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Effect of concanavalin A on the electrogenic transfer of some amino acids and hexose sugars across rat small intestine

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ABSTRACT

1. The effect of concanavalin A (Con A) on the electrogenic transfer of glucose, galactose, glycine and valine was investigated *in vitro* using everted sacs of the rat jejunum. The short circuit current (s.c.c.), potential difference (p.d.) and tissue resistance were also measured in the presence and absence of the drug.

2. Con A increased apparent Km for glucose, galactose, glycine and valine, decreased s.c.c. and p.d. in the presence of glucose, and inhibited sodium transport.

3. In view of the fact that oxygen consumption was unaffected, it is proposed that Con A did not act by a non-specific mechanism.

INTRODUCTION

Con A is a globular protein normally extracted from lectin (from Jack beans). According to So & Goldstein (1976a, b), Con A possesses four saccharide-binding sites and generally binds to saccharides containing D-mannose or D-glucose residues. The detailed manner in which Con A interacts with saccharides is of considerable interest, in as much as the biological activity of Con A appears to require binding to the cell surface via glycoprotein or polysaccharide receptors (Becker *et al.* 1976).

Apart from the work of Dhaunsi *et al.* (1985) which showed that pea lectin and lentil lectins affected the transport of some nutrients, there are few reports in the literature on the effect of Con A on intestinal transport. In the present study the effect of different concentrations of Con A on the transport of a selected group of monosaccharides and amino acids was investigated in the rat intestine using an electrical method for measuring changes in trans-intestinal potential and the s.c.c. associated with the active transport of substrates.

MATERIAL AND METHODS

ANIMALS

Laboratory-bred male Wistar rats weighing 230–270 g were used. They were maintained *ad libitum* on a commercial diet (No. 86, Dixon & Sons, London, U.K.) and had free access to water. They were acclimatized at a temperature of $20 \pm 3°C$

and a photoperiod of approximately 12 h light–dark cycle. The rats were anaesthetized by injecting 60 mg sodium pentobarbital per kg body weight intraperitoneally.

MEASUREMENT OF THE ACTIVE TRANSPORT

Everted sacs (each 6 cm long) were prepared from the middle of combined jejunum and ileum. They were filled with 0·7 ml of Krebs bicarbonate solution (Krebs & Henseleit 1932) and incubated in vessels similar to those used by Barry *et al.* (1964). The transfer p.d. was measured by 2 agar salt bridges placed in the serosal and mucosal solution. These bridges were connected to 2 calomel half cells arranged back-to-back across the input terminal of a digital electrometer. Before beginning the experiments the sacs were allowed to achieve a stable potential. This usually occurred 10 min after the beginning of the incubation. For the transfer of non-metabolized hexose and amino acids, mannose (164 mM) was added to the serosal side.

Increasing additions of D-glucose, D-galactose, glycine and valine were made to the mucosal solution bathing the sac in order to produce a final mucosal fluid concentration of 2, 4, 8, 16 and 32 mM. In the experimental group the sac was incubated with a Krebs' solution containing 10 μg/ml Con A, and the changes in the p.d. after each addition of the substrates were measured as in the control group.

Further details of the experimental procedures and the methods of estimation of the electrogenic transfer can be found in Syme & Levin (1976) and Al-Balool (1987).

MEASUREMENT OF S.C.C., P.D. AND TISSUE RESISTANCE

The s.c.c., p.d. and tissue resistance were measured *in vitro* using a sheet of jejunum (approximately 2 cm^2) clamped between two perspex chambers. The preparation was incubated in Krebs' bicarbonate containing 28 mM glucose on both sides and gassed with 95% O_2/5% CO_2. A current was applied across the tissue using Ag–AgCl electrodes that contacted mucosal and serosal solutions *via* wide-bore (2 mm) salt bridges. The preparation was left until a steady transmural p.d. was reached (10–15 min). The current was applied to clamp the p.d. at zero and other values in order to obtain the s.c.c. and the resistance of the intestinal wall. Values for the intestinal resistance and the s.c.c. were obtained from a graphic plot of p.d. against the current applied in the presence and absence of tissue. The difference between the two slopes was the resistance of the intestine and the intercept of the two resistance lines gave the value of the s.c.c. (Clarkson & Toole 1964). Results were expressed as ohms/cm^2 sac and $\mu A/cm^2$ for the resistance and the s.c.c. respectively. The Krebs' bicarbonate saline bathing the mucosal side was then removed from the chamber by suction and a new solution containing the drug was introduced. After 10 min, a current was applied and the s.c.c. and p.d. were measured every 10 min for approximately 30 min.

MEASUREMENT OF SODIUM TRANSFER

For the measurement of sodium transfer, intestinal sacs (6 cm long) were taken from the distal ileum. These sacs were initially filled with 0·7 ml Krebs' bicarbonate saline containing 28 mM glucose (serosal fluid) and were incubated in 25 ml of the same

solution (mucosal fluid). In the experimental sac, 10 μg/ml or 20 μg/ml Con A were added to the mucosal solution only. For the first 5 min of incubation the incubation solution was gassed continuously with 95% O_2/5% CO_2. At the end of the incubation period the sac was removed and the final serosal fluid and sample of the initial Krebs' bicarbonate were used for the estimation of sodium concentration using a flame photometer (Corning 400).

MEASUREMENT OF OXYGEN CONSUMPTION

Intestinal tissue oxygen consumption was measured using the YSI biological oxygen monitor (Model 53). The oxygen probe is a specially designed Clark type polarographic electrode which fits in one of the four sample chambers to make a closed system. The everted sac (about 1 cm in length) of mid-jejunum was suspended from the oxygen electrode by its ligatures and then placed in the chamber which contained 5 ml of Krebs' bicarbonate. Glucose (28 mM) was present in both the serosal and mucosal solutions. In the experimental group Con A (20 μg/l) was added to the mucosal solution. The solution was gassed with 95% O_2/5% CO_2 for 5 min. The gassing was stopped and the decrease in P_{O_2} measured for 5 min. The change in P_{O_2} was converted into O_2 consumption/weight/min.

EXPRESSION OF THE RESULTS

The results were expressed as the mean (± the standard error, SEM) with the number of observations in parentheses. Where a comparison was made between two sets of results, the unpaired Student's *t*-test was applied to establish the level of significance with confidence limits of 95%.

RESULTS

EFFECT OF CON A ON THE ELECTROGENIC TRANSFER OF AMINO ACIDS AND SUGARS

The 'apparent Km' and the $p.d._{max}$ as calculated with Lineweaver–Burk transformation of the saturation curve are shown in Tables 1 and 2. Con A increased

Table 1. Effect of Con A on $p.d._{max}$ of amino acids and hexoses. Con A was added to the mucosal solution only. The results (mean ± SEM) of 6-cm everted sacs with the number of animals in parentheses. The values were calculated from Lineweaver–Burk's (16) transformation of the saturation kinetics curve obtained for the substrate range of 2, 4, 8, 16, 32 mM which were corrected for osmotic-induced p.d. using mannitol

	$p.d._{max}$ (mV)							
Con A conc. (μg/ml)	Glucose	% Change	Galactose	% Change	Glycine	% Change	Valine	% Change
0	8·61 ± 0·57 (10)		9·53 ± 0·56 (11)		4·33 ± 0·32 (8)		4·60 ± 0·22 (8)	
10	8·20 ± 0·24 (11)	NS	7·89 ± 0·78 (6)	NS	4·30 ± 1·60 (14)	NS	4·76 ± 0·26 (11)	NS

Table 2. Effect of Con A on the apparent Km of amino acids and hexoses. Con A was added to the mucosal solution only. The results (mean ± SEM) of 6-cm everted sacs with the number of animals in parentheses. The values were calculated from Lineweaver–Burk's (16) transformation of the saturation kinetics curve obtained for the substrate range of 2, 4, 8, 16, 32 mM which were corrected for osmotic-induced p.d. using mannitol

	Apparent Km (mM)							
Con A conc. (μg/ml)	Glucose	% Change	Galactose	% Change	Glycine	% Change	Valine	% Change
0	2·29 ± 0·10 (10)		3·47 ± 0·16 (11)		8·89 ± 0·88 (8)		3·10 ± 0·23 (8)	
10	4·87 ± 0·49* (11)	+112	11·38 ± 0·81* (6)	+228	16·35 ± 1·60* (11)	+84	4·89 ± 0·38 (11)	+58

* $P < 0·001$.

the 'apparent Km' of glucose, galactose, glycine and valine, but had no significant effect on the $p.d._{max}$ of substrates.

EFFECT OF CON A ON THE S.C.C., P.D. AND TISSUE RESISTANCE

Con A caused a dose-dependent decrease in s.c.c. and p.d. and had no significant effect on the tissue resistance (Fig. 1). After 2 min of the addition of the drug solution (5 μg/ml) the s.c.c. decreased by 19% ($P < 0·005$) and the p.d. decreased by 15% ($P < 0·05$). The decrease in both parameters continued slightly with time. When the concentration of Con A was increased by 10 μg/ml and 20 μg/ml a gradual decrease in the s.c.c. and p.d. was observed (Fig. 1). When the relation between the concentration of Con A and the percentage decrease in s.c.c. and p.d. was plotted, the percentage decrease in the s.c.c. and p.d. reached saturation at high concentrations of the drug (Fig. 2). Using the Lineweaver & Burk (1934) plot, the Ki of Con A was 9·56 ± 0·86 for the s.c.c. and 7·92 ± 0·79 for the p.d.

EFFECT OF CON A ON SODIUM TRANSFER

To test whether the changes in the s.c.c. reflect an alteration in sodium transport, the effect of Con A on sodium transfer by rat ileum was investigated.

The ileal mucosal sodium transfer from large incubatory volume is small and technically difficult to measure accurately. However, because the serosal volume is small, the changes in sodium concentration are large and can be assessed by flame photometry. Therefore serosal transfer was measured in the presence and absence of Con A. At 10 μg/ml and 20 μg/ml, Con A inhibited the ileal serosal sodium transfer by 39% ($P < 0·001$) and 47% ($P < 0·001$) respectively (Table 3).

EFFECT OF CON A ON OXYGEN CONSUMPTION

The oxygen consumption of control sacs was 1·39 ± 0·27 μl/mg wet weight/h (mean ± SEM of six experiments), while in the presence of Con A the value was

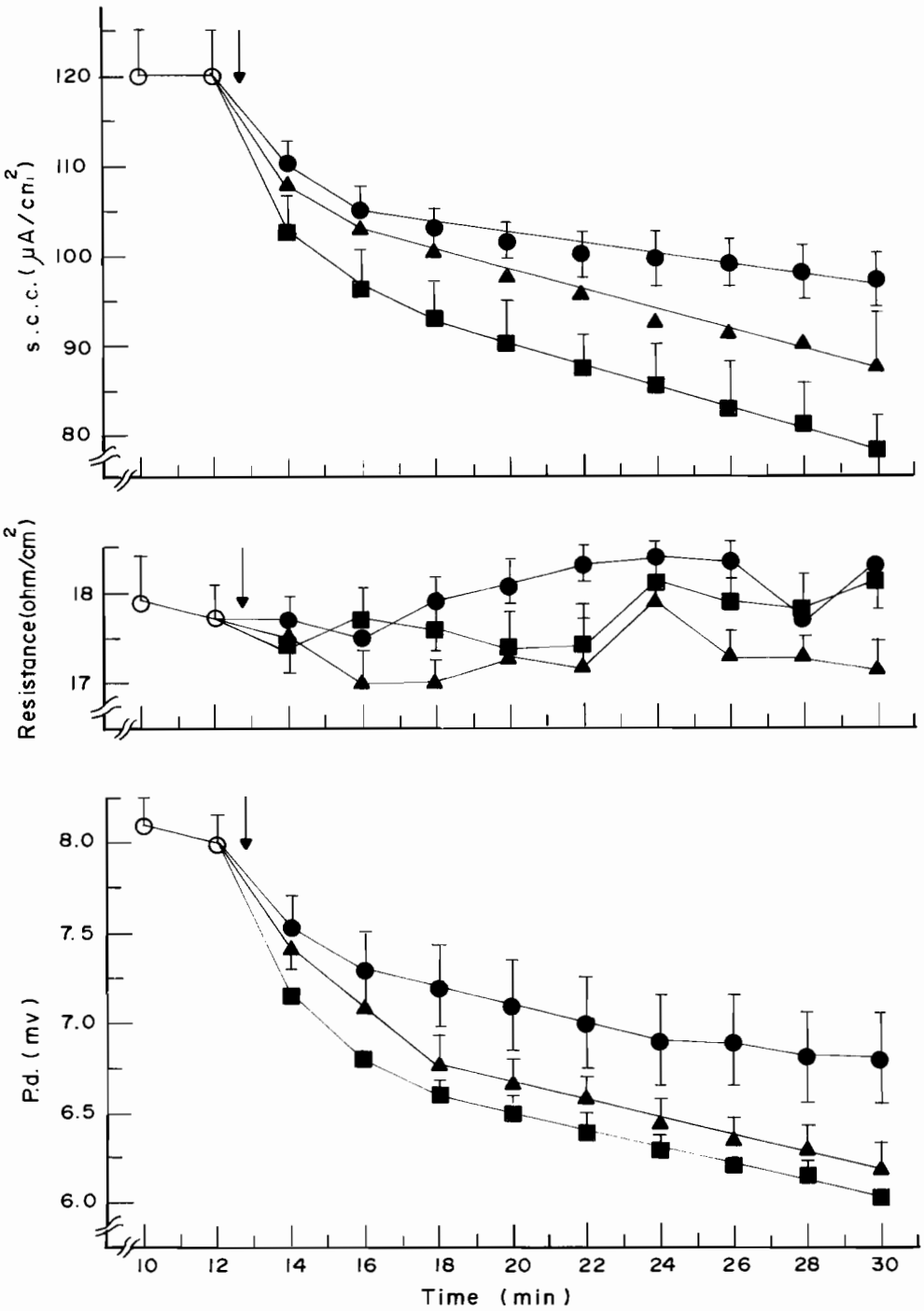

Fig. 1. Time course of the effect of Con A on the s.c.c., p.d. and tissue resistance generated by 28 mM glucose across a sheet of rat jejunum. Control (○), 5 μg/ml Con A (●), 10 μg/ml Con A (▲) and 20 μg/ml (■). Con A added at the time indicated by arrows. Vertical lines represent SEM ($n = 6$).

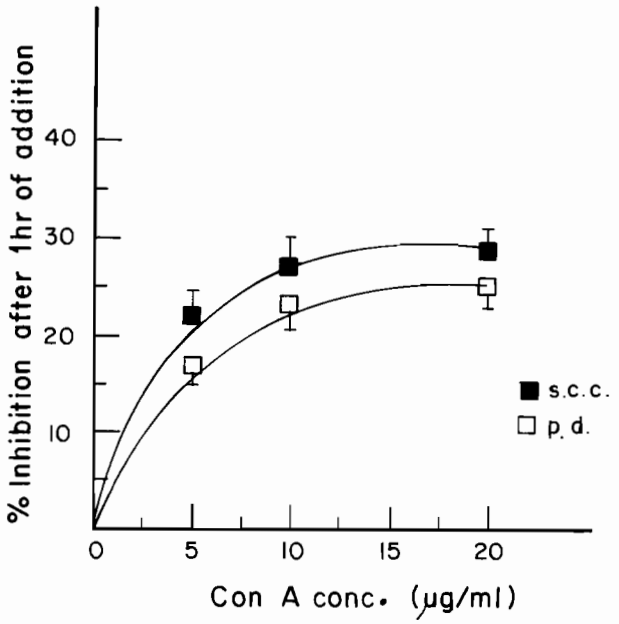

Fig. 2. Relationship between concentration of Con A and percentage decrease in s.c.c. and p.d. across the rate jejunum.

Table 3. Effect of Con A on sodium transfer by everted rat ileum. ISA = initial serosal amount; FSA = final serosal amount; SNaT = serosal sodium transfer = final serosal amount − initial serosal amount. Con A was added to the mucosal side only. Six cm of everted distal ileum was used. The sac contained 0·7 ml of bicarbonate containing 28 mM glucose and was suspended in 25 ml of the same solution. The results are given as mean ± SEM with the number of animals used in parentheses

Con A conc. (μg/ml)	ISA (μEq)	FSA (μEq)	SNaT (μEq)	% Change	
0	150·1 ± 1·6	215·7 ± 5	65·5 ± 4·6		(8)
10	149·5 ± 0·82	189·9 ± 2·3	39·9 ± 2·3*	−39	(8)
20	148·5 ± 0·88	184·5 ± 2·0	34·5 ± 2·0*	−47	(7)

* $P < 0{\cdot}001$.

1·2 ± 0·28 μl/ml wet weight/h (mean ± SEM of six experiments). The two values are not significantly different from each other.

DISCUSSION

Con A was found to increase significantly the 'apparent Km' for glucose, galactose, glycine and valine without inducing a significant change in the $p.d._{max}$ observed for

the transport of these substances across the rat jejunum (Tables 1 and 2). This suggests that Con A binds to similar sites on the amino acid and monosaccharide carriers. Since the sodium site is common to both carriers, it is tempting to think that this is the site that is affected by Con A.

In the present study Con A was also found to decrease the electrogenic capacity of the rate jejunum in the presence of glucose. The kinetics of the effect of Con A on the s.c.c. and p.d. revealed that the inhibition was dose-dependent at high concentrations (Fig. 2). This probably indicates the presence of only a limited number of sites for Con A attachment. Schultz & Zalusky (1964) attributed the increase in the s.c.c. induced by the addition of glucose to the increase in the rate of active sodium transport across the rabbit ileum. A similar effect on the rate of active sodium transport could account for the inhibitory action of Con A on the s.c.c. and p.d. in the present study.

The rat ileum has been shown to accumulate sodium against its electrochemical and concentration gradient (Curran & Solomon 1957; Clarkson & Rothstein 1960). Serosal sodium transfer was found to be significantly inhibited by Con A (Table 3). Since the drug produced no significant changes in tissue resistance (Fig. 1), indicating a lack of effect on the passive permeability of sodium through the intercellular pathway, Con A probably inhibits sodium transfer by inhibiting transcellular transport of sodium ions. This may take place by blockage of the entry of sodium across the luminal membrane, or it may be due to a decrease in the rate of pumping of sodium out of the cell across the serosal or basolateral membrane, or both.

The short latent period for the action of Con A on the s.c.c. and p.d. observed in the present study seems to indicate that the drug probably produced its initial effect at the brush border. In addition, Con A was found to have no effect on the oxygen consumption of the rat small intestine, indicating that the drug had little action on the metabolism of the intestinal epithelium. Therefore, it seems likely that Con A affected ileal sodium transport by its action on the mechanism of entry of sodium into the cells on the brush border side rather than by affecting cellular energy metabolism.

Speculation of the possible action of Con A is rendered difficult by a rather limited number of studies on its effects on intestinal transport. Sjolander *et al.* (1984) found that Con A treatment (1 mg/ml) decreased intestinal permeability to large dextran molecules without affecting the passage of small molecules. They attributed the decrease in intestinal permeability to large molecules to the 'mucotractive' effect suggested by Freed & Buckley (1978) and Freed (1979). Freed (1979) found that Con A stimulated the discharge of mucus from goblet cells and suggested that the mucus thus released could form an additional barrier to the absorption of large molecules and thereby reduce the transmural passage. However, since Con A produced no significant change in the ultrastructure of the small intestine, Sjolander *et al.* (1984) suggested that this could be the reason why Con A has no significant effect on the passage of small molecules. Moreover, Dhaunsi *et al.* (1985) studied the effect of pea and lentil lectins on the transport of D-glucose, L-alanine and L-phenylalanine using brush border vesicles of rat intestine. They found that the transport of D-glucose was unaffected by pea lectin but increased significantly in the presence of lentil lectins. On the other hand, in the presence of pea lectin the transport of L-alanine was significantly increased, while lentil lectin had no effect. Both lectins caused a significant decrease in the transport of L-phenylalanine.

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دراسة تأثير الكونكاناڤالين أ على التغيرات الكهربائية التي تحدث عند مرور السكريات والأحماض الأمينية خلال الأمعاء الدقيقة للفأر

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خلاصة

في هذا البحث تمت دراسة تأثير مادة الكونكاناڤالين أ على التغيرات الكهربائية التي تحدث عند مرور السكريات والأحماض الأمينية خلال الأمعاء الدقيقة للفأر باستخدام طريقة الكيس المقلوب ، فقد تمت دراسة التغيرات التي تحدث في فرق الجهد وكمية الكهرباء اللازمة التي يتلاشى معها فرق الجهد ، والمقاومة الكهربائية للأمعاء الدقيقة ، ومقارنتها في حالتي وجود وعدم وجود مادة الكونكاناڤالين أ . وقد وجد أن المادة تزيد من « ثابت ميشيل للسكريات والأحماض الأمينية » ، كما أنها تقلل من فرق الجهد وكمية الكهرباء اللازمة لكي يتلاشى فرق الجهد في حالة وجود الجلوكوز ، وكذلك تقلل من سرعة مرور الصوديوم خلال الأمعاء الدقيقة . واعتمادا على أن احتياج الأمعاء الدقيقة للأكسجين لم يتغير في وجود مادة الكونكاناڤالين فأنه يمكن الاستنتاج أن تأثير هذه المادة نوعي وليس تأثيراً شاملا .