component as long as tissue pressure was maintained low by the secretion itself. The secretion observed with neurotensin is about 50% greater than that observed with vasoactive intestinal peptide, which is a potent stimulant of active secretion (Mailman, 1978), and this extra secretion may reflect fluid leakage from the plasma.

Local intraarterial infusion of tetrodotoxin blocked only the increased net absorption caused by neurotensin but not the net secretion nor the cardiovascular effects. The main effect was exerted on the unidirectional absorptive fluxes which were decreased to a greater extent by neurotensin in the presence of tetrodotoxin thus resulting in a relative increase in net secretion. The decrease in the absorptive Na flux was twice as great during neurotensin infusion after tetrodotoxin as compared to the decrease during neurotensin alone. This would suggest that neurotensin can cause a degree of stimulation of sodium absorption through a local nerve-dependent mechanism and although this stimulation is not sufficient to override the secretory effect of neurotensin it may be responsible for the initial transient increase in absorption which was observed. The close intraarterial infusion of tetrodotoxin presumably causes a relatively localized inhibition of intrinsic nerve activity. The transient decrease in arterial pressure indicates that there may have been additional effects caused by the escape of tetrodotoxin into the general circulation although stimuli from the gut may have been responsible for the decreased pressure. The failure of tetrodotoxin to block the neurotensin-induced secretion and cardiovascular effects suggests that these effects are initiated by cholinergic nerves located outside the gut. It is not known why tetrodotoxin did not block the

neurotensin-induced secretion *in vivo*, as it did *in vitro* (Miller *et al.*, 1981), but different doses and the considerable technical differences may account for the discrepancy. Also, there may be more than one neurotensin-sensitive pathway operating differentially under the different experimental conditions. The neurotensin-induced absorption may be stimulated through intrinsic cholinergic nerves since both local tetrodotoxin and peripheral atropine block the absorption.

Distinct differences in results from the present experiments were found in a recent study (Eklund *et al.*, 1987). In their experiments, intraarterial tetrodotoxin did block the increased secretion induced by intraarterial neurotensin. There were a number of technical differences between the present experiments and those of Eklund *et al.* (1987). They used cats anaesthetized with chloralose and it is known that anaesthetic and species differences can markedly influence the effects of neurotensin on blood pressure (Rioux *et al.*, 1982). In addition, the intestine and adrenals were denervated. Perhaps a more important difference was that the animals were also pretreated with atropine. A study of the interaction of atropine and local nerve blockade may reveal some other aspects of the mechanism of action of neurotensin. In these same studies, it was found that neurotensin had no effect on intestinal blood flow which was similar to the observations presented here. A 4–10 min lag period before neurotensin increased secretion was also observed but no transient increase in absorption was detected (based on inspection of a representative figure which showed a measurement of continuous absorption).

Rosell *et al.* (1980) considered intravenous neurotensin in the range of 6–30 pmol $kg^{-1} min^{-1}$ to be physiologically relevant in the dog although more recent studies suggest that this range might be too high (Reasbeck *et al.*, 1984). The dose of neurotensin employed in these studies (0.68 $\mu g\, min^{-1}$) is about 20 pmol $kg^{-1} min^{-1}$ which probably represents a high physiological range for the humoral effects of neurotensin. It has been suggested that neurotensin releases noradrenaline from canine ileum when injected locally (Sakai *et al.*, 1984). Adrenergic stimuli increase intestinal absorption (Miller *et al.*, 1981; Fondacaro, 1986) and might be responsible for the transiently increased absorption observed here. Both atropine and tetrodotoxin increased absorption by themselves as has been observed in some studies both *in vivo* and *in vitro* (Hubel, 1985). Both these drugs may cause an inhibition of cholinergic effects relative to adrenergic effects either directly or indirectly. They may affect different steps in a single pathway or different pathways. Although atropine and tetrodotoxin had different qualitative and quantitative effects on neurotensin-induced absorption and secretion, the additional complication of the cardiovascular changes makes a choice between one or more neurotensin-sensitive pathways difficult. The possibility of other nerves or regulatory agents being involved in the effects of neurotensin and its microcirculatory effects require further study.

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