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EFFECTS OF GLUCAGON ON CANINE INTESTINAL SODIUM AND WATER FLUXES AND REGIONAL BLOOD FLOW

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SUMMARY

1. Glucagon (0·05 or 0·5 μg/kg.min) was infused into a mesenteric artery of a canine ileal segment from which transport was measured (direct infusion) or into a mesenteric artery of an adjacent non-perfused segment (indirect infusion). Unidirectional Na and H_2O fluxes and arterial and mesenteric vein pressures and total and absorptive site blood flows were measured.

2. Direct glucagon infusion increased the absorptive and secretory fluxes of Na and H_2O and absorptive site blood flow, and decreased absorptive site resistance and arterial and mesenteric vein pressure. Indirect glucagon infusion had the opposite effects.

3. Neither the direct arterial infusion of histamine (0·1–53 μg/kg.min) nor the I.V. infusion of glucose (0·2 g/min) or insulin (0·1 u./kg) or glucose plus insulin, mimicked the effects of glucagon.

4. The unidirectional secretory and absorptive fluxes of both Na and H_2O were linearly related to the calculated capillary pressure during glucagon infusion.

5. It was concluded that the effects of glucagon on gut transport were due to effects exerted through the cardiovascular system.

INTRODUCTION

The functions of the intestine include absorption, motility and digestive secretion. Gastrointestinal hormones have long been known to control the latter two of these functions but recently absorption has also been shown to be affected (reviewed in Makhlouf, 1974). The effects on absorption are variable and depend on the experimental preparation and species. Absorption is decreased or unaffected by hormones such as gastrin, vasoactive intestinal peptide, gastric inhibitory peptide (Barbezat & Grossman, 1971*a*; Bynum, Jacobson & Johnson, 1971), cholecystokinin and secretin (Hubel,

1972*a*, *b*; Bussjaeger & Johnson, 1973). Glucagon is an exception, in that, I.V. glucagon at 4–256 μg/kg increases absorption in the rat (Hubel, 1972*a*) but I.V. glucagon at 0·5 μg/kg . min decreases absorption in the dog (Barbezat & Grossman, 1971*b*). The different effects of glucagon and other hormones on absorption in different preparations suggest that these hormones have several effects some of which increase absorption and others which decrease absorption. Certain effects could be more prominent in one preparation as compared to another.

Glucagon, in particular, has several effects which could directly or indirectly alter gut absorption. For example, glucagon increases both blood glucose and insulin (Samols, Marri & Marks, 1966) which have effects on gut absorption (Aulsebrook, 1965). Of particular interest was the possibility that glucagon could affect gut transport by altering capillary blood pressure through its vasodilatator activity which can be selective for the gut (Ross, 1970; Ulano, Treat, Shanbour & Jacobson, 1972). Altered capillary blood pressure in turn can alter the movement of salt and H_2O across the gut through effects on Starling forces (Mailman & Jordan, 1975; Hakim & Lifson, 1969) or through changes in blood flow (Ochsenfahrt & Winne, 1968). Any cardiovascular effects on absorption could be due to direct effects on the gut or indirect effects through baroreceptor mediated autonomic activity. These studies were designed to determine if any of the above effects of glucagon were responsible for altered gut absorption.

METHODS

The method for measuring total and absorptive site blood flow has been described in detail elsewhere (Mailman & Jordan, 1975). Briefly, dogs were anaesthetized with Na pentobarbitone (30 mg/kg), a segment of ileum was cannulated at both ends and the lumen perfused with saline (2 ml./min) containing ^3H_2O, [^{14}C]inulin and ^{22}Na (New England Nuclear). After a 60 min equilibration period, the effluent was collected for nine 20 min periods. A branch of the mesenteric vein draining the segment was cannulated and a sample of mesenteric vein blood collected during the middle of each period. Samples of femoral artery blood were collected every 40 min. Femoral artery and mesenteric vein pressures were measured with mercury and saline manometers, respectively. Radioisotope concentrations in plasma and gut effluent were determined by liquid scintillation counting. Unidirectional Na and H_2O fluxes were measured by the method of Berger & Steele (1958). Total blood flow (TBF) was calculated as (1) TBF = ^3H_2O absorbed/($^3H_2O_V - {}^3H_2O_A$), and absorptive site blood flow (ASBF) as (2) ASBF = ^3H_2O absorbed/($^3H_2O_L - {}^3H_2O_A$) where ^3H_2O represents the ^3H_2O concentration and V, A and L represent the solutions in vein, artery and lumen, respectively.

Several different substances were infused either into a mesenteric artery or into a femoral vein. These infusions consisted of (*a*) direct (see below) mesenteric arterial glucagon, (*b*) indirect (see below) mesenteric arterial glucagon, (*c*) direct mesenteric arterial histamine, (*d*) I.V. glucose, (*e*) I.V. insulin and (*f*) I.V. insulin plus glucose.

A branch of a mesenteric artery was cannulated for the infusion (0·3 ml./min) of

either saline, glucagon or histamine. The cannulated mesenteric arterial branch was either to the perfused gut segment (direct infusion) or to the mesenteric artery of an adjacent non-perfused gut segment (indirect infusion). During indirect infusion the infused substance had to pass through the non-perfused gut segment and then through the general circulation before it circulated through the perfused gut segment. At the end of the experiments placement of the mesenteric arterial cannula was checked by dye injection.

During the first 60 min in which gut absorption was measured, saline was infused through the arterial cannula. During the next 60 min period, glucagon (E. Lilly) was infused at 0·05 μg/kg.min and during the third 60 min period, glucagon at 0·5 μg/kg.min was infused. Indirect glucagon infusions were carried out in six experiments and direct glucagon infusions were carried out in seven experiments. Histamine (0·1, 1·0, 5·9 or 53 μg/kg.min) (Sigma) was directly infused for 60 min following the first 60 min period of gut sampling. One experiment was done for each dose. In five experiments, insulin (E. Lilly) (0·1 u./kg) was injected through a femoral vein at the end of the first 60 min of gut sampling and a continuous infusion of glucose (0·2 g/min) begun through a femoral vein. After 60 min the glucose infusion was stopped and a second injection of insulin was given. In four experiments, heat denatured insulin was used and the glucose infusion carried out as above. There are no significant effects of time on this preparation as shown in previous studies (Mailman & Jordan, 1975) and by control experiments in these studies ($n = 4$, data not included).

Na and K concentrations in the gut effluent and plasma were determined by flame photometry (Brinkman). Radioisotope concentrations were determined by liquid scintillation counting (Beckman).

Statistical analysis was by paired t test between the control periods and the subsequent experimental periods in each animal. Comparisons between different experimental groups was by unpaired t test. All data are expressed as the mean difference ($\pm$ S.E. of mean) between the control and experimental periods. Control period values are given in each Figure legend.

Capillary pressures were calculated by the method of Pappenheimer & Soto-Rivera (1948). Blood flow resistance was calculated from the total and absorptive site blood flows and the arterial–mesenteric vein pressure differences. The control pre/post capillary resistance was estimated as 5/1 and any change in total resistance was assumed as due to changes in precapillary resistance (Folkow, 1967). All measurements are expressed with respect to wet gut weight.

RESULTS

Indirect glucagon infusion decreased the net and both unidirectional Na fluxes at 0·05 and 0·5 μg/kg.min (Fig. 1*A*). Direct glucagon infusion increased the unidirectional secretory and absorptive Na fluxes at both dose levels but there was no significant effect on the net Na fluxes. However, when the direct and indirect glucagon infusions were compared at the same dose levels the direct glucagon infusion increased the net and unidirectional Na fluxes as compared to the indirect infusion.

The effects of glucagon, both direct and indirect, on the H_2O fluxes (Fig. 1*B*) were similar to those on the Na fluxes, with the exception that there was no significant difference between the effect of direct and indirect glucagon infusion at 0·05 μg/kg.min on the net H_2O fluxes and there was

no significant effect of indirect glucagon infusion at 0·5 μg/kg.min on the unidirectional secretory flux.

Total blood flow (Fig. 2) was significantly decreased at the lower dose of indirect glucagon infusion and significantly increased at the higher dose of direct glucagon infusion and the increase was significantly greater than that

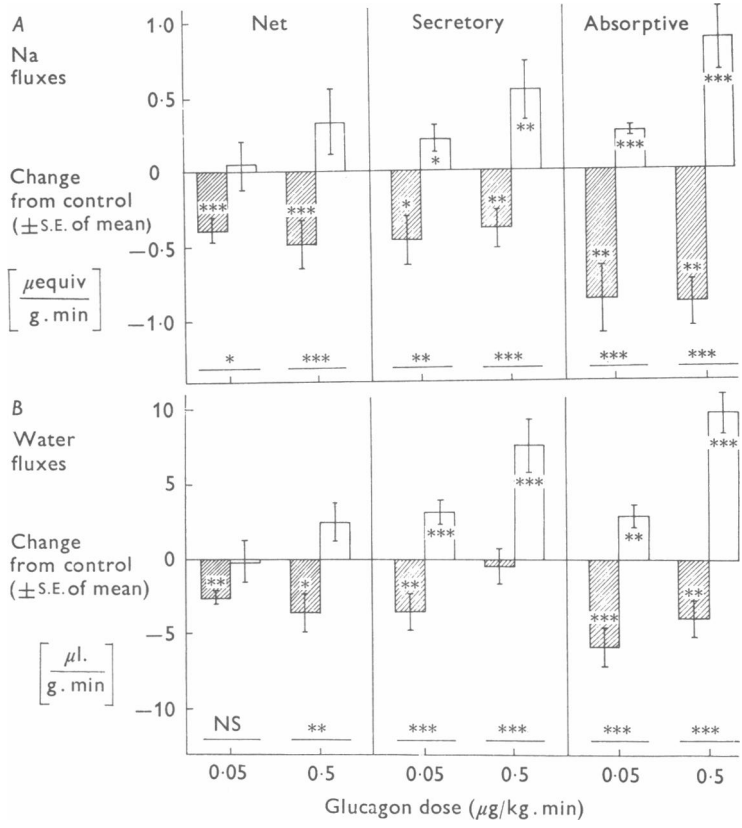

Fig. 1. *A*, effects of glucagon (0·05 or 0·5 μg/kg.min) on net and unidirectional Na fluxes (mean ± S.E. of mean) across canine ileum. Control period values were 0·83 (± 0·10), 1·46 (± 0·18) and 2·29 (± 0·17) μequiv/g.min for the Na net, secretory and absorptive fluxes, respectively. *B*, effects of glucagon (0·05 or 0·5 μg/kg.min) on net and unidirectional H_2O fluxes (mean ± S.E. of mean) across canine ileum. Control period values were 6·9 (± 0·6), 16·6 (± 1·6) and 23·5 (± 1·6) μl./g.min for H_2O net, secretory and absorptive fluxes, respectively. Glucagon was infused directly into the mesenteric artery of the perfused gut segment (n = 7) or indirectly into the mesenteric artery of an adjacent non-perfused gut segment (n = 6). *, **, *** represent differences between the control and experimental periods significant at the 5, 1 and 0·1 % level, respectively. ▨, indirect infusion; □, direct infusion.

due to indirectly infused glucagon. Absorptive site blood flow (Fig. 2) was significantly decreased at both levels of indirectly infused glucagon and significantly increased at both levels of directly infused glucagon. The effects of the direct glucagon infusions were significantly different from those of the indirect glucagon infusions. There was a similarity between the changes in absorptive site blood flow and the changes in the unidirectional Na and H_2O fluxes since these all increased or decreased in parallel.

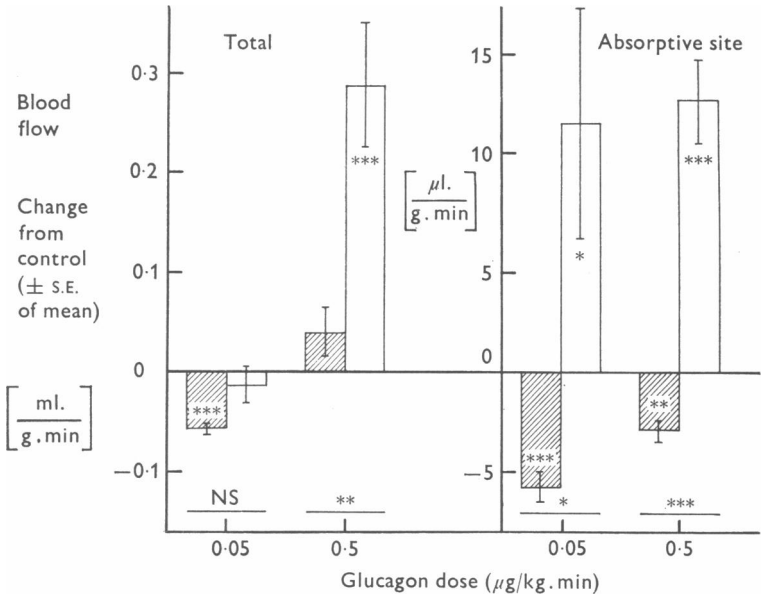

Fig. 2. Effect of glucagon (0·05 or 0·5 μg/kg.min) on total and absorptive site segmental blood flow in canine ileum. Glucagon was infused directly into the mesenteric artery of the perfused segment (n = 7) or indirectly into the mesenteric artery of an adjacent non-perfused segment (n = 6). *, **, *** represent differences between control and experimental periods significant at the 5, 1 and 0·1% level, respectively. Control period values were 0·44 (± 0·03) ml./g.min and 25·0 (± 1·9) μl./g.min for total and absorptive site blood flow, respectively. ▨, indirect infusion; □, direct infusion.

Arterial blood pressure was decreased by both the direct and indirect glucagon infusions and to the same extent for each dose (Fig. 3). This was to be expected since glucagon would affect blood pressure only after it reached the general circulation and the same dose would be delivered by both the direct and indirect infusion. Mesenteric venous pressure (Fig. 3) of the perfused segment was significantly increased by both doses of indirectly infused glucagon but significantly decreased only at the lower dose of directly infused glucagon. A significant difference between mesenteric

venous pressure due to the direct and indirect glucagon infusions was present only at the lower dose.

Total blood flow resistance was significantly decreased both by direct and indirect infusion of glucagon at both dose levels but there was no significant difference between the direct and indirect glucagon infusions (Fig. 4). Absorptive site blood flow resistance was decreased by indirect infusion of glucagon only at the higher dose but by both doses of directly infused glucagon (Fig. 4). The decreased absorptive site resistance due to directly infused glucagon was significantly greater than the decrease due to indirectly infused glucagon.

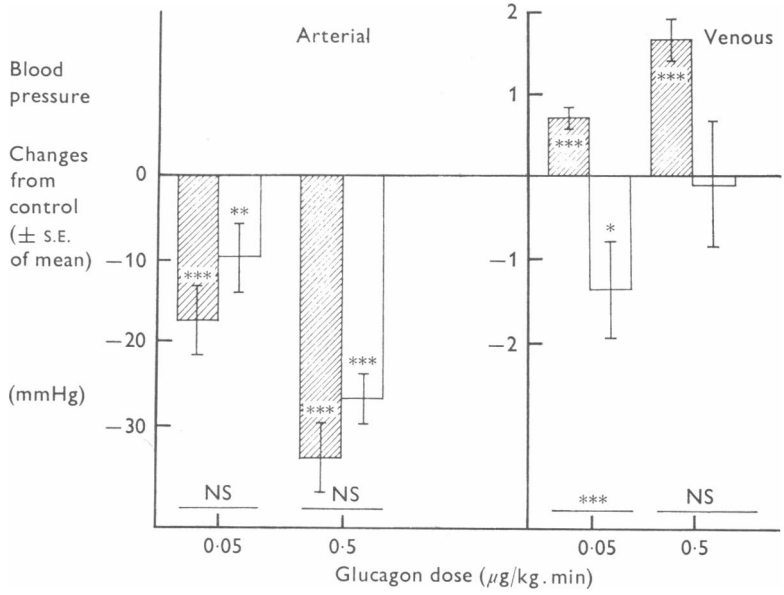

Fig. 3. Effects of glucagon (0·05 or 0·5 μg/kg.min) on arterial and mesenteric venous pressure (mean ± S.E. of mean) of canine ileum. Glucagon was directly infused into the mesenteric artery of the perfused gut segment (n = 7) or indirectly into the mesenteric artery of an adjacent non-perfused gut segment (n = 6). *, **, *** represent differences between control and experimental periods significant at the 5, 1 and 0·1 % level, respectively. Control period values were 112 (± 3) and 10·7 (± 0·9) mmHg for arterial and mesenteric vein pressure, respectively. ▨, indirect infusion; □, direct infusion.

There was a linear relationship between capillary pressure and each of the unidirectional fluxes (Fig. 5). However, the direct and indirect glucagon infusions did not have the same relationship to the unidirectional fluxes. Thus, capillary pressure could not have been the sole determinant of the unidirectional fluxes.

Because of the dose and infusion site dependency of the glucagon effects, a wide range of histamine infusion rates were studied to determine if direct infusion of this vasodilator compound could mimic the effects of directly infused glucagon. There were several points of interest in the histamine effects but the main point pertinent to this study was that directly infused histamine did not mimic the effects of directly infused glucagon (Fig. 6). Indeed, direct histamine infusion caused effects more similar to those of

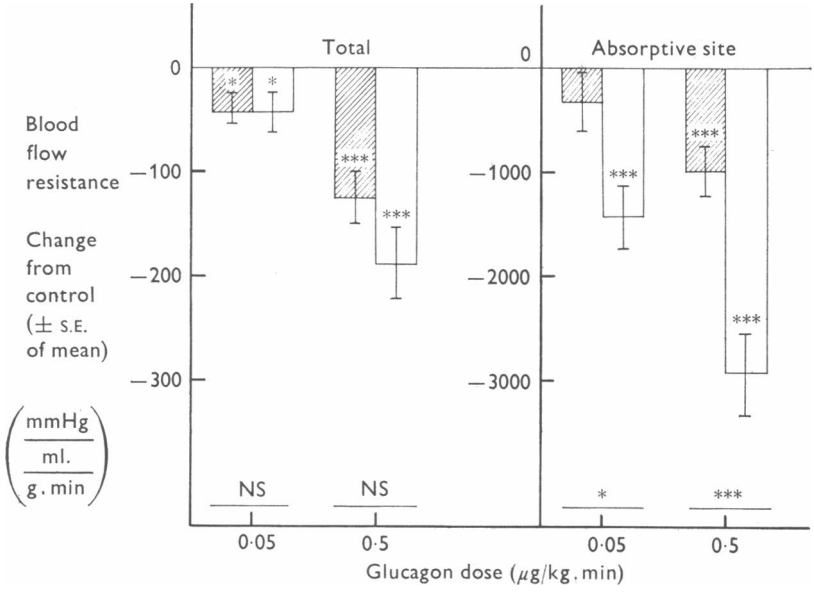

Fig. 4. Effect of glucagon (0·05 or 0·5 μg/kg.min) on total and absorptive site blood flow resistance (mean ± S.E. of mean) in the canine ileum. Glucagon was infused directly into the mesenteric artery of the perfused gut segment (n = 7) or indirectly into the mesenteric artery of an adjacent non-perfused gut segment (n = 6). *, **, *** represent differences between the control and experimental periods significant at the 5, 1 and 0·1 % level, respectively. Control period values were 232 (± 36) and 4085 (± 371) mmHg/ml./g.min for total and absorptive site blood flow resistance, respectively. ▨, indirect infusion; □, direct infusion.

indirectly infused glucagon, in that the absorptive Na and H_2O fluxes and absorptive site blood flow were decreased and mesenteric vein pressure was increased at all levels of histamine. Arterial pressure was decreased 1–23 mmHg by the histamine infusions and absorptive site blood flow resistance changed by −1420, −14, 211 and 360 mmHg/ml./g.min at 0·1, 1·0, 5·9 and 53 μg histamine/kg.min, respectively, i.e. the net effect of histamine was to decrease absorptive site resistance at the two lower doses and to increase it at the two higher doses.

Fig. 7 represents the effects of glucose (0·2 g/min) or glucose plus insulin (0·1 u./kg) infused I.V. which would mimic the site of release of these substances into the circulation by glucagon. Again, there were several effects of both glucose and insulin plus glucose but the germane point is that there

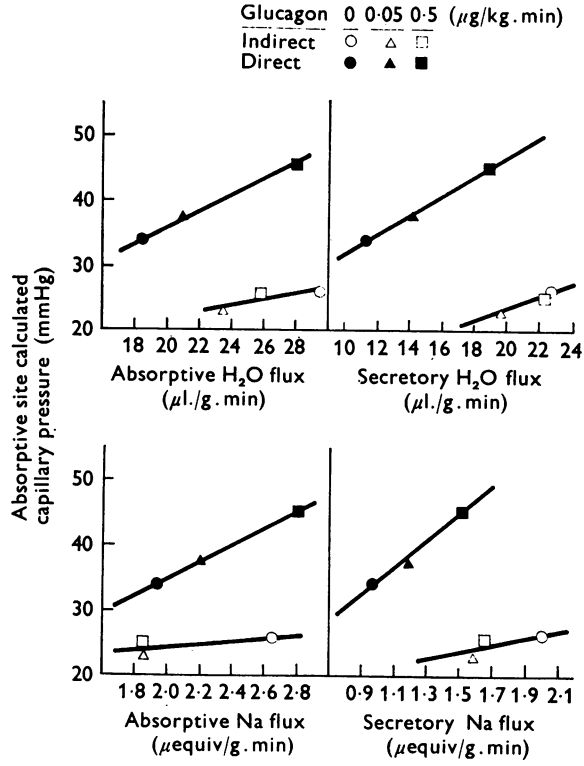

Fig. 5. The relationship between the average calculated capillary pressures and unidirectional Na and H_2O fluxes across canine ileum during glucagon infusion (0·05 or 0·5 μg/kg.min) directly into the mesenteric artery of the perfused gut or indirectly into the mesenteric artery of an adjacent non-perfused gut segment.

was no obvious similarity between these effects and those of glucagon either directly or indirectly infused. The effects of insulin alone were similar to those of insulin plus glucose (data not included). Therefore, insulin and/or glucose released by glucagon could potentiate or antagonize some of the glucagon effects but did not seem directly responsible for them.

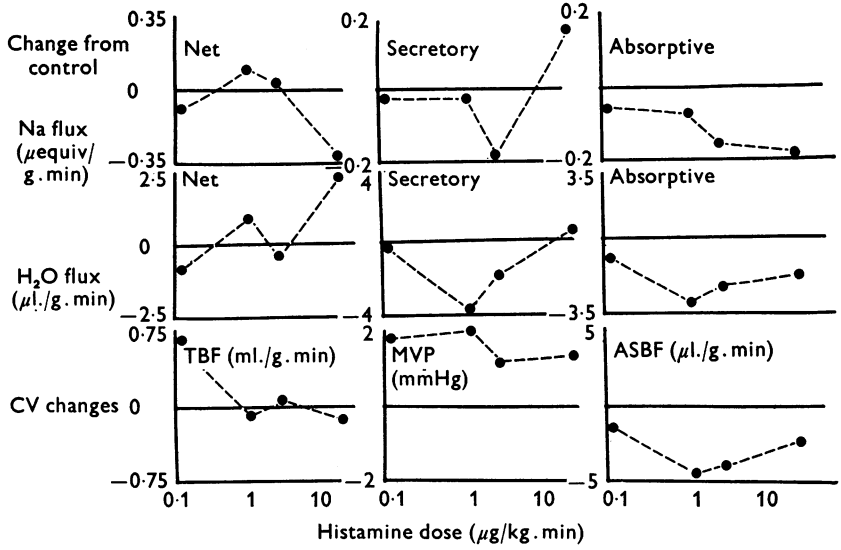

Fig. 6. Effects of histamine (0·1, 1·0, 5·9 and 53 μg/kg.min) infused directly into a mesenteric artery of a segment of canine ileum on Na and H_2O fluxes and cardiovascular (CV) parameters. $n = 1$ for each dose. Average values (± S.E. of mean) for the control periods of the four animals were 1·22 (± 0·16), 1·42 (± 0·16) and 2·64 (± 0·18) μequiv/g.min for Na net, secretory and absorptive fluxes and 8·7 (± 1·1), 17·4 (± 1·1) and 26·1 (± 1·9) μl./g.min for H_2O net, secretory and absorptive fluxes and 0·45 (± 0·09) ml./g.min, 9·0 (± 0·4) mmHg, and 28·5 (± 2·0) μl./g.min for total blood flow, mesenteric venous pressure and absorptive site blood flow, respectively.

DISCUSSION

It was pointed out above that I.V. glucagon (4–256 μg/kg) injection in the rat increased intestinal absorption (Hubel, 1972*a*) but I.V. glucagon infusion (0·5 μg/kg.min) in the dog increased intestinal secretion (Barbezat & Grossmann, 1971*b*). Our results indicate that glucagon (0·05–0·5 μg/kg.min) can indeed have opposite effects on gut transport depending on the effective dose delivered to the intestine relative to the dose delivered to the rest of the body. Higher doses of glucagon reaching the gut do not affect net Na and H_2O transport, as compared to control, but increase gut absorption when compared to the concurrent indirect effects. If the indirect effects are less pronounced or the direct effects more pronounced because of species differences or higher doses, a net increase in absorption could occur. The changes in gut absorption, particularly those following direct glucagon infusion, do not seem to be due to non-specific vasodilatation since histamine does not mimic the effects of glucagon, nor due to the release of

glucose or insulin by glucagon (Samols *et al.* 1966) since these substances also do not mimic the effects of glucagon.

Cardiovascular effects can, at least partly, account for the changes in gut transport. The effects seem to be exerted through the antagonistic actions of centrally mediated vasoconstriction and local vasodilatation due to

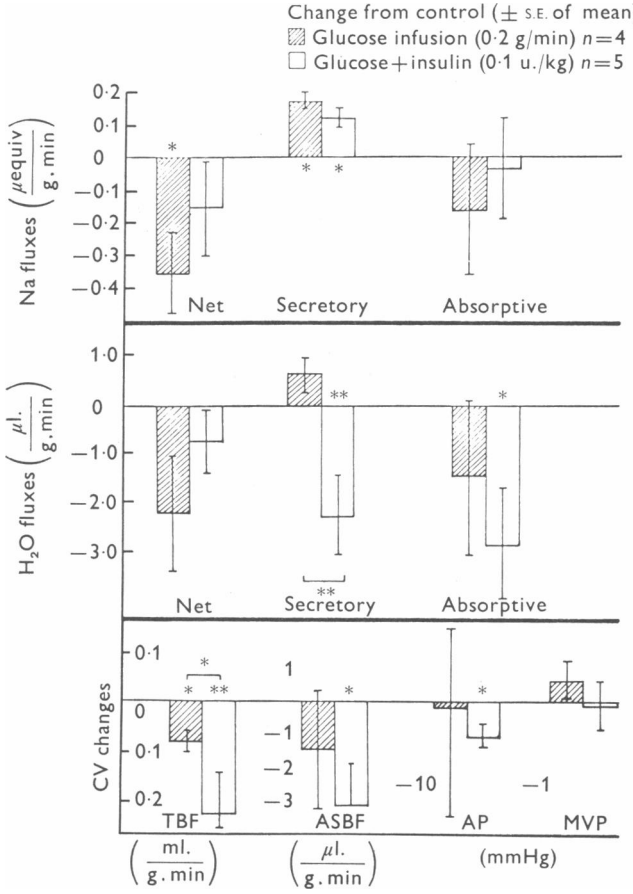

Fig. 7. Effects of glucose (0·2 g/min) or glucose plus insulin (0·1 u./kg) on Na and H_2O fluxes and cardiovascular (CV) parameters (mean ± S.E. of mean) of canine ileum. Glucose and insulin were administered I.V. *, **, *** represent differences between the control and experimental periods significant at the 5, 1 and 0·1 % level, respectively. Control period values were 1·58 (± 0·13), 1·14 (± 0·09) and 2·72 (± 0·14) μequiv/g.min for Na net, secretory and absorptive fluxes, 10·1 (± 0·9), 15·5 (± 1·2) and 25·8 (± 1·5) μl./g.min for H_2O net, secretory and absorptive fluxes, 0·55 (± 0·05) ml./g.min, 27·8 (± 1·6) μl./g.min, 114 (± 4) and 8·9 (± 0·4) mmHg for total and absorptive site blood flow and arterial and mesenteric vein pressure, respectively.

glucagon. The indirect effects of glucagon presumably are due to vasodilatation and a decrease in blood pressure and a consequent compensatory generalized vasoconstriction mediated by baroreceptors and the sympathetic nervous system. Since glucagon reduced both total and absorptive site resistance of the perfused gut segment, whether directly or indirectly infused, the gut vasculature is relatively more sensitive to the vasodilatating action of glucagon than the vasculature of other organs, as concluded by others (Ross, 1970). In turn, the vasculature of the absorptive site is more sensitive to glucagon than the vasculature of the remainder of the gut since a direct glucagon infusion reduces absorptive site resistance more than an indirect infusion but there is no difference in the effects on total resistance. The changes in absorptive site resistance and in arterial and venous pressure allow an increased blood flow to the absorptive site during direct glucagon infusion and a decrease in blood flow during indirect infusion.

Changes in Starling forces have been shown to affect gut transport. During saline infusion, absorptive site blood flow remains constant but colloid osmotic pressure decreases and capillary pressure increases and the unidirectional secretory fluxes of Na and H_2O are linearly correlated with Starling forces. However, the absorptive fluxes are unchanged (Mailman & Jordan, 1975). During glucagon infusion both blood flow and Starling forces change and both the unidirectional absorptive and secretory fluxes change, suggesting that blood flow also has an effect on gut transport. Absorptive site blood flow is associated primarily with changes in absorptive fluxes since during histamine infusion only these change in parallel. Capillary pressure, during glucagon infusion, is linearly related to the absorptive and secretory fluxes of Na and H_2O but the relationship is not the same for the direct and indirect infusion. Therefore, other effects must also be exerted on gut transport and the parallels between absorptive site blood flow and the unidirectional fluxes during glucagon and histamine infusion suggest that they may be related.

One possible mechanism of this relationship could be that glucagon has a direct effect on gut transport and in turn blood flow could parallel the resulting changes in metabolism. Another possibility could be that the blood flow changes are primary and result in different rates of 'wash-out' of transported materials at the absorptive site (Dobson, Sellers & Thoracius, 1971; Ochsenfahrt & Winne, 1968) or could increase the supply of O_2 and nutrients (Granger & Shepherd, 1973) which in turn allow an increase in metabolism. The changes in absorptive site blood flow may also be associated with changes in the number of perfused capillaries which could change the surface area across which the diffusion of both the secretory and absorptive fluxes occur (Shepherd, Mailman, Burks & Granger, 1973; Mellander & Johansson, 1968).

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