Intestinal Transport of Glucose and Sodium: Stimulation by Reserpine and the Humoral Mechanism Involved.* (30189)

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Gaunt and his associates found that acute reserpine treatment inhibited renal excretion of water, Na, and K(1). Plummer reported that the drug increased heart rate in the dog heart-lung preparation(2), and Paasonen showed that this increase is associated with release of bound norepinephrine from storage sites in the heart(3). Recent studies in this laboratory(4) have shown that epinephrine and norepinephrine *in vitro* stimulate transport of glucose and Na by everted segments of rat small intestine. The present report is concerned with effects of reserpine on intestinal transport of glucose and Na and experiments to test the hypothesis that, as in the heart, reserpine action is consequent to release of bound catecholamines. The results show that reserpine stimulates intestinal transport of glucose and Na, and suggest that the drug acts through release of intestinal catecholamines.

Methods and materials. Male Holtzman rats weighing 270-300 g were used. Animals treated with the large doses of reserpine† used in these experiments do not feed for many hours, and since absorption is influenced by the amount of food eaten, both control and experimental rats were deprived of chow at the beginning of treatment, or 18 hours before sacrifice in the experiments with *in vitro* reserpine. One segment of upper jejunum about 15 cm in length and a second of lower jejunum and upper ileum of the same size were taken from each rat and incubated for 90 minutes in a Dubnoff shaker. The buffer was a modified Krebs-bicarbonate solution with 137 mM of Na, 5 of K, 1 of Ca and 17 of glucose. Segments were prepared and incubated according to the procedure previously reported(5). Sodium in the serosal (absorbed) fluid was analyzed by flame photometry and glucose by the Somogyi modification of the Nelson method (6). Segments were dried and weighed and solute transport expressed as μmoles/g tissue/hr. Data from corresponding regions of the gut were analyzed by a pairing technique and subjected to the "t" test.

Animals given reserpine by injection received the drug subcutaneously in doses of 1.5 mg/kg body weight at 18 hours and again at 2 hours before sacrifice, except for one experiment in which reserpine was also given at 42 hours. Dichloroisoproterenol (DCI)‡ was given subcutaneously at 18 and 2 hours in doses of 5 mg/kg. In experiments with reserpine *in vitro* the drug was present in the mucosal fluid at a concentration of 2×10^{-5} M. Adrenal demedullation was performed by slitting the capsule of the gland, expressing the medulla and part of the cortex and returning the gland to the body cavity. Demedullated animals were used 18 days after surgery or sham operation, an interval sufficient to allow functional regeneration of the cortex(7). Histological examination of demedullated glands taken at sacrifice showed regrowth of cortical tissue.

Results. Animals treated with reserpine exhibited the usual signs which follow large amounts of the drug, including lethargy and diarrhea. At the time of sacrifice the small intestine was virtually empty. The data appear in Table I, and show that segments from reserpinized animals absorbed increased amounts of glucose and Na. In particular glucose transport was more than doubled. In previous studies on effects of catecholamines on glucose and Na transport by intestinal segments it was found that DCI, an adrenergic blocking agent, reduced the stimulating effects of epinephrine(4). Therefore, it appeared that if reserpine produced its effects by releasing catecholamines, DCI should block the effects of reserpine. Reserpine was thus given to 8 animals, and 4 of

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† Obtained from Vitarine Co., N. Y.

‡ Purchased from Aldrich Chemical Co., Milwaukee, Wis.

TABLE I. Effects of Reserpine on Glucose and Na Absorption.

Exp	Treatment	No. segments	Absorption, μmoles/g/hr Glucose	Na
1	None	8	250 ± 39	404 ± 22
	Reserpine	8	617 ± 46*	640 ± 35*
2	Reserpine	8	583 ± 37	600 ± 44
	" , DCI	8	405 ± 18*	476 ± 51*
3	None	8	244 ± 35	396 ± 44
	DCI	8	272 ± 29	391 ± 17
4	Reserpine, sham-operated	8	494 ± 50	735 ± 48
	Reserpine, de-medullated	8	521 ± 63	788 ± 61
5	None	12	250 ± 38	444 ± 39
	Reserpine, *in vitro*	12	511 ± 52*	648 ± 39*
6	None	4	384 ± 75	356 ± 87
	Reserpine	4	732 ± 71	693 ± 66

Means ± S.E. * $P < .05$.

In Exp 1, 2 and 4, 1.5 mg/kg reserpine given at 18 and 2 hr before sacrifice; in Exp 6, at 42, 18 and 2 hr. DCI 5.0 mg/kg at 18 and 2 hr. Reserpine *in vitro* 2×10^{-6} M.

these were also treated with DCI. Segments from rats receiving both drugs absorbed less glucose and Na than those from animals which had been treated with reserpine alone, suggesting that catecholamines were in fact involved in the response to reserpine (Exp. 2). Administration of DCI alone produced no effects, showing that the drug had no independent influence on transport at this dose level (Exp. 3).

Since reserpine causes release of adrenal catecholamines in the rat(8), the responses of adrenal-demedullated and sham-operated animals to reserpine were compared. Segments from both groups absorbed large amounts of glucose and Na, indicating that medullary catecholamines are not essential to the effects of reserpine on sugar and Na transport (Exp. 4). In view of these results, it seemed that reserpine might act directly on the intestine, and the drug was therefore studied for its effects *in vitro*. As in earlier experiments, stimulation of glucose and Na transport was observed (Exp. 5).

Paasonen has shown that pre-treatment with reserpine depletes norepinephrine from dog heart and that hearts from such animals are then unresponsive to levels of reserpine that ordinarily produce an increase in rate (3). To determine whether a similar effect might be observed in the intestine, a group of rats was given the drug at 42, 18 and 2 hours before sacrifice. Segments from these animals, however, still absorbed increased amounts of glucose and Na (Exp. 6).

Discussion. The effects of injected reserpine on glucose and Na transport in these experiments might possibly depend on one or more mechanisms. The drug releases a number of humoral substances including ACTH(9), histamine, serotonin(10) and catecholamines(8) from storage sites in various tissues in the rat. However, Moran has shown that reserpine does not release serotonin from rat intestine, and while intestinal histamine release follows reserpine treatment, the drug does not produce histamine liberation *in vitro*(10). Our data show that the adrenal medulla is not required for the effects of injected reserpine on transport, and the activity of the drug *in vitro* appears to exclude ACTH and histamine as requisite mediators and indicates a direct action on the intestine. Furthermore, the blocking of reserpine action by DCI suggests that catecholamines were involved.

Mussachia *et al*(11) reported that glucose uptake by segments of duodenum and jejunum from the hamster was reduced 24 hours after intraperitoneal injection of a single large dose of reserpine (2.5 mg/kg). Intestinal catecholamines decreased from 14 μg/g tissue to zero at 24 hours. These effects of reserpine on absorption are in contrast to those reported here. The differences may perhaps be due to species variation in rates of release and/or inactivation of catecholamines, or accountable to the different routes and times of reserpine treatment.

Soulairac appears to have been the first to suggest that sympathetic nerves may stimulate intestinal sugar transport(12). The present experiments and those reported earlier(4) seem to support his hypothesis.

Summary. Reserpine stimulates transport of glucose and Na by everted segments of rat small intestine. The drug is effective both *in vivo* and *in vitro*. Adrenal demedullation does not diminish the response to reserpine

injections, but dichloroisoproterenol blocks the effects of reserpine. It is concluded that reserpine acts directly on the gut, and that its effects are at least in part consequent to release of catecholamines from storage sites in the intestine.

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Influence of Vasopressors on Survival After Traumatic, Intestinal Ischemia and Endotoxin Shock in Rats.* (30190)

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PLV-2 (2-phenylalanine-8-lysine vasopressin) a synthetic vasoexcitor-pressor polypeptide, has been reported to increase survival of rats in hemorrhagic shock in which norepinephrine and angiotensin were ineffective(1, 2). Improved survival after PLV-2 administration was attributed to its selective vasomotor effects as observed in the microcirculation of the rat mesoappendix. Unlike norepinephrine which produced unselective intense constriction of all microvessels, or angiotensin which resulted in marked venular dilation and stasis, PLV-2 predisposed to improved true capillary inflow and outflow and reduced vascular sensitivity to epinephrine.

The use of vasoexcitor-pressor drugs in shock, as distinct from the hemodynamic principle of such therapy to improve tissue blood flow, is currently a matter of considerable uncertainty. It was of interest, therefore, to determine whether the favorable effects of PLV-2 in hemorrhagic shock also applied in other types of experimental shock. The present study compared the influence of norepinephrine, angiotensin and PLV-2† on survival of rats in traumatic, intestinal ischemia, and endotoxin shock.

Materials and methods. (A) Traumatic shock. Wistar strain female rats, 130-180 g body wt were fasted for 18-20 hours, except for water, and anesthetized with pentobarbital sodium, 2.0 mg/100 g body wt I.M. Normal saline, 1.0 ml/100 g was injected I.P. and one hour later the rats were subjected to 700 r at 40 rpm in a Noble-Collip drum. On removal from the drum a femoral vein was cannulated and 30 minutes post removal saline, 1.0 ml/100 g, alone or containing the various doses of the drugs‡ used (Table I) was infused I.V. over a 56-60-

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‡ Norepinephrine used in this study was Levophed® Bitartrate; 0.2% sol. = 0.1% base. All doses were calculated as the base. Angiotensin used was Hypertensin®. PLV-2 doses used were calculated on the basis of 20 μg of active polypeptide/I.U. (pressor).