Dopamine Stimulation of Active Na and Cl Absorption in Rabbit Ileum

INTERACTION WITH α_2-ADRENERGIC AND SPECIFIC DOPAMINE RECEPTORS

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ABSTRACT The effects of dopamine on active intestinal ion transport have been evaluated. An epithelial sheet preparation of rabbit ileum was used in vitro with the Ussing chamber-voltage clamp technique. Dopamine, in the presence of 1 mM ascorbic acid, added to the serosal bathing solution caused a dose-dependent decrease in short-circuit current, with a half-maximal effect at 1.2 μM and maximal effect of -50 $\mu A/cm^2$ at 50 μM; dopamine decreased the potential difference, and increased the conductance and net Na and net Cl absorption. There was no effect on the residual ion flux. Dopamine did not alter the change in short-circuit current caused by mucosal glucose (10 mM) or serosal theophylline (10 mM). Mucosal dopamine had no effect. The effect of dopamine on short-circuit current was inhibited by the dopamine antagonists haloperidol and domperidone and the α_2-adrenergic antagonist yohimbine; there was no effect of the α_1-antagonist prazosin and the β-antagonist propranolol. In addition, the α_2-adrenergic agonist clonidine, but not the α_1-agonist methoxamine caused a dose-dependent decrease in short-circuit current. The ileal effects of dopamine did not occur via conversion into norepinephrine or release of norepinephrine from the peripheral nerves since "peripheral sympathectomy" with 6-hydroxydopamine did not alter the dopamine-induced change in ileal short-circuit current. The dopamine effects were not associated with a change in basal ileal cyclic AMP content but were associated with a decrease in total ileal calcium content as measured by atomic absorption spectrometry and as estimated by $^{45}Ca^{++}$ uptake. The decrease in calcium content could be attributed to a dopamine-induced decrease in $^{45}Ca^{++}$ influx from the serosal surface. Because of the presence of dopamine in ileal mucosa and these effects on ileal electrolyte transport, it is possible that dopamine may be involved in the physiologic regulation of active intestinal electrolyte absorption.

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INTRODUCTION

In previous studies of the neurohumoral control of intestinal transport, catecholamines have been shown to stimulate electrolyte absorption. In rabbits, the α-adrenergic agonists epinephrine and norepinephrine stimulate active Na and Cl absorption in the small intestine, whereas β-adrenergic agonists are without effect (1, 2). In rat colon, both α- and β-adrenergic agonists stimulate active Na and Cl absorption (3). Dopamine is another catecholamine found in significant amounts in the intestine, particularly the mucosal layer (4). The effect of dopamine on intestinal transport has not been well characterized to date; only a single negative study of the effects of dopamine on intestinal transport has been reported (5). In preliminary in vivo studies of the effect of parenteral dopamine on release of hormones from the gastrointestinal mucosa of the rat, a striking stimulation of in-

testinal water, Na, and Cl absorption was observed (M. Donowitz, unpublished observation) (6).

Thus, in the present studies we have expanded these observations with an evaluation and characterization of the dopamine effects on intestinal active electrolyte transport.

METHODS

Male New Zealand albino rabbits weighing 2–2.5 kg were maintained on a standard rabbit chow diet with free access to water. The animals were anesthetized with Na pentobarbital, the distal ileum removed, and stripped epithelial sheets prepared for study as previously described (7, 8).

In vitro transmural electrolyte transport. The methods used to determine transport have been described previously (7, 8). In brief, ileal mucosa was mounted between lucite modified Ussing chambers having an aperature of 1.13 cm^2, gassed with 95% O_2-5% CO_2, and maintained at 37°C. Transmural potential difference (PD),[1] short-circuit current (Isc), conductance (G), and unidirectional fluxes of Na and Cl were determined; in some experiments only electrical measurements were performed. An automatic voltage clamp was used to correct for fluid resistance between PD sensing bridges and provided continuous short-circuiting of the tissue except for the brief periods during which the PD was measured. Unidirectional fluxes of Na and Cl were measured using ^{22}Na and ^{36}Cl on paired tissue differing in conductance by <25%. A negative sign before a net ion flux indicates net secretion; a positive sign, net absorption.

Unless specified, the bathing solutions consisted of Ringer's-HCO_3 composed of (in mM): 115 NaCl; 25 $NaHCO_3$; 2.4 K_2HPO_4; 0.4 KH_2PO_4; 1.2 $CaCl_2$; 1.2 $MgCl_2$: osmolality 275 mosmol/kg; pH 7.4 after gassing with 95% O_2–5% CO_2. A HCO_3-free bathing solution was made by replacing HCO_3 with isethionate. This solution was gassed with 100% oxygen and the pH was adjusted to 7.4. 10 mM glucose was added to the serosal, and 10 mM mannitol to the mucosal bathing fluid at the time of mounting the tissue. Ascorbic acid (1 mM) was present in the bathing solution to which dopamine was added to reduce oxidation of dopamine, unless stated otherwise.

6-Hydroxydopamine studies. Animals were treated with 6-hydroxydopamine 30 mg/kg i.v., 41 h before study, and 20 mg/kg, 21 and 17 h before study by the method of Fozard (9, 10). This technique has been estimated to decrease tissue catecholamine levels by 80% (11). Ileum from untreated control and 6-hydroxydopamine treated animals were compared as to the maximum change in Isc in response to epinephrine (10 μM), tyramine (0.1 mM), and dopamine (0.1 mM).

Calcium content studies. Total ileal calcium was estimated as previously described using $^{45}Ca^{++}$ (7) and also measured directly with atomic absorption spectrometry. $^{45}Ca^{++}$ content was determined in epithelial sheets of ileum, similar to those used in the Ussing chamber experiments, and gassed with 95% O_2–5% CO_2. After preincubation of tissue for 30 min in Ringer's-HCO_3 (2 ml/100 mg of tissue), net retention of $^{45}Ca^{++}$ was determined in the presence of [^{3}H]polyethylene glycol (PEG), 900 mol wt (New England Nuclear, Boston, MA), as an extracellular space marker. After a 60-min exposure to $^{45}Ca^{++}$ the tissue was exposed for a further 25 min to dopamine (0.1 mM) or Ringer's-HCO_3 alone, both containing 1 mM ascorbic acid. Then tissue was washed three times in iced isotonic mannitol, blotted lightly, weighed, placed into 1 ml Protosol (New England Nuclear), and incubated overnight at 55°C to dissolve the tissue. $^{45}Ca^{++}$ and ^3H in tissue and bathing medium were determined by liquid scintillation spectrometry. By subtracting the $^{45}Ca^{++}$ in the extracellular fluid from the total tissue $^{45}Ca^{++}$, the intracellular $^{45}Ca^{++}$ was determined. Uptake values of $^{45}Ca^{++}$ were determined by dividing intracellular $^{45}Ca^{++}$ by external fluid specific activity. $^{45}Ca^{++}$ uptake was expressed in nanomoles per milligram wet weight.

In addition, ileal calcium content was directly measured by atomic absorption spectrometry by a previously described technique (12). Epithelial sheets of ileum were treated identically as described for the experiments with $^{45}Ca^{++}$, while the extracellular calcium was determined using [^{3}H]PEG on duplicate tissue from the same animals. The tissues were removed 25 min after exposure to either dopamine (0.1 mM) or to Ringer's-HCO_3 alone, both containing 1 mM ascorbic acid. They were washed in iced isotonic mannitol, blotted, homogenized in 0.3 mM $LaCl_3$ in 5 M HCl with a Polytron; after centrifugation in a Beckman microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA), the supernatant was diluted 1:5 (vol/vol) with $LaCl_3$ and calcium content determined using IL Atomic Absorption Spectroscope, model 551 (IL Instrumentation Laboratory, Lexington, MA). Standards were ultrapure $CaCl_2$ (Alfa Products, Danvers, MA). Calcium in the extracellular fluid was subtracted from total calcium content to give intracellular calcium. Calcium content was expressed in nanomoles per milligram wet weight.

^{45}Calcium influx studies. The rate of Ca^{++} influx from the serosal bathing solution into the ileal epithelial sheet was determined using an influx chamber as described by Nellans and Schultz (13). Tissue was not short-circuited during these studies. Tissue was mounted in this chamber mucosal surface down, gassed with 95% O_2–5% CO_2, and maintained at 37°C. The exposed surface area was 0.33 cm^2, and circulation on the mucosal surface by the gas lift system was augmented with a stirring bar. Bathing solutions consisted of Ringer's-HCO_3 with 10 mM glucose on the serosal and 10 mM mannitol on the mucosal surface. After 25 min of exposure to Ringer's-HCO_3, the serosal bathing solution was changed to one containing Ringer's-HCO_3, 10 mM glucose plus 0.03 μCi/ml $^{45}Ca^{++}$ and 1 μCi/ml [^{3}H]PEG. In addition, half the tissue was exposed to dopamine (0.1 mM) on the serosal surface. 1.5, 2, and 3 min later, the serosal bathing solution was rapidly removed, the tissue flushed with cold isotonic mannitol, punched out, blotted, and weighed. The tissue was dissolved in Protosol, and $^{45}Ca^{++}$ and ^3H determined in both tissue and serosal bathing solution. Calcium in the extracellular fluid was subtracted from total calcium to give intracellular calcium. Calcium influx was expressed in nanomoles per milligram wet weight per minute. The rate of $^{45}Ca^{++}$ influx was determined for each experiment by performing linear regression analyses of data obtained at the four times studied. In preliminary experiments, it was determined that $^{45}Ca^{++}$ influx in untreated control tissue was linear between 30 s and 3 min after exposure to $^{45}Ca^{++}$. In addition, up to 5 min after addition of $^{45}Ca^{++}$ to the serosal surface, no $^{45}Ca^{++}$ was detectable in the mucosal bathing solution. Thus, over this period of time, Ca^{++} influx and $^{45}Ca^{++}$ influx are equivalent; and will be referred to as Ca^{++} influx.

Cyclic AMP content determinations. The effect of dopamine on rabbit ileal cyclic AMP content was determined. Rabbit ileal mucosal sheets were preincubated at 37°C for 30 min in Ringer's-HCO_3 containing 10 mM glucose and

[1] *Abbreviations used in this paper*: Isc, short circuit current; PD, potential difference; PEG, polyethylene glycol.

gassed with 95% O_2–5% CO_2. Then the ileal sheets were exposed in vitro to dopamine without ascorbic acid, theophylline, which served as a positive control, or to Ringer's-HCO_3 alone. Tissue was removed 5, 10, and 15 min later and assayed for cyclic AMP.

Cyclic AMP was determined by the competitive protein-binding assay of Gilman (14) using a commercial kit (Amersham/Searle Corp., Arlington Heights, IL). Mucosal scrapings from the rabbit ileum were frozen rapidly in liquid nitrogen, homogenized with a cold glass homogenizer, and the cyclic AMP extracted into acid ethanol (0.2 N HCl). After centrifuging, the supernatant was decanted off. The protein content of the pellet was determined by the method of Lowry et al. (15). The supernatant was evaporated to dryness with nitrogen, and the residue redissolved in 0.05 M Tris buffer and 4 mM EDTA for assay.

Agents used in these studies include dopamine, ascorbic acid, 6-hydroxydopamine, tyramine, epinephrine, polyethylene glycol, and yohimbine (Sigma Chemical Co., St. Louis, MO); haloperidol (McNeil Laboratories, Fort Washington, PA); prazosin HCl (Pfizer, Inc., New York); clonidine HCl (Boehringer Ingelheim, Ltd., Ridgefield, CT); domperidone (Janssen Pharmaceutics, Beerse, Belgium); methoxamine HCl (Burroughs Wellcome Co., Research Triangle Park, NC); and theophylline (Eastman Kodak Co., Rochester, NY).

Statistical analyses were performed by Student's *t* test for paired and unpaired data and were two-tailed; linear regression analyses were performed by the method of least squares. All results were expressed as mean±SE.

RESULTS

Dopamine effect on Isc. Dopamine (0.1 mM) added to the solution bathing the ileal serosal surface caused a decrease in Isc that reached a maximum within 5–10 min. This decrease in Isc was dose dependent (Fig. 1) with threshold for a significant decrease at 0.5 μM, a half-maximal decrease at 1.2 μM and a maximum decrease of 50 μA/cm² at 50 μM.

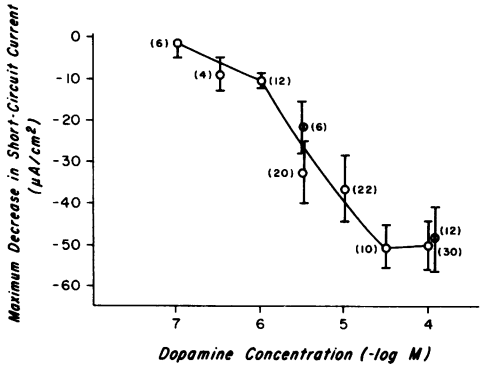

FIGURE 1 Dose-response curve of the decrease in ileal Isc caused by dopamine. Dopamine was added to the solution bathing the serosal surface and the maximum decrease in Isc that occurred within 5–10 min after addition plotted. Numbers in parentheses represent the number of animals studied. Symbols represented by × refer to the dopamine effect in the absence of ascorbic acid; all other values are in the presence of 1 mM ascorbic acid on the serosal surface.

Addition of dopamine to the mucosal bathing solution at concentrations up to 0.1 mM did not alter Isc. The duration of the maximum decrease in Isc varied with the concentration of dopamine though at all concentrations, in the presence of ascorbic acid, the maximum decrease in Isc was constant for at least 35 min. With 0.1 mM dopamine, in the absence of ascorbic acid, the maximum decrease in Isc lasted for at least 45 and generally for 60 min. The Isc then gradually increased but did not return to base line even after 90 min.

Because of the possibility of dopamine oxidation in this vigorously gassed system, the effect of addition of ascorbic acid at a final concentration of 1 mM was determined. As demonstrated in Fig. 1, the presence of ascorbic acid slightly, but significantly, increased the peak Isc response to dopamine at 5 μM but not at 0.1 mM. In addition, the presence of ascorbic acid prolonged the duration of the maximum decrease in Isc caused by 0.1 mM dopamine to >90 min. In all other experiments involving dopamine, including flux studies, ascorbic acid (1 mM) was present in the serosal bathing solution. This concentration of ascorbic acid did not alter any parameter of basal ileal electrolyte transport (Table I).

Dopamine effect on active electrolyte transport. Dopamine (0.1 mM) was added to the solution bathing the serosal surface of rabbit ileum and the effect on electrolyte transport determined 5–35 min later. During the period of the flux studies, the Isc in tissue exposed to dopamine was constant. As demonstrated in Table I, dopamine significantly decreased the Isc and PD, increased conductance and significantly increased net Na and Cl fluxes. The increase in net Cl absorption was significantly greater than that of Na; this accounted for the decrease in Isc, since dopamine did not alter the residual ion flux. Of note, the dopamine stimulation of the mucosal-to-serosal fluxes of Na and Cl were not significantly different in magnitude. However, although dopamine significantly increased both the mucosal-to-serosal and serosal-to-mucosal Na fluxes, it only increased the mucosal-to-serosal movement of Cl (Table I). The effect of dopamine (0.1 mM) on net Na and Cl fluxes did not change when studied during six consecutive 15-min flux periods (data not shown). Addition of dopamine (0.1 mM) to the solution bathing the mucosal surface did not alter any parameter of ileal electrolyte transport (data not shown). As demonstrated in Table I, the dose dependence of the dopamine effect on ileal electrolyte transport was similar to that on Isc. There was no significant effect at 0.1 μM; and the significant dopamine effects demonstrable at 5 μM were less than the maximal effect seen with 0.1 mM dopamine in terms of changes in Isc, conductance, and net Cl absorption.

Exposure to 0.1 mM dopamine, in the absence of

TABLE I
*Effect of Dopamine on Rabbit Ileal Electrolyte Transport**

	Time period	Isc	PD	G	J_{ms}^{Na}	J_{ms}^{Na}	J_{net}^{Na}	J_{ms}^{Cl}	J_{ms}^{Cl}	J_{net}^{Cl}	J^{R}
Base line	20–60	56.0±4.6	−3.0±0.4	15.8±1.3	6.46±0.21	6.71±0.29	−0.25±0.21	5.65±0.38	7.01±0.32	−1.35±0.24	0.97±0.21
Dopamine											
0.1 mM	65–95	14.5±4.7	−0.9±0.2	23.5±1.8	9.15±0.43	8.31±0.60	0.83±0.42	8.44±0.53	6.88±0.35	1.56±0.44	1.27±0.62
(n = 14)	Δ	−41.2±4.1	2.1±0.3	7.6±0.7	2.68±0.39	1.60±0.41	1.08±0.24	2.78±0.61	−0.13±0.24	2.91±0.45	0.30±0.38
	P	<0.01‡	<0.01	<0.01‡	<0.02	<0.02	<0.05	<0.01	NS	<0.01‡	NS
Base line	20–60	55.6±4.1	−3.7±0.5	15.2±2.0	6.64±0.51	7.41±0.70	−0.76±0.71	5.39±0.42	6.37±0.24	−0.97±0.41	1.85±0.51
Dopamine											
5 μM	65–95	36.8±2.9	−2.4±0.3	18.2±1.9	8.95±0.66	8.77±0.66	0.18±0.80	7.22±0.70	6.45±0.33	0.76±0.42	1.94±0.70
(n = 12)	Δ	−18.8±4.9	1.4±0.4	3.0±1.4	2.30±0.58	1.36±0.55	0.95±0.44	1.83±0.66	0.08±0.31	1.74±0.39	−0.09±0.82
	P	<0.05	<0.025	<0.05	<0.025	<0.05	<0.05	<0.05	NS	<0.02	NS
Base line	20–60	52.7±4.0	−3.8±0.4	14.1±0.6	6.84±0.21	7.71±0.64	−0.87±0.44	6.15±0.38	7.59±0.49	−1.43±0.40	1.32±0.21
Ascorbic acid	65–95	54.9±3.4	−3.5±0.4	14.6±0.7	6.27±0.24	8.09±0.71	−1.82±0.58	6.19±0.34	7.68±0.56	−1.49±0.28	1.50±0.42
(n = 9)	Δ	2.2±2.0	0.3±0.4	0.5±0.3	−0.57±0.38	0.38±0.61	−0.95±0.49	0.04±0.29	0.09±0.53	−0.06±0.36	0.18±0.41
	P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Units of: Isc, μA/cm²; PD, mV; G (conductance), mmhos/cm²; fluxes, μeq/cm²-h; positive indicates net absorption, negative, net secretion; dopamine in several concentrations plus ascorbic acid (1 mM) or ascorbic acid alone were added to the serosal bathing solution at 60 min; fluxes were compared before (20–60 min) and after (65–95 min) dopamine addition. Δ represents 65–95 min flux period minus 20–60 min flux period. Results are mean±SE; n represents number of animals studied; P values represent comparisons of 65–95 and 20–60 min flux periods on the same tissue (paired t test).

‡ Represents dopamine effect (dopamine-base line) at 0.1 mM, which is significantly different from that at 5 μM compared by an unpaired t test.

ascorbic acid, did not alter the glucose-dependent (10 mM) increase in Isc (47.3±10.3 vs. 50.9±8.4 $\mu A/cm^2$, in dopamine exposed and control tissue, respectively) or the theophylline-induced (10 mM) increase in Isc (89.1±17.7 vs. 79.4±13.5 $\mu A/cm^2$, in dopamine-exposed and control tissue, respectively).

The effects of several stimulators of active electrolyte absorption have previously been demonstrated to depend on HCO_3 in the external bathing solution (1, 21, 24, 25). To examine the dependence on bathing solution HCO_3 of the dopamine-induced change in Isc, this effect was determined in the presence and absence of HCO_3 studied over 90 min, a time during which bathing solution pH remained 7.4. Dopamine (0.1 mM) caused a significantly smaller decrease in Isc (-14.8 ± 4.1 $\mu A/cm^2$) in tissue exposed to HCO_3-free bathing solutions than in tissue from the same animal exposed to standard Ringer's-HCO_3 (-42.1 ± 5.4 $\mu A/cm^2$, $n = 5$, $P < 0.05$).

Effect of catecholamine antagonists on the dopamine-induced change in Isc. We studied whether the effects of dopamine on ileal electrolyte transport were related to specific dopamine receptors or involved interaction with other catecholamine receptors by comparing the effects of dopamine on ileal Isc in the absence and presence of a series of catecholamine antagonists. Haloperidol and domperidone were used as specific dopamine antagonists; prazosin as an α_1-adrenergic receptor antagonist; yohimbine as an α_2-adrenergic receptor antagonist; and propranolol as a β-receptor antagonist (19, 22). Prazosin and yohimbine were used to separate α_1 and α_2 effects because, in our hands, phentolamine, a more widely used α-receptor antagonist had agonist properties at concentrations as low as 0.1 μM and at lower concentrations did not inhibit the epinephrine-induced changes in rabbit ileal Isc (M. Donowitz, unpublished observations). When these antagonists were added to the serosal bathing solution, none altered the Isc compared to control tissue, and under all circumstances, the Isc was constant within 20 min of addition of the antagonists, and remained so for at least the next 20 min.

The effect of dopamine (5 μM) on ileal Isc was determined 25 min after addition of the catecholamine antagonists. The greatest decrease in Isc caused by dopamine was most affected by yohimbine. As demonstrated in Fig. 2, yohimbine at as low a concentration as 10 nM significantly inhibited the maximum decrease in Isc. This inhibition was dose dependent, and at 1 μM yohimbine the dopamine effect was inhibited by 84±3%. Haloperidol at 1 μM significantly inhibited the dopamine effect by 10%, at 5 μM by 40% and at 10 μM by 45%. Domperidone at 10 μM significantly inhibited the dopamine effect by 24% (-48.8 ± 4.1 vs. -37.0 ± 4.5 $\mu A/cm^2$, in the absence and presence of domperidone, respectively, $P < 0.05$). Prazosin and propranolol did not affect the dopamine-induced decrease in Isc. These data suggest an effect of dopamine via both α_2-adrenergic and dopamine receptors.

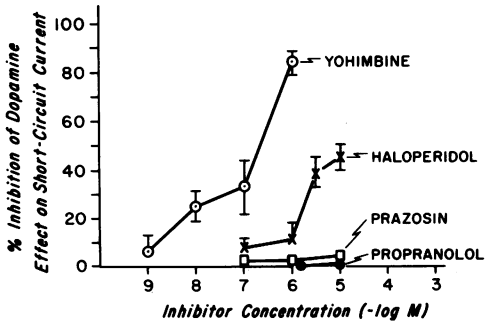

FIGURE 2 Inhibition of dopamine (5 μM)-induced decrease in ileal Isc caused by varying concentrations of catecholamine antagonists. Dopamine plus ascorbic acid was added to the solution bathing the ileal serosal surface 25 min after exposure to the catecholamine antagonists. The greatest change in Isc was determined. Data plotted are the percent of the greatest change which occurred in control tissue exposed only to dopamine plus ascorbic acid, and simultaneously studied tissue from the same animals initially exposed as well to catecholamine antagonists. Number of animals studied at each concentration of inhibitor varied between 5 and 10.

Effect of the α_1-agonist methoxamine and α_2-agonist clonidine on Isc. The α_2-antagonist inhibition of the dopamine stimulation of absorption suggested that α_2-receptors might be involved in regulation of ileal electrolyte transport. Consequently, the effects of the α_1-agonist methoxamine and α_2-agonist clonidine were determined on ileal Isc. As demonstrated in Fig. 3, clonidine caused a dose-dependent decrease in Isc with a threshold at 10 nM; half-maximal effect at 2.6 μM and a maximal effect of -80 $\mu A/cm^2$ at 0.1 mM. This effect of clonidine began within 2 min after addition and reached a maximum in ~5 min. In contrast, there was no difference in the effect of methoxamine at 10 μM, 0.1 mM, and 1 mM with mean changes in Isc < 10 $\mu A/cm^2$.

Use was made of the clonidine-induced decrease in Isc to further suggest that dopamine might be altering ileal electrolyte transport by interacting with both α_2-adrenergic and specific dopamine receptors. It was initially demonstrated that clonidine did not appear to act partially through dopamine receptors. The maximum decrease in Isc caused by 1 μM clonidine was not significantly different in the presence or absence of 5 μM haloperidol (-35.6 ± 16.7 $\mu A/cm^2$ vs. -37.6 ± 10.5, in the absence and presence of haloperidol, respectively). Then, the maximum changes in Isc caused by clonidine (0.1 mM), dopamine (50 μM), and

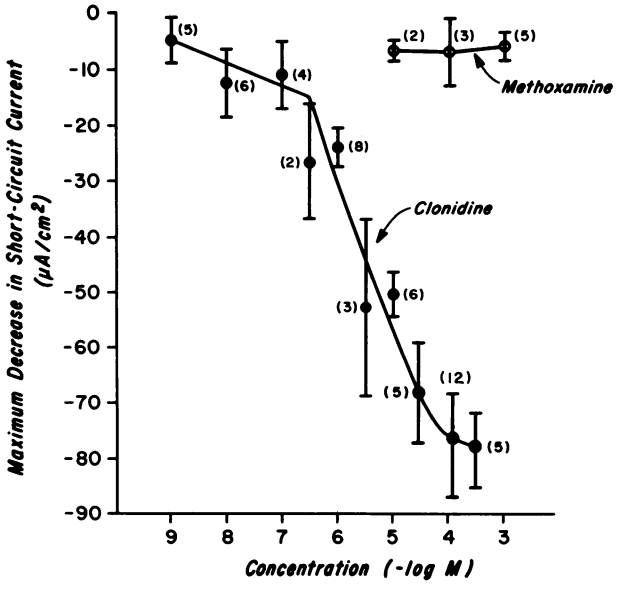

FIGURE 3 Dose-response curve of maximum decrease in ileal Isc caused by clonidine and methoxamine. The maximum decrease in Isc, which occurred ~5 min after addition of the α-adrenergic agonists to the solution bathing the ileal serosal surface, was recorded. Numbers in parentheses represent the number of animals studied.

the combination of clonidine plus dopamine at these concentrations (all concentrations caused maximum changes in Isc) were compared in tissues from the same animals. The combination of clonidine plus dopamine caused a significantly greater change in short-circuit current (−82.7±5.4 $\mu A/cm^2$) than that caused by either clonidine alone (−69.1±7.9) or dopamine (−53.6±8.4) alone ($P < 0.05$ for clonidine alone or dopamine alone vs. clonidine plus dopamine in tissues from same animal studied simultaneously, $n = 14$). That the effect of dopamine plus clonidine exceeded the clonidine effect suggests that dopamine acts on receptors in addition to α_2-receptors. Using similar concentrations of dopamine and clonidine, a similar conclusion was reached by adding dopamine to untreated control tissue or to tissue initially exposed to clonidine. The maximum clonidine effect was constant for at least 25 min and dopamine was added 10 min after clonidine. Dopamine caused a maximal decrease in Isc in untreated control tissues of 52.8±11.5 $\mu A/cm^2$ and a statistically significant further decrease in tissues initially exposed to clonidine of 6.0±1.5 ($n = 7$).

6-Hydroxydopamine treatment on dopamine-induced changes in Isc. Because of the possibility that dopamine might be converted into or release other catecholamines in intestinal nerves to alter intestinal electrolyte transport only indirectly, the effect of addition of 0.1 mM dopamine was compared in animals treated with 6-hydroxydopamine for 2 d and in untreated control animals. In these studies (Table II) the effects of epinephrine and dopamine to decrease rabbit ileal Isc were not significantly altered by treatment with 6-hydroxydopamine, while the effect of tyramine to lower Isc was significantly inhibited. Tyramine is thought to act by releasing norepinephrine from nerve endings.

Effect of dopamine on calcium content and Ca^{++} influx from the serosal surface. Dopamine lowered ileal calcium content. Dopamine caused a significant decrease in ileal ^{45}calcium content compared to tissue exposed to Ringer's-HCO_3 throughout (0.78±0.07 nmol/mg wet wt vs. 0.66±0.06 in control and dopamine-exposed tissue, respectively, $P < 0.02$). Exposure to dopamine did not alter the extracellular space (13.8±1.4% vs. 13.2±1.1%). In separate experiments, the effect of dopamine was determined on ileal calcium content as measured by atomic absorption spectrometry. Exposure to dopamine significantly decreased ileal total calcium content (0.99±0.06 nmol/mg wet wt vs. 0.89±0.05 in control and dopamine exposed tissue, respectively, $P < 0.05$).

In addition, the effects of dopamine (0.1 mM) on the serosal surface was determined on the rate of Ca^{++} influx from the serosal surface into the tissue. The rate of $^{45}Ca^{++}$ influx was linear for both control and dopamine-exposed tissue when measured between 1.5 and 3 min after exposure to $^{45}Ca^{++}$. The rate of $^{45}Ca^{++}$

TABLE II
*Effect of Pretreatment with 6-Hydroxydopamine on the Effect of Dopamine to Decrease Ileal Isc**

	Change in Isc ($\mu A/cm^2$)				
	Untreated	n	6-Hydroxydopamine treated	n	P
Control	−4.6±2.6	6	−4.4±2.7	5	NS
Tyramine	−27.2±5.5	6	−12.3±4.4	5	<0.05
Epinephrine	−98.0±22.2	6	−78.0±9.4	5	NS
Dopamine	−50.2±4.7	6	−55.4±10.9	5	NS

* Rabbits were injected with 6-hydroxydopamine according to the method of Fozard (9,10). 6-Hydroxydopamine was injected 30 mg/kg i.v. ~41 h before study and 20 mg/kg 21 and 17 h before study. After allowing the ileal mucosa to stabilize in standard Ringer's-HCO_3 for 30 min, tyramine (0.1 mM), epinephrine (10 μM), or dopamine (0.1 mM) was added to the serosal surface and ileal Isc compared to control tissue to which no addition was made. In all experiments the maximum decrease in Isc that occurred in the 15 min after addition of agents was recorded. *P* values represent comparisons of tissue from 6-hydroxydopamine-treated animals and untreated control animals exposed to the same addition (unpaired *t* tests). *n*, number of animals studied.

influx was significantly decreased by dopamine (0.042±0.006 vs. 0.024±0.008 nmol/mg wet wt per min, in control and dopamine-exposed tissue, respectively, $P < 0.02$). Exposure to dopamine did not alter the extracellular space as determined from the serosal surface (4.43±0.48% vs. 3.83±0.54%).

Effect of dopamine on cyclic AMP content. As demonstrated in Table III, ileal cyclic AMP was not significantly different in control and dopamine exposed mucosa at any of the times studied. Theophylline caused a two- to threefold increase in cyclic AMP content.

DISCUSSION

These studies demonstrate that dopamine stimulates active Na and Cl absorption in rabbit ileum by interacting with α_2-adrenergic receptors and probably also with specific dopaminergic receptors. Because the studies were performed in vitro, the data suggest that the stimulation of Na and Cl absorption represents a peripheral effect of dopamine independent of the central nervous system. Dopamine does not appear to cause these effects primarily by being converted into other catecholamines or releasing other catecholamines from nerves since these ileal effects were very similar before and after chemical peripheral sympathectomy with 6-hydroxydopamine.

The dopamine-induced increase in net Na and Cl absorption was associated with quantitatively similar stimulation of the mucosal-to-serosal fluxes of Na and Cl. However, while dopamine also increased the serosal-to-mucosal flux of Na, it did not significantly change this unidirectional Cl flux. This caused the increase in net Cl flux to exceed the increase in net Na flux and accounts for the dopamine-induced decrease in Isc.

TABLE III
Effect of Dopamine on Rabbit Ileal Cyclic AMP Content[*]

	Cyclic AMP content, *pmol/mg protein*		
	Minutes after exposure		
	5	10	15
Untreated Control (n = 8)	7.3±1.1	7.2±2.6	6.2±0.5
Dopamine (0.1 mM) (n = 8)	6.4±1.3	7.3±1.4	6.7±0.9
P	NS	NS	NS
Theophylline (10 mM) (n = 5)	14.1±2.3	19.3±6.9	21.6±4.0
P	<0.05	<0.05	<0.02

[*] Tissue stabilized in Ringer's-HCO_3 for 30 min and then exposed to dopamine, theophylline, or untreated control for 5, 10, and 15 min. Results are mean±SE; n represents number of animals studied. P values represent comparison of cyclic AMP content at each time in comparison with untreated control tissue (unpaired t test).

This pattern of change in electrolyte transport caused by dopamine is similar to changes in active Na and Cl absorption previously described in rabbit ileum with exposure to other neurohumoral substances including epinephrine, norepinephrine, somatostatin, and the enkephalins (1, 2, 16, 23, 24); and to the drugs aspirin and chloroquine applied to the ileal serosal surface (5, 17). It is also similar to the pattern of change brought about by exposure of ileum to calcium-free bathing solutions on mucosal plus serosal surfaces or to verapamil, a "calcium channel" blocker, which presumably lowers cell calcium (18). Where studied, this stimulation of Na and Cl absorption was associated with an increase in Na and Cl influx across the brush border and most probably occurs via the so-called neutral NaCl absorptive process (26). This process may be made up of a combination of two neutral exchange processes (Na^+/H^+ and Cl^-/HCO_3^- or OH^-) (27, 28). The dependence on bathing solution HCO_3 of the Isc effect of dopamine reported here and that previously reported for norepinephrine (1) and enkephalinamide (16) may be related to the dependence of either or both of the above neutral exchange processes on bathing solution HCO_3 or on the intracellular mechanism of their linkage.

The evidence that dopamine acts on both intestinal α_2- and dopamine receptors is based on the specificity of the agonists and antagonists used; and it is not possible to be certain from these studies that receptors other than the primary receptor (α_2) are involved. That dopamine alters ion transport in the intestine through α_2-adrenergic receptors was firmly established by these studies using yohimbine, a specific α_2-adrenergic antagonist. That yohimbine is a more effective inhibitor of these dopamine effects than haloperidol is consistent with more of the dopamine effect on ileal transport occurring via α_2-adrenergic receptors. Furthermore, the fact that dopamine is only a partial agonist for α_2-adrenergic receptors is made clear since a maximum concentration of clonidine induced a greater change in Isc than a maximum concentration of dopamine, even though dopamine appears to act on both dopamine and α_2-receptors.

That dopamine appears to act on both α-adrenergic and dopamine receptors in the ileum has precedence from tissues of central nervous system and muscle origin. For instance, dopamine-induced prolactin secretion by rabbit brain was inhibited by haloperidol and the α-adrenergic antagonist phentolamine; in the rat brain striatum, dopamine stimulation of adenylate cy-

clase activity was inhibited by phentolamine (29). In addition, dopamine-induced contractions in the opossum duodenal muscularis propria were blocked by both haloperidol and phentolamine or phenoxybenzamine (30). It previously has been questioned whether the phentolamine inhibition of the dopamine effects indicates that phentolamine interacts with specific dopamine receptors rather than indicating an α-adrenergic agonist effect of dopamine (29). However, the failure of the clonidine effect on ileal Isc to be inhibited by haloperidol makes this a less likely explanation in the intestine. There have been no previous studies, however, to determine whether it is α_1- or α_2-receptors through which dopamine partially acts.

This study also demonstrates that catecholamines alter active ileal ion transport largely through α_2-adrenergic receptors; and extends the previous observation that in rabbit ileum catecholamine-induced absorption represents an α-adrenergic and not a β-adrenergic effect (1, 4). The evidence that it is the α_2- and not the α_1-adrenergic receptors that affect ileal electrolyte absorption include the dose-dependent decrease in Isc caused by the α_2-adrenergic agonist clonidine, but not by the α_1-adrenergic agonist methoxamine; in addition, the α_2-antagonist yohimbine, but not the α_1-antagonist prazosin inhibited the dopamine-induced changes in ileal Isc.

This study does not make clear the cellular mechanism by which dopamine stimulates Na and Cl absorption though intracellular calcium may be involved. The studies demonstrating that dopamine decreased total ileal calcium content were done on epithelial sheets, which prevents concluding that the changes occurred specifically in the intestinal epithelial cells. In addition, a change in total ileal calcium content, which represents primarily intracellular calcium stores, is only an indirect reflection of a change in cytosolic-free calcium, which is thought to be the effective calcium pool (31). However, lowering of intracellular calcium and stimulation of Na and Cl absorption, as caused by exposure to dopamine, are the same changes in total ileal calcium and ion transport caused by ileal exposure to l-verapamil, a calcium channel blocker, and to calcium-free bathing solutions (18). In addition, the decrease in total ileal calcium content caused by dopamine could have been caused either by a decrease in influx of Ca^{++} or an increase in efflux. When measured, dopamine significantly decreased the $^{45}Ca^{++}$ influx by 44%; in contrast, the calculated calcium efflux (estimated from: change in calcium content = calcium influx minus calcium efflux [29]) was decreased as well. This suggests that dopamine affected the cellular calcium content by changing the basal-lateral membrane permeability to calcium rather than by primarily affecting the intracellular calcium stores. Involvement of a decrease in cellular calcium as part of a dopamine effect has not previously been demonstrated, though recently the dopamine-induced depolarization of mouse neuroblastoma cells was shown to be inversely related to the calcium concentration in the external bathing solution, also compatible with dopamine action via lowering intracellular calcium (32).

Dopamine receptors are characterized as D_1 and D_2, based largely on central nervous system studies, with D_1 but not D_2 receptors linked to stimulation of adenylate cyclase activity (33–35). Since exposure of ileal mucosa to dopamine is associated with a stimulation of absorption rather than secretion, it seems unlikely that dopamine is acting on D_1 receptors. This pattern of a neurohumoral substance which, in the central nervous system acts through some receptors that are linked to the adenylate cyclase-cyclic AMP system, but in the intestine acts independently of this system, is a pattern that we have seen previously with serotonin (36). It may be that classical central nervous system neurotransmitters do not act via the adenylate cyclase-cyclic AMP system in their intestinal effects.

These studies suggest that dopamine stimulates active ileal Na and Cl absorption. Because of the significant amount of dopamine present in the gastrointestinal tract in many species (4) and with the distribution appearing to be primarily in the mucosa, it is possible that dopamine may be involved in the physiologic regulation of active intestinal ion absorption.

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