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JEJUNAL SODIUM TRANSPORT IN THE RAT: EFFECTS OF ALLOXAN DIABETES

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SUMMARY

A previous study has shown that, in the presence of relatively high concentrations of glucose (16 mM), net transport of Na^+ and glucose is greater in jejunal sacs prepared from diabetic than control rats. This increase in Na^+ transport may be caused by (a) the increased transport of glucose, or (b) differences in glucose metabolism between controls and diabetics. We examined effects of alloxan diabetes on jejunal Na^+ transport using a low concentration (1 mM) of a non-metabolized hexose, 3-*O*-methyl-D-glucose. Net transport of Na^+ into the serosal medium (all data μmoles/g wet wt of sac per h) was greater in everted sacs from diabetic (30) than control (15) rats. Mucosal-to-serosal flux of Na^+ was also greater in sacs from diabetic rats (213 vs 176), but serosal-to-mucosal fluxes did not differ significantly (151 vs 142). Serosal transport of 3-*O*-methyl-D-glucose was three times greater in sacs from diabetic rats (6.2 vs 2.4). Thus, jejunal Na^+ transport is increased in diabetes, independently of the presence of high concentrations of glucose. In these studies, the increase in Na^+ transport is of sufficient magnitude to explain the stimulation of hexose absorption in diabetes. Alternatively, the magnitude of the stimulation of hexose transport could not explain effects on Na^+ movements.

INTRODUCTION

In a preliminary communication Aulsebrook [1] has reported that net serosal transport of Na^+ is greater in everted jejunal sacs prepared from alloxan diabetic than from control rats. The increased Na^+ transport was observed during studies of net glucose transport, which was also increased in sacs from diabetic rats. Glucose was present at relatively high concentrations (16 mM) for an in vitro study. Glucose is rapidly metabolized by intestinal tissue, but rate of metabolism and metabolic pathways are altered by diabetes [2], and might affect Na^+ transport. We, therefore, examined Na^+ transport using a non-metabolized hexose (3-*O*-methyl-D-glucose) at relatively low concentration (1 mM). In addition to net Na^+ and hexose absorption, we measured both mucosal-to-serosal and serosal-to-mucosal fluxes of Na^+.

MATERIALS AND METHODS

We used male albino rats (Simonsen Labs, Minneapolis, Minn.) fed ad libitum (Wayne Lab Blox, Allied Mills). Transport was studied using everted sacs of mid-jejunum prepared by the technique of Crane and Wilson [3]. Animals fasted for 18 h were anesthetized with intraperitoneal sodium pentobarbital (40 mg/kg), and four sacs, each 5 cm in length, were prepared from each animal as previously described [4]. Blood was drawn from the inferior vena cava immediately after removal of the intestine. Approximately 5 min was required to prepare a sac, and the remaining segments were gassed with O_2/CO_2 (95 : 5, v/v) in Krebs–Ringer bicarbonate buffer [5] at 4 °C during the interval. Sacs were incubated for 30 or 60 min at 37 °C in 15 ml of buffer [5] containing 1 mM 3-*O*-methyl-D-glucose (Calbiochem.). The serosal medium was 1 ml of the same solution. For Na^+ transport studies, $^{22}Na^+$ (New England Nuclear, spec. act. 230 Ci/mole, 0.1 μCi/ml) was added to either the mucosal or serosal medium. For 3-*O*-methyl-D-glucose transport studies, 3-*O*-[^{14}C]methyl-D-glucose (New England Nuclear, 10 Ci/mole, 0.04 μCi/ml) was present in mucosal and serosal media.

Radioactivity was measured by liquid scintillation counting of initial and final mucosal and serosal solutions. For $^{22}Na^+$, 0.1 ml sample and 1.9 ml water were added to plastic counting vials with an auto-dilutor (Research Specialties), followed by 10 ml of Bray's solution [6]. For 3-*O*-[^{14}C]methyl-D-glucose, sample volume was 0.2 ml with 1.8 ml water. Counting efficiency was 49 % for $^{22}Na^+$ and 68 % for 3-*O*-[^{14}C]methyl-D-glucose. Na^+ and K^+ were analyzed by atomic absorption spectrometry (Perkin-Elmer 303) on initial and final mucosal and serosal solutions. Blood glucose was analyzed by the method of Somogyi [7]. Initial wet weight of sac is used for expressing transport activity because percent dry weight of mucosal scrapings of mid-intestine of control and diabetic rats differs only by 2 % (control = 24.6 %, diabetic = 22.1 %) [8]. Expressed on a dry weight basis, the increase in transport activity would be slightly greater in diabetics.

CALCULATIONS

In the following formulas, subscripts "i" and "f" refer to initial and final values. cpm is corrected radioactivity in 1 ml sample volume; wt(g) refers to initial wet wt of sac, and V is serosal volume in ml unless specified otherwise.

(1) Net serosal sodium transport (μmoles/g wet wt per h):

$$\frac{(C_f \times V_f) - (C_i \times V_i)}{\text{wt(g)} \times 1\ \text{h}},$$

where C is serosal Na^+ concentration, μmoles/ml. Net serosal K^+ transport was calculated in the same way.

(2) Net serosal 3-*O*-methyl-D-glucose transport (μmoles/g wet wt per h):

$$\frac{\{(\text{cpm}_f \times V_f) - (\text{cpm}_i \times V_i)\} C_i}{\text{wt(g)} \times 1\ \text{h}},$$

where cpm are for 3-*O*-[^{14}C]methyl-D-glucose, and C_i is initial μmoles/ml per cpm.

(3) Mucosal-to-serosal flux of Na^+ (μmoles/g wet wt per h):

$$\frac{\left(\frac{cpm_f \times V_f}{cpm_i \times V_i}\right) \times \left(\frac{C_i + C_f}{2}\right) \times V_i}{wt(g) \times 1\ h}$$

where cpm_f and V_f refer to serosal medium and all other terms relate to mucosal medium.

(4) Serosal-to-mucosal flux of Na^+ is calculated by the same formula, but cpm_f and V_f refer to mucosal medium and all other terms relate to serosal medium.

(5) Net serosal water transport (g/g wet wt per h) is obtained from the increase in weight of serosal medium.

Standard statistical methods [9] were used and differences between data examined by the Student's t test.

RESULTS

Animal populations studied and data on everted sacs are shown in Table I. Initial and final concentrations of Na^+, K^+ and 3-*O*-methyl-D-glucose in mucosal

TABLE I

POPULATIONS STUDIED AND SAC WEIGHTS

	Mean ±S.E.	
	Control	Diabetic
Na^+ transport studies		
Body wt (g)*	314 ±10 (19)***	241 ±14 (17)***
Blood glucose (mg/100 ml)**	104 ± 6	307 ±41
Sac wt (g)		
Initial	0.529± 0.012 (19, 52)†	0.512± 0.015 (17, 44)†
Final††	0.611± 0.015	0.593± 0.016
3-*O*-Methyl-D-glucose transport studies		
Body wt (g)*	335 ± 8 (6)***	278 ±15 (12)***
Blood glucose (mg/100 ml)**	94 ± 4	326 ±54
Sac wt (g)		
Initial	0.463± 0.017 (6, 21)†	0.484± 0.02 (12, 45)†
Final††	0.473± 0.018	0.493± 0.02

* At the time of study 5–8 days after injection. Initial body weights were 240–280 g. Controls and animals to be made diabetic were weight matched initially when controls received distilled water and diabetics a solution of alloxan in distilled water (210 mg/kg of a 100 mg/ml solution of monohydrate) by intraperitoneal injection. Body weight of controls is greater, $P < 0.001$.

** Blood drawn after 18 h fast with water ad libitum. Blood glucose of diabetics is greater, $P < 0.001$.

*** Number of animals.

† Number of animals, number of sacs.

†† All sacs were blotted after incubation. Final sac weight for 3-*O*-methyl-D-glucose studies is weight after draining and blotting a second time. For Na^+ studies it is the weight after draining only.

and serosal media are given in Table II. Table III shows that net serosal transport of Na^+, K^+, 3-*O*-methyl-D-glucose and water are greater in sacs from diabetic than from control rats, but the serosal-to-mucosal fluxes of Na^+ are the same in both groups. Fig. 1 shows that mucosal-to-serosal fluxes of Na^+ are greater in sacs from diabetic than from control animals at 60 min of incubation, although the increase at 30 min is not significant. The time course of mucosal appearance for $^{22}Na^+$ in serosal-to-mucosal Na^+ flux studies is shown in Fig. 2, and Fig. 3 shows the time course for mucosal disappearance of 3-*O*-methyl-D-glucose. The correlation between transport of Na^+ and water into the serosal medium is shown in Fig. 4.

TABLE II

CONCENTRATIONS OF SODIUM, POTASSIUM AND 3-*O*-METHYL-D-GLUCOSE

Initial and final mucosal and serosal concentrations, mM ±S.E. Number of animals and sacs as in Table I. Initial concentration of 3-*O*-methyl-D-glucose was 1 mM.

	Control	Diabetic
Na^+ transport studies		
Na^+ concentration		
Initial	140 ±1	143 ±1
Final		
Mucosal	143 ±1	145 ±1
Serosal	142 ±1	142 ±1
K^+ concentration		
Initial	6 ±0.1	7 ±0.3
Final		
Mucosal	7 ±0.3	10 ±0.3
Serosal	9 ±0.5	11 ±0.6
3-*O*-Methyl-D-glucose transport studies		
Final		
Mucosal	0.85±0.02	0.70±0.01
Serosal	2.03±0.01	3.25±0.15

TABLE III

TRANSPORT OF SODIUM, POTASSIUM, 3-*O*-METHYL-D-GLUCOSE AND WATER

All data μmoles (mg for water)/g wet wt of sac per h ±S.E. Number of animals and sacs as in Table I.

	Control	Diabetic
Net serosal transport		
Na^+	15 ±2	30 ± 3*
K^+	6 ±1	10 ± 1*
3-*O*-Methyl-D-glucose	2.42±0.30	6.17± 0.48*
Water	74 ±8	231 ±23*
Serosal-to-mucosal Na^+ flux	142 ±6	151 ±11

* Differs significantly from control, $P < 0.001$.

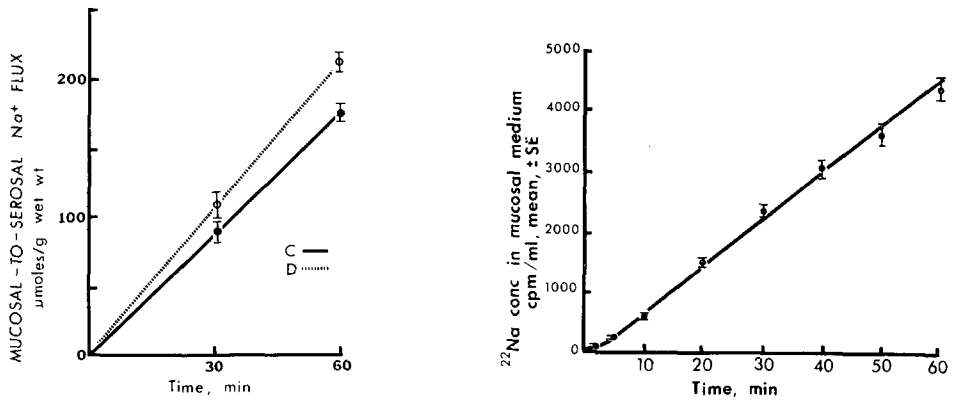

Fig. 1. Mucosal-to-serosal flux of Na^+. Everted jejunal sacs were incubated for 30 or 60 min in Krebs–Ringer bicarbonate buffer containing 1 mM 3-*O*-methyl-D-glucose in both mucosal and serosal media. $^{22}Na^+$ present initially in mucosal medium only. Data are mean ±S.E. Control (●), 30 min: 7 sacs from 3 rats; 60 min: 17 sacs from 6 rats; diabetic (○), 30 min: 11 sacs from 4 rats; 60 min: 18 sacs from 6 rats. Flux rates are linear at 30 and 60 min. The flux rate in diabetics is not significantly greater at 30 min, but the increase is significant at 60 min ($P < 0.001$).

Fig. 2. Appearance of $^{22}Na^+$ in mucosal medium during serosal-to-mucosal Na^+ flux studies. Incubation conditions as described in Fig. 1. $^{22}Na^+$ present initially in serosal medium only. Each point is mean cpm/ml ±S.E. of 7 sacs from 2 control rats. Mucosal medium sampled at 2, 5 and 10 min and every 10 min thereafter. $^{22}Na^+$ enters the mucosal medium rapidly and its concentration increases linearly with time after 5 min.

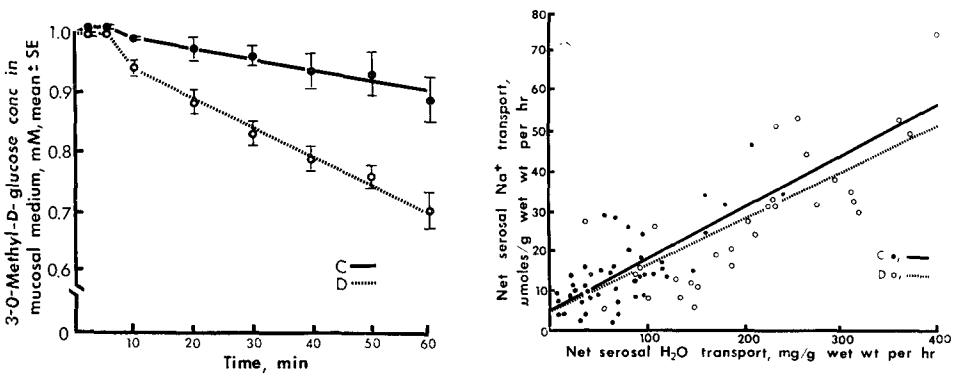

Fig. 3. Decline in 3-*O*-methyl-D-glucose concentration in the mucosal medium of everted sacs from control (●) and diabetic (○) rats during 60 min incubation as described in Fig. 1. After 10 min the concentration of 3-*O*-methyl-D-glucose declines linearly with time.

Fig. 4. Net transport of Na^+ and water into serosal medium by everted jejunal sacs incubated as described in Fig. 1. The correlation (r) between Na^+ and water transport is 0.732 at the slower rate in controls (●) and 0.858 at the more rapid rate in diabetics (○), and is statistically significant ($P < 0.01$ for data from both controls and diabetics).

DISCUSSION

These studies demonstrate that net Na^+ transport and mucosal-to-serosal flux of Na^+ are increased in jejunal sacs from diabetic as compared with

control rats. Serosal-to-mucosal Na^+ fluxes are the same in both groups. Transport of 3-*O*-methyl-D-glucose, a non-metabolized hexose, is also increased in sacs from diabetic rats. These findings agree with those of Aulsebrook [1], who demonstrated increased net transport of Na^+ and glucose in sacs from diabetic as compared with control rats. However, alloxan and streptozotocin diabetes in the rat is associated with significant increases in hexokinase and glucose-6-phosphate dehydrogenase activities in jejunal mucosa [2, 10, 11]. Glucose utilization and lactate production by jejunal mucosa is also increased. These increases in glycolytic rate could provide some of the energy required for increased jejunal Na^+ transport in the presence of the high concentrations of glucose used by Aulsebrook [1]. The present studies demonstrate that Na^+ transport is increased in sacs from diabetics in the presence of a low concentration (1 mM) of non-metabolized hexose (3-*O*-methyl-D-glucose), and does not require high concentrations (16 mM) of glucose. In addition, the present studies demonstrate that the increased net Na^+ absorption in diabetes is the result of greater mucosal-to-serosal Na^+ flux.

With respect to these concurrent increases in transport: (1) Na^+ and hexose transport might each be increased independently; (2) the increase in Na^+ transport might be responsible for the stimulation of hexose transport; (3) the increase in hexose transport might be responsible for stimulation of Na^+ transport. Net serosal transport of Na^+ in sacs from diabetics, 30 μmoles/g wet wt per h (Table III), is twice as great as corresponding control values. Mucosal-to-serosal Na^+ flux is 37 μmoles/g wet wt per h greater in diabetics (Fig. 1). Although hexose transport in diabetics (6.2 μmoles/g wet wt per h) is nearly three times greater than in controls, the magnitude of the increase in terms of μmoles transported into the serosal medium (3.8 μmoles/g wet wt per h) is small in relation to effects on Na^+. Thus, it appears most likely that Na^+ transport is increased in diabetics independently of hexose transport, and it would be difficult to explain the increase in Na^+ transport as secondary to hexose effect. On the other hand, since transport of Na^+ and hexose may be coupled [12, 13], the stimulation of hexose transport could readily be explained as an effect of transported Na^+. The increased transport of both hexose [4, 14–16] and amino acid [5, 17] previously observed in diabetes may be secondary to effects on Na^+ transport. Alternatively, transport of Na^+ and hexose and amino acid might be increased independently.

The cause for increased absorption of Na^+ and hexose in diabetes is unknown. Urinary glucose excretion by diabetic rats approaches carbohydrate intake [18]. Hence, despite hyperglycemia, diabetics are carbohydrate depleted. This glycosuria produces an osmotic diuresis, with urine volumes equal to or exceeding body weight. The resulting increase in urinary Na^+ excretion in diabetics would produce Na^+ depletion unless compensated by increased absorption. Thus, absorption is enhanced for two substances (Na^+ and hexose) whose urinary excretion in diabetes is excessive.

Data on K^+ concentrations (Table II) and net K^+ transport (Table III) are difficult to interpret. Final serosal and mucosal medium concentrations increased in both diabetics and controls, but the increase was greater in diabetics, particularly in the mucosal medium. Whether the greater serosal K^+ transport in diabetics (Table III) reflects an actual increase in transport activity or simply greater loss from tissue cannot be determined from these studies.

Serosal water transport is three times greater in diabetics than controls (Table

III). This is in accord with greater solute (Na^+, K^+ and 3-*O*-methyl-D-glucose) transport in diabetics. Serosal water transport correlated with that of Na^+ (Fig. 4) at both the lower rates with controls and the higher rates with diabetics.

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