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Possible Role of Nitric Oxide in 5-HT-Induced Intestinal Fluid and Electrolyte Secretion, In Rats

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5-Hydroxytryptamine is an important neurotransmitter and hormone/paracrine agent mediating various enteric functions. The precise physiological and pathophysiological role of nitric oxide in 5- hydroxytryptamine-induced diarrhea remains unclear. Evidence is accumulating on the importance of nitric oxide in 5-hydroxytryptamine-induced secretion, but its role in fluid accumulation and electrolyte transport in jejunum and colon is not well established. This study therefore investigated *in vivo*, the effect of a nitric oxide synthase inhibitor and a nitric oxide precursor on intestinal fluid and electrolyte secretion induced by 5-hydroxytryptamine. Subcutaneous administration of 5-hydroxytryptamine (2-8 mg/kg) produced a dose-related increase in the intestinal fluid and electrolyte secretion in jejunum and in colon of the anaesthetized rats. 5-Hydroxytryptamine-induced (6.0 mg/kg, s.c.) jejunal and colonic fluid and electrolyte accumulation was inhibited by N^G-nitro-L-arginine (10-40 mg/kg, i.p.). The inhibitory effect of N^G-nitro-L-arginine was reversed by L-arginine (600-1500 mg /kg, i.p.) dose-dependently but not by D-arginine (900 mg/kg, i.p.). These results demonstrate that, 5- hydroxytryptamine-induced fluid and electrolyte secretion in the jejunum and colon may involve nitric oxide.

5-Hydroxytryptamine (5-HT) is an important neurotransmitter in the gastrointestinal tract[1]. 5-HT is present in the enteric nervous system[2], in enterochromaffin[3] cells and in some species, in mast cells, in the intestinal epithelium[4]. Within the enteric nervous system, 5-HT is stored in the myenteric neurons and in axons, which extend to innervate other neurons in myenteric and submucosal ganglia[5]. Exogenous5-HT elicits intestinal secretion of water and electrolytes in animals and man, both *in vitro* and *in vivo*[6]. Endogenous 5-HT is involved in the pathophysiology of diarrhea associated with the carcinoid syndrome and other hypersecretory states such as cholera[7]. 5-HT is a potent intestinal secretagogue for water and electrolytes[8]. A role for 5-HT in secretory diarrhea produced by carcinoid syndrome[9], enteric infection[10] and inflammatory bowel diseases[11] has been suggested. The precise role of 5-HT in the physiology and pathophysiology of the gastrointestinal tract remains unclear, luminally administered 5-HT has been demonstrated to induce intestinal secretion in the conscious dog[12] and the anaesthetized rat[13]. Beubler *et al.*[14] had shown the secretion of fluid, by subcutaneous administration of 5-HT.

Nitric oxide (NO) a free radical produced from guanidine nitrogen of L-arginine[15] by the enzyme NO synthase has a wide variety of physiological effects. These include the relaxation of vascular smooth muscle[16], inhibition of platelet[17], mediation of cytotoxicity by phagocytic cells[18], effects of cytokines and endotoxins and neurotransmission[19]. Many cells and tissues synthesize NO including cytotoxic macrophages[20], neutrophils[21], mast cells[22], brain and neural cells[23], hepatocytes[24], renal messangial[25], and epithelial cells[26]. NO has been identified as a nonadrenergic-

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noncholinergic neurotransmitter causing smooth muscle relaxation, including many gastrointestinal tract location[27]. NO may be an important mediator of processes affecting intestinal epithelial function. This may be due to the ability NO to stimulate soluble guanylate cyclase resulting in the production of the secondary messenger cyclic GMP[28], a potent intestinal secretor agent[29], *in vitro* studies, applying Ussing chamber technique, have shown that serosal administration of NO or NO donating compounds to stripped intestinal tissue increases short circuit current, this is an indication of prosecretory and/or antiabsorptive role of NO[30-32].

It has been recently reported that NO is involved in 5-HT-induced intestinal fluid accumulation[33,34], in 5-HT-evoked chloride secretion[35] and also in 5-HT-modulated behavior changes[36] but, the precise role of NO in 5-HT-induced secretory mechanism unknown. In this study we tried to find mechanism of 5-HT-induced fluid and electrolyte accumulation in jejunum and colon and mechanism of inhibitory effect of N^G-nitro-L-arginine (L-NNA) on 5-HT-induced fluid and electrolyte accumulation in jejunum and colon in vivo, in anaesthetized rats.

MATERIALS AND METHODS

Albino rats of either sex, weighing about 150-180 g were used. The animals, which did not show any signs of diarrhea, were selected for the study. The rats were fasted for 24 h, before experiments but were given free access to water. All the experiments were carried out between 2.00-7.00 pm to avoid circadian effects. 5-HT sulfate and L- arginine were purchased from Sigma Chemical Co., St. Louis, USA. N^G-nitro-L-arginine and D-arginine from Fluka Chemical Co. were used in this study; these compounds were dissolved in saline before being used.

Methodology:

General anesthesia was induced by intraperitoneal injection of pentobarbitone 30 mg/kg and maintained throughout the experiment by administering 5 mg/kg of pentobarbitone whenever required. A midline abdominal incision was made and the abdomen was opened and a polythene catheter was placed in the jejunum and fixed by ligation. A second ligature was tied approximately 20 cm distal to the first ligature. The colon was cannulated proximally about 5 cm distal to the ileo-caecal junction with polythene catheter and a second ligature was placed at the distal end of the colon. The jejunum and colon, were rinsed carefully with 10 ml and 20 ml of warm sterile (0.9%) NaCl solution (37°), respectively to remove the contents followed by blowing air with the help of a syringe. The distal end of both jejunum and colon were ligated and the abdomen was closed and stitched. One hour after the preparation, 2 ml of prewarmed (37°) Tyrode solution (composition, g/l); NaCl-8.0, KCl-0.2, $CaCl_2$-0.26, $MgSO_4.7H_2O$-0.26, $NaHCO_3$-1.0, $NaH_2PO_4.7H_20$-0.05 and glucose-1.0 was instilled in both jejunum and colon and the catheters were withdrawn before tying off the proximal end. After filling, the loops were returned to the peritoneal cavity, and the abdomen was closed. The drug to be tested was administered just before instilling Tyrode solution. After 30 min, the loops were removed from the animal and the volume of fluid retained was measured.

The effect of 5-HT on jejunal and colonic secretion was studied by subcutaneous administration after 1 h of the animal preparation. The effect of L-NNA on 5-HT-induced secretion was studied by intra peritoneal administration 30 min prior to administration of 5-HT. The effect of L-arginine and D-arginine on L-NNA-inhibited 5-HT-induced secretion was studied by intraperitoneal administration after 15 min of the administration of L-NNA, which was administered 30 min prior to the administration of 5-HT. 5-HT was administered just before instillation of Tyrode solution. After 30 min time period, the loops were removed from the animal and the volume of fluid present was measured. The empty loops were weighed, fluid and electrolyte secretion was taken as the difference between the initial and final volume of the fluid in the loops, and the accumulated fluid was measured by using micropipettes and was expressed as μl/g wet weight of the loop. The concentration of Na^+ and K^+ were determined using a flame photometer and chloride ions were estimated by the method of Schales and Schales. The ion concentrations were expressed in mEq/l. After removing the loop from the animals, the animals were sacrificed by an overdose of pentobarbitone.

Statistical analysis:

Results were expressed as mean±SEM. and they were analyzed by unpaired student 't' test, probability values of $P<0.01$ were considered as significant.

RESULTS

Basal fluid and electrolyte transport:

The control rats were administered 2 ml/kg, s.c. saline. Under control conditions there was net absorption of fluid and electrolytes in both jejunum and colon, respectively, absorption of fluid is more in colon than in jejunum but the concentration of Na^+ and Cl^- absorbed are equal. In the accumulated fluid the concentration of K^+ in colon was more

than the concentration of K^+ in jejunal secretion.

Effect of 5-HT on fluid and electrolyte transport:

Subcutaneous administration of 5-HT (4-8 mg/kg) significantly reversed fluid absorption to net secretion dose-dependently, in both jejunum and colon. Minimum dose (2 mg/kg, s.c.) of 5-HT significantly inhibits fluid absorption and absorption of Na^+, K^+ and Cl^- in both jejunum and colon. The maximum dose of 5-HT (8 mg/kg, s.c.) has produced an increase in secretion of fluid, Na^+, K^+ and Cl^- significantly ($P<0.01$) in both jejunum and colon, as compared to control (saline-treated). The dose, which reversed the fluid absorption into fluid secretion, was 4 mg/kg (s.c.) in both jejunum and colon but, the concentration of K^+ was not increased significantly in jejunum as compare to concentration of K^+ in colon. However, the level of Na^+ and Cl^- increased significantly in both jejunum and colon. The dose of 5-HT selected for the study was 6.0 mg/kg (s.c.) as it has increased the concentration of electrolytes and fluid significantly, in both jejunum and colon. (Table 1)

Inhibitory effect of L-NNA on 5-HT-induced fluid and electrolyte secretion:

L-NNA exhibited an inhibitory effect on 5-HT-induced fluid and electrolyte secretion in colon and jejunum, but it was observed only at higher doses of L-NNA (20 and 40 mg/kg, i.p.) and this inhibition was more in colon than in jejunum. Intraperitoneal administration of L-NNA (20.0 mg/kg) significantly inhibited fluid and Na^+ and Cl^- secretion in jejunum, whereas in colon it significantly inhibited only electrolyte (Na^+, K^+ and Cl^-) secretion without any effect on fluid secretion. Maximum dose of L-NNA (40 mg/kg, i.p.) significantly ($P<0.01$) inhibited 5-HT-induced fluid and electrolyte secretion in both jejunum and colon, except for K^+ secretion in jejunum. The inhibitory effect of L-NNA on fluid secretion was more in colon (110%) as compare to jejunum (45%) (Table 2)

Reversal by L-arginine of the inhibitory effect of L-NNA:

Administration of L-arginine intraperitoneally (600-1500 mg/kg) had reversed the inhibitory effect of L-NNA (40 mg/kg, i.p.) on 5-HT-induced fluid and electrolyte secretion in a dose-dependent manner. Maximum dose of L-arginine (1500 mg/kg, i.p.) had reversed completely the L-NNA (40 mg/kg, i.p.) inhibitory effect but the effect on concentration of K+ in jejunum was not changed significantly. The minimum dose of L-arginine (600 mg/kg, i.p.) had no significant effect on inhibitory effect of L-NNA (40 mg/kg, i.p.) except the level of fluid secretion in colon. A moderate dose of L-arginine (900 mg/kg, i.p.) had reversed the inhibitory effect of L-NNA

TABLE 1: 5-HT-INDUCED INTRALUMINAL FLUID AND ELECTROLYTE SECRETION.

	Jejunum				Colon			
Treatment (s.c.)	wet weight (μl/g)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	wet weight (μl/g)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)
Saline	-536.5	-127.5	4.54	122.5	-606.9	128.1	7.6	122.5
(2 ml/kg)	±51.11	±1.41	±0.53	±3.01	±63.30	±1.03	±0.18	±1.38
5-HT	-209	147.3	5.73	126.0	-132.6	146.1	9.9	122.6
(2 mg/kg)	±15.10*	±0.23*	±0.25	±3.77	±8.63*	±2.49*	±0.30*	±0.50
5-HT	15.7	150.3	5.96	139.5	24.0	152.8	10.9	132.5
(4 mg/kg)	±1.55*	±0.95	±0.13	±2.11	±1.78*	±1.42*	±0.48*	±1.16
5-HT	119.9	166.9	6.86	16.7	164.0	189.6	13.0	160.0
6 (mg/kg)	±4.89*	±1.87*	±0.24	±2.44*	±7.39*	±1.75*	±0.76*	±1.24
5-HT	215.4	191.3	7.63	180.3	436.5	198.6	15.8	171.8
(8 mg/kg)	±7.78*	±4.03*	±0.24*	±1.59	±48.35*	±1.92*	±0.23*	±1.98

5-HT was administered 1 hour after the preparation of animal and just before instillation of Tyrode solution . The results were analyzed by unpaired student 't' test and expressed as mean±S.E. saline treated group was considered as control, for coming to conclusion, *$P<0.01$.

TABLE 2: INHIBITORY EFFECT OF N^G–NITRO-L-ARGININE (L-NNA), ON 5-HT-INDUCED INTRALUMINAL FLUID AND ELECTROLYTE SECRETION.

	Jejunum				Colon			
Treatment (s.c.)	wet weight (µl/g)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	wet weight (µl/g)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)
5-HT	119.9	166.8	6.8	166.7	164.0	189.6	13.0	16.0
(6 mg/kg)	±4.89	±1.87	±0.24	±2.44	±7.39	±1.75	±0.76	±1.24
L-NNA	102.39	163.2	6.1	161.1	172.1	183.0	12.0	153.5
(10 mg/kg)	±8.54	±2.13	±0.20	±1.71	±9.90	±1.78	±0.298	±2.42
L-NNA	94.3	152.1	7.5	153.5	124.2	162.5	9.5	150.8
(20 mg/kg)	±4.28*	±1.86*	±0.20	±1.59*	±12.69*	±1.42*	±0.51*	±1.78*
L-NNA	65.7	150.2	6.8	148.2	-16.8	144.8	7.9	132.8
(40 mg/kg)	±4.54*	±1.85*	±0.17	±1.98*	±1.39*	±1.92*	±0.15*	±1.76*

L-NNA was administered 30 min. prior to administration of 5-HT. The results were analyzed by unpaired student 't' test and expressed as mean±S.E. 5-HT (6 mg/kg) treated group was considered as control for coming to conclusion. *P<0.01.

(40 mg/kg, i.p.) significantly in colon but not in jejunum of both fluid and electrolyte transport. However, it was found to have a significant effect on fluid secretion in jejunum but not on, electrolytes transport. D-arginine (900 mg/kg, i.p.) had no significant effect on inhibitory effect of L-NNA on 5-HT-induced fluid and electrolyte secretion. (Table 3)

DISCUSSION

5-HT can induce net ion and fluid secretion in intestine of a variety of different species[37]. *in vitro* experiments in the rats have shown that 5-HT-induced secretion is due to simultaneous inhibition of neutral NaCl absorption and electrogenic Cl^- secretion[38]. In present study 5-HT at low dose (2 mg/ kg) inhibited intraluminal fluid absorption but at higher dose (4-8 mg/kg) induced net fluid secretion and secretion was more in colon as compared to jejunum region. These results support the previous report of 5-HT induces a dose dependent rise in transintestinal potential difference in both jejunum and the colon with maximum response, observed in the colon[39]. Similarly Na^+ and Cl^- contents in both jejunum and colonal secretion were increased after 5-HT (2-8 mg/ kg) administration indicates and supports 5-HT-induced electrolyte secretion.

The mechanism for this induced secretion is unknown but it may be hypothesized that most of the residual anion movement (JNa^+-JK^+-JCl^-) can be accounted for by bicarbonate[40]. 5-HT inhibits Na^+ absorption on largely in villi and probably stimulates Cl^- channels in the apical membrane predominantly of crypt cells. This Cl^- secretion by, inserting or activating Cl^- channels in the apical membrane, predominantly of crypt cells. This Cl^- secretion in turn, facilitates HCO_3^- secretion by Cl^-. HCO_3 exchange by enabling Cl^- to recycle across the apical membrane[41]. The high concentration of K^+ could be explained by K^+ release from damaged epithelial cells[42]. Physiological control of colonic ion transport in either the absorptive or the secretory direction depends upon the system of neural, endocrine and paracrine components[43]. Previous studies have shown that, the activation of basolaterally situated K^+ conductance mediated by intracellular Ca^{+2} increases the driving force for secretion, where as the increase in intracellular cAMP concentration activates a luminal Cl^- conductance and a basolateral K^+ conductance[44]. Previous studies have also shown that, increase in the level of endogenous 5-HT mediates intestinal water and electrolyte transport without any change in adenylate cyclase activity, cyclic nucleotide phosphodiesterase or Na^+-K^+- ATPase[45,46].

The effect of NO on fluid transport in the intestine reported to be dependent upon the condition under the study which in turn relate to physiological vs. pathophysiological states. In normal anaesthetized rats N^G-nitro-L-arginine methyl ester reversed jejunal secretion[47] and in normal tissue nitric oxide synthase inhibitors can be secretagogue[48]. These findings suggest that physiologically NO may promote fluid absorption. However, in pathological states, NO may be produced at higher concentrations capable of evoking

TABLE 3: INHIBITORY EFFECT OF NG-NITRO-L-ARGININE (L-NNA), ON 5-HT-INDUCED INTRALUMINAL FLUID AND ELECTROLYTE SECRETION.

	Jejunum				Colon			
Treatment (mg/kg)	wet weight (μl/g)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	wet weight (μl/g)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)
5-HT (6 mg/kg)	65.7	150.2	6.8	148.2	-16.8	144.8	7.9	132.8
+ L-NNA (40 mg/kg)	±4.54	±1.85	±0.17	±1.98	±1.39	±1.92	±0.15	±1.76
L-Arginine	77.9	152.8	6.8	150.1	25.8	146.9	7.5	138.0
(600 mg/kg)	±11.20	±0.13	±0.13	±1.16	±2.56	±2.10	±0.36	±2.13
L-Arginine	120.3	163.7	7.5	169.2	166.4	171.3	12.8	160.2
(900 mg/kg)	±6.51	±3.73	±0.34	±1.8	±3.68	±3.68	±0.50	±1.12
D-Arginine	65.4	144.8	7.1	147.7	-13.9	147.4	7.9	132.9
(900 mg/kg)	±3.60	±2.94	±0.17	±1.74	±1.04	±2.16	±0.32	±1.83

L-NNA (40 mg/kg, i.p.) was administered 30 min. prior to 5-HT (6 mg/kg, s.c.) administration and L-Arginine/D-Arginie was administered 15 min. after L-NNA. The results were analyzed by unpaired student 't' and were expressed as mean±S.E. L-NNA (40 mg/kg) treated group was considered as control, for coming to conclusion. *P<0.01.

net secretion. Support for this contention comes from the reversal by NO synthases inhibitor of castor oil[49], *Escherichia coli*[50] and sodium choleate-induced[51] diarrhea.

In our study 5-HT-induced secretion was observed at higher doses (2-8 mg/kg) but not at lower doses. Rat's gastrointestinal tract is innervated by NO synthase containing neurons[52]. Administration of 5-HT above 2 mg/kg may stimulate synthesis of NO from enteric neurons, and it may mediate fluid and electrolyte secretion. The inhibitory effect of L-NNA may suggest the release of NO from enteric neurons after the administration of 5-HT. Which may acts on Villi and which may leads to an increase in the concentration of Na^+[53]. NO may also acts on crypt cells of apical membrane, may involves the insertion and activation of Cl^- channels which may leads to increased Cl^- secretion[30]. NO may damage the epithelial cell thereby increase the concentration of K^+ in jejunum and colon[53].

We conclude from this study that 5-HT-induced fluid and electrolyte secretion may partially involve nitric oxide. The present study demonstrates that, 5-HT releases NO into the lumen which may cause fluid accumulation by increasing the level of electrolytes Na^+, K^+ and Cl^- in the rat jejunum and colon. The basic mechanism of inhibitory effect of L-NNA on fluid and electrolyte secretion in the jejunum and colon induced by 5-HT cannot be explained from these experiments. Further studies are needed to completely understand the role of NO in 5-HT-induced luminal fluid and electrolyte secretion.

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