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Effect of Cortisone on Postnatal Development of Ion Transport in Rabbit Small Intestine

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Summary

To study the effect of corticosteroids on postnatal maturation of Na^+ transport in the small intestine, we studied 10–12-day-old suckling rabbits after they had received cortisone acetate, 20 mg/kg SC on days 3, 4, and 5 of life. When killed, the cortisone-injected animals weighed significantly less than saline-injected controls. In jejunal villus enterocytes isolated from this cortisone-treated group, the specific activities of sucrase and Na^+-K^+-ATPase were significantly greater than those in control enterocytes. Studied in Ussing chambers, a significant electrical and ion-flux response to glucose was observed in the jejunal epithelium of the treated group, but not in controls. We conclude that exogenous cortisone, administered early in life, can stimulate the precocious development not only of certain epithelial enzymes but also glucose-facilitated Na^+ transport in the jejunum of the rabbit.

Certain features of the small intestinal capacity to transport sodium appear to mature during early postnatal life in the rabbit (18). We studied the impact of injections of corticosteroid given in high dosage soon after birth on this developmental process. Although its mechanisms of action are not known, exogenous cortisone has been shown to induce the premature maturation of numerous small bowel enzymes and transport pathways in several species (9).

MATERIALS AND METHODS

Litters of New Zealand White rabbits were reduced in size to 6–8 animals on day 3 of life when half those in each litter were given cortisone acetate, 20 mg/kg SC, and the rest, controls, received the same volume of saline. These injections were repeated on days 4 and 5 and all rabbits were killed without fasting when they were 10–12 days old. Blood was taken from the heart for measurement of serum Na concentration just before the animals were killed by an intracardiac injection of phenobarbital. At death the adrenal glands were removed and prepared for light microscopic study by standard techniques. A 15-cm segment of jejunum, starting 5–8 cm distal to the ligament of Treitz was quickly removed for *in vitro* transport studies and epithelial cell isolation. In villus cells, isolated from the proximal 10 cm of the jejunal segment by a vibration technique that excludes crypt cells (5), we measured the activities of several enzymes: sucrase by the method of Dalhqvist (3), Na^+-K^+-ATPase by a modification of the method of Kelly *et al.* (11), and thymidine kinase by a modification (6) of the method of Klemperer and Haynes (13) and Breitman (1); protein was measured by the method of Lowry *et al.* (15). The presence of intact crypts was confirmed by light microscopy of the tissue remaining after the isolation procedure.

Four adjacent segments of unstripped jejunal mucosa were mounted in small Ussing short-circuited chambers, exposing 0.4 cm^2 mucosal and serosal surfaces to 15 ml of warmed, oxygenated Krebs bicarbonate-phosphate buffer to measure steady state, mucosa to serosa (J_{ms}), serosa to mucosa (J_{sm}), and net (J_{net}) Na^+ and Cl^- fluxes under basal conditions and in the presence of 30 mM glucose (12). Spontaneous transepithelial potential differences (PD) were measured at intervals, and an external short circuit current, sufficient to bring the PD to zero, was introduced as previously described (17). After equilibration periods of 30 min in the basal state, and 15 min in the presence of glucose, unidirectional and net ion fluxes were measured with tracer quantities of ^{22}Na and ^{36}Cl added either to the serosal or mucosal side of paired tissues. Reservoirs were sampled at 10-min intervals and ion fluxes were calculated using standard equations (12). The intestinal tissue remained viable during these transport studies, as shown by the stability of the electrical characteristics of the tissue during the experiments and the lack of histologic abnormalities after completion of the 2-h experiments.

Statistical analyses were performed by Student's *t* test or paired *t* test where appropriate. Our analysis of the enzyme data was based on a logarithmic distribution and the data expressed as antilogarithms.

RESULTS

Cortisone-treated rabbits at 10–12 days appeared lethargic and ill; they weighed much less, 140 ± 9 g, than controls, 235 ± 16 g, (mean ± S.E.) ($P < 0.001$) but all animals survived. Serum sodium concentrations were similar in both groups, 124 ± 2.9 mEq/liter in the controls, and 126 ± 3.6 mEq/liter (mean ± S.E.) in the cortisone-treated animals. In serial sections of adrenal glands studied by light microscopy no significant differences were seen between the two study groups.

The activities of three epithelial enzymes assayed in villus enterocytes are summarized for the two study groups in Table 1. Six-fold, highly significant increases in sucrase and Na^+-K^+-ATPase specific activities were found in cells from the cortisone-treated group compared with controls, but thymidine kinase activities were similar in both groups.

Table 1. *Enzyme activities[1] in jejunal villus enterocytes from 10–12-day-old rabbits*

	Saline-injected controls	Cortisone-injected	P^2
Number of animals	13	15	
Enzymes			
Sucrase-units/g protein	2.3 ± 0.8	14.0 ± 4.6	< 0.001
Na^+-K^+-ATPase-units/g protein	0.53 ± 0.56	3.12 ± 0.80	< 0.005
Thymidine kinase-units/mg protein	6.8 ± 0.8	5.8 ± 0.8	NS

[1] Each value represents the antilog of log mean and range of 1 S.E. Sucrase activity is expressed as μmoles substrate hydrolysed per min, Na^+-K^+-ATPase as μmoles of inorganic phosphorus per min and thymidine kinase as pmoles of thymidine phosphate per min.

[2] Treated group compared with control, NS = not significant.

Electrical data obtained from the Ussing chamber studies are summarized in Figure 1. Throughout the 60-min basal period, PD and short circuit currents remained relatively stable in both study groups but significantly higher in controls than in cortisone-treated animals ($P < 0.001$). In the presence of glucose, 30 mM, PD and short circuit currents rose significantly ($P < 0.001$) but no significant increments occurred in controls. Tissue conductance across the epithelium was not significantly different in the two groups and did not change in the presence of glucose.

Under basal conditions unidirectional and net Na^+ and Cl^- fluxes were similar in the two study groups (Table 2). Significant increments in mucosa to serosa fluxes J^{Na}_{ms}, J^{Cl}_{ms}, and net fluxes J^{Na}_{net} and J^{Cl}_{net} were observed in the cortisone-treated group but not in controls after glucose was added.

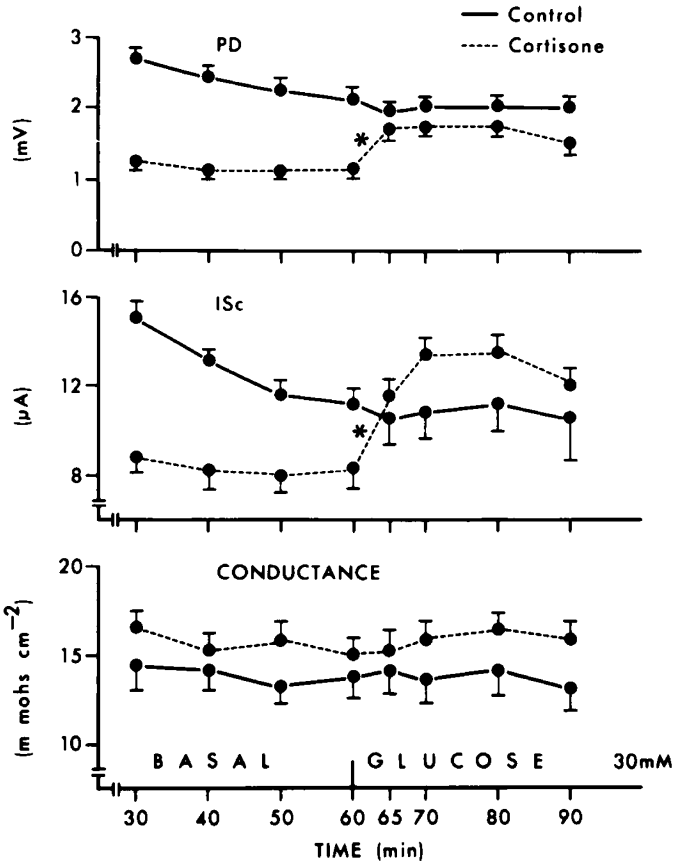

Fig. 1. Jejunal epithelium from two groups of 10–12 day-old rabbits studied in Ussing chambers. After glucose 30 mM, significant increments (*) in PD and short circuit current (Isc) occurred only in cortisone-treated animals. Conductances were stable and similar in two groups. Mean values, showing 1 S.E. at intervals during course of experiments.

DISCUSSION

Cortisone, given shortly after birth in a high dose, caused the premature appearance of glucose-stimulated sodium transport in the jejunum of the suckling rabbit. Although the exact nature of this induction cannot be determined from our data, some effect of the drug on the brush border membrane glucose-sodium carrier is suggested. Adrenal corticoids, administered either during pregnancy or soon after birth, have been shown to induce widespread maturational changes in the small intestine (8, 12, 14, 17, 20) including the precocious development of brush border digestive enzyme activity (4). Furthermore, the activity of the brush border disaccharidase, sucrase, increases sharply in the suckling rat shortly after the spontaneous normal surge of circulating free corticosterone at 12 days of age (9). In our cortisone-injected animals, sucrase, which may be closely related spatially to the glucose-Na^+ carrier in the brush border membrane (2), increased in activity as expected (9). Sodium-potassium-dependent ATPase activity, closely associated with the sodium pump at the basolateral membrane was also significantly higher in enterocytes from cortisone-injected rabbits than in control cells. The response of Na^+-K^+-ATPase to cortisone may reflect a direct impact of the drug or it may reflect an adaptive response to altered intracellular sodium concentrations resulting from other changes in transport function (19). A similar response of this ubiquitous enzyme to administered corticosteroid has been observed in the gill of the mature eel (16). Thymidine kinase activity is associated with proliferating cells and in the small intestine of mature animals it is largely confined to the crypts. In certain pathologic states and in very young animals, villus cells can be relatively rich in the enzyme, presumably because either cell division is occurring not just in crypts but on the villi, or the cells that divide in the crypts are failing to differentiate as they migrate from crypt to villus (12, 18). Because thymidine kinase activity was relatively high in our control group, and unaffected by injected cortisone, the impact of the drug appears not to have been one of a general stimulus to cell differentiation.

When *in vitro* studies of this nature are applied to very small animals with fragile tissues, concern rises for possible artifacts caused by damage from manipulating the intestinal mucosa. In the present experiments, it was reassuring to find that it was the mucosa from the smaller animals, not the mucosa from the larger, better nourished control rabbits that responded in a mature fashion to glucose. Cortisone-treated rats were sickly and weighed much less than controls. A similar general effect of the drug on young animals has been noted previously (8). The lack of significant abnormalities of serum sodium concentration and the observed normal adrenal cortical structure in the cortisone-treated group suggests that these rabbits, killed 5–7 days after they received their last dose of cortisone, did not suffer from adrenal insufficiency. Undoubtedly the cortisone-treated group was relatively undernourished but chronic malnutrition would be expected to delay rather than accelerate maturation (7). In everted jejunal sacs from adult rats and guinea pig, but not from the hamster, D-glucose transport was enhanced after semistarvation (10).

Table 2. *Ion flux in short circuit jejunal epithelium; control and cortisone-treated 10–12-day-old rabbits μEq $cm^{-2}h^{-1}$ (mean ± S.E.)*

			Na flux			Cl flux		
Group	No	Study	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}
Control	11	Basal	6.91 ± 0.50	6.70 ± 0.50	+0.21 ± 0.51	6.21 ± 0.82	6.35 ± 0.79	−0.13 ± 0.87
		Glucose 30 mM	7.30 ± 0.60	7.20 ± 0.51	+0.10 ± 0.70	6.85 ± 0.80	7.11 ± 0.72	−0.17 ± 0.63
		Δ Glucose	+0.39 ± 0.32	+0.50 ± 0.39	−0.11 ± 0.44	+0.64 ± 0.44	+0.76 ± 0.23	−0.04 ± 0.60
		P^1	NS	NS	NS	NS	NS	NS
Cortisone	11	Basal	8.06 ± 1.16	7.98 ± 1.64	+0.08 ± 0.98	8.29 ± 1.26	8.03 ± 0.80	+0.16 ± 0.86
		Glucose 30 mM	9.79 ± 1.40	8.66 ± 1.63	+1.13 ± 0.84	11.32 ± 1.47	9.60 ± 1.11	+1.72 ± 1.21
		Δ Glucose	+1.73 ± 0.43	+0.68 ± 0.39	+1.05 ± 0.29	+3.03 ± 0.65	+1.57 ± 0.59	+1.56 ± 0.66
		P^1	< 0.005	NS	< 0.01	< 0.001	< 0.025	< 0.05

[1] Paired *t* test of increments after 30 mM glucose in two study groups; NS = not significant.

Our earlier study of normal suckling rabbits, 3–5 days of age, with body weights comparable to the cortisone-injected 10–12-day-old animals in the present study, did not show enhanced glucose stimulated efflux compared with 10–12-day-old controls (18).

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