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Role of Nitric Oxide in 5-HT-induced Intestinal Motility and Diarrhea

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5-Hydroxytryptamine (5-HT) is an important neurotransmitter and hormone/paracrine agent mediating various enteric functions. Its precise physiological, pathological and pathophysiological role remains nuclear. Nitric Oxide (NO) is involved in the physiological modulation of peristalsis and intestinal transit by interacting with the part of neuronal mechanisms and affecting the gastrointestinal musculature. This study investigated the role of Nitric Oxide in 5-HT-induced intestinal motility and diarrhea. Intraperitoneal administration of 5-HT, produced a dose-related increase in the incidence of diarrhea in fasted mice. 5-HT (0.1 mg/kg i.p.) induced diarrhea was inhibited by N^G-Nitro-L-arginine (L-NNA) (1-30 mg/kg) dose dependently. The inhibitory effect of L-NNA was reversed by L-arginine (50-300 mg/kg i.p.) dose dependently but not by D-arginine (100 mg/kg i.p.). Subcutaneous administration of 5-HT (0.5-2.0 mg/kg) produced a dose-related increase in intestinal transit, 5-HT (1.0 mg/kg, s.c.) induced increased intestinal motility was not modified by L-NNA (1.0-40 mg/kg, i.p.) L-arginine (50-300 mg/kg i.p.) dose dependently inhibited 5-HT-induced intestinal motility, which had been reversed by L-NNA (5-20 mg/kg, i.p.). L-NNA inhibited the effect of L-arginine, without modifying the 5-HT-induced intestinal motility. These results provide evidence that nitric oxide may play a role in diarrhea, but not that of the 5-HT -induced intestinal motility in the rat *in vivo*.

Most of the endogenous 5-HT is found in the enterochromaffin cells of the gastrointestinal mucosa and also located within neurons in the enteric nervous system[1]. All intrinsic neurons of the gut for which 5-HT serves as a neurotransmitter are interneurons, terminating on the ganglion cells of the submucosal and myenteric plexuses[2,3]. The precise role of 5-HT in the physiology and pathophysiology of the gastrointestinal tract remains unclear. It is likely, however, that 5-HT has numerous physiological actions, possibly regulating intestinal motility and modulating intestinal secretion of water and electrolytes[4].

The role of 5-HT in the gut is very complicated owing largely to the presence of multiple 5-HT receptor subtypes. A number of 5-HT receptor sub types have been identified using biochemical, electrophysiological and pharmacological techniques. The following 5-HT receptor types are known to exist. The heterogenous 5-HT_1 (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1P}), 5-HT_2, 5-HT_3 and 5-HT_4. 5-HT_2 receptor appears to be present on smooth muscle, while the others, 5-HT_{1A}, 5-HT_{1P}, 5-HT_3, and 5-HT_4 are neuronal[5]. 5-HT acts on synaptic cholinergic excitatory and NANC inhibitory motor neurons to modulate the occurrence of phasic contraction. These actions involve 5-HT_{2A}, 5-HT_{2C} and 5-HT_4 receptor, 5-HT_{2A} and 5-HT_{2C} receptors are located on postsynaptic cholinergic neurons[6] and 5-HT_4 receptors on non-odgeneric non-cholinergic (NANC) inhibitory neurons[7]. 5-HT_4 receptor may mediate the release of NO by the action of endogenous or exogenous 5-HT.

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Nitric oxide (NO) generated from L-arginine is believed to be an inhibitory NANC neurotransmitter that mediates gastrointestinal motility physiologically and in certain pathophysiological states like trinitrobenzene sulfonic acid (TNBS)-induced ileitis, spontaneous colitis seen in human leukocyte antigen (HLA)-B27 transgenic rats and in chronic colitis model in the rhesus monkey[8-10]. NO has been postulated to be involved in small intestinal motility in rats[11] and dogs[12] and colonic motility in dogs[12] and humans[13]. In addition to its action on intestinal motility, NO affects intestinal electrolyte transport[14]. The muscle of the rat and canine intestine is innervated by nitric oxide synthase (NOS) containing neurons[15,16], supporting a role for NO in regulating intestinal motility and secretion. NO is involved in diarrhea induced by castor oil[17], bisacody1 and phenolphthalein[18], carbachol[19], sodium choleate [20] and magnesium sulphate[21]. These laxatives may reduce the active Na^+ and K^+ absorption and decreasing NA^+-K^+-ATPase in the small intestine and colon[22] and change the intestinal permeability and histology[23]. These reports further suggest regulatory effects of NO in fluid secretion and transit of small intestine and colon. NO a free radical produced by the enzyme NO synthase from the terminal guanidine nitrogen on L-arginine, has been recently shown to have a role in 5-HT- induced relaxation of gastronintestinal smooth muscle[24] and 5-HT - induced fluid secretion in the gut[25].

A recent study has confirmed the invlovment of nitric oxide in 5-HT-induced behavioral changes and the function was mediated by 5-HT2 receptors[26]. The purpose of the present study was to establish whether NO is involved in 5-HT-induced diarrhea and intestinal motility.

MATERIALS AND METHODS

Animals:

Male swiss albino mice weighing 16-20 g were used, for the study of diarrhea. Albino rats of either sex weighing 150-180 g were used for the study of gastrointestinal transit. The animals with diarrhea were excluded from the study. Before experiments, rats were housed under standard controlled conditions and food and water provided *ad libitum*. The animals were allowed atleast 1 w to acclimatize to the environment before the experiments were performed. All experiments were carried out between 14:00 and 19:00 h to avoid circadian influence on gastronintestinal function.

5-HT-induced diarrhea:

The effect of different agents on 5-HT induced diarrhea was studied by using a modified Kadowaki method[27]. Diarrheal activity of 5-HT were examined in groups consisted of 10 male mice of 16-20 g weight. Mice were deprived of food over night before the experiment, but were allowed free access to water. Animals with diarrhea were excluded from the study. Evaluation of diarrhea was made 15 min within the iterperitioneal administration of 5-HT. After administration of 5-HT each animals were allocated at individual cages, with clean filter paper at the bottom. In the second series of experiments we evaluated the abilities of various drugs on the response to the dose of 5-HT. Animals were pretreated with the test drugs intraperitioneally 30 pin prior to the administration of 5-HT and the effects of the drugs were determined 15 min after 5-HT administration. The extent of diarrhea were expressed by percent incidence scored on all or none basis.

5-HT induced small intestinal motility:

Adult albino rats of either sex weighing between 150-180 g were fasted overnight and tested for intestinal transit. After 15 min the administration of 5-HT, the charcoal meal consisting of 10% of activated charcoal and 5% gum acacia and 5% gum acacia were given orally (1ml/rat). The rats were killed by cervical dislocation, after the 20 *min* time period of administration of charcoal meal. The small intestine was removed from the pyloric sphincter to the ileo-caecal junction. The pyloric end of the stomach was tied to a glass rod and suspended with 1*gm* weight at the ileo-caecal junction for 20*s*. The distance travelled by the charcoal meal was noted and expressed as percentage of intestinal transit using the following formula. To evaluate the effect of different agents on 5-HT induced transit, these agents were administered 30 *min* prior to the administration of 5-HT.

5-Hydroxytryptmine creatinine sulfate and L-arginine were purchased from Sigma Chemicals Company, St. Louis, MO. N^G-Nitro-L-arginine and D-arginine purchased from Fluka, Germany were used for the study. These compounds were dissolved in saline before being used.

Statistics:

The chi-square test was used to determine the

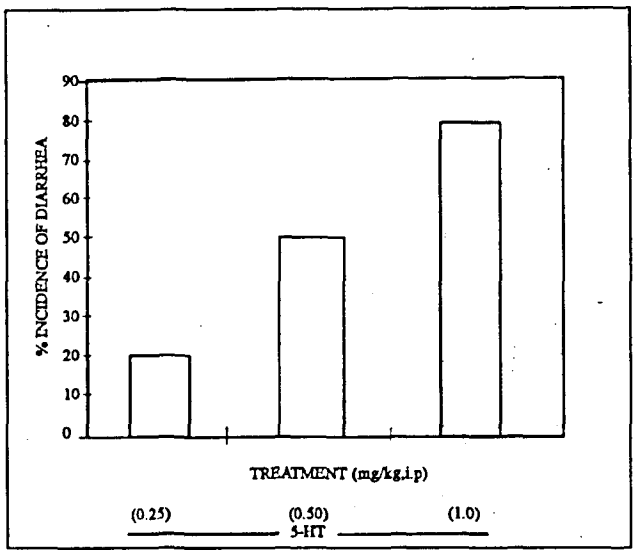

Fig. 1: 5HT-induced diarrhea in fasted mice

Dose-dependent (0.25, 0.5 and 1.0 mg/kg i.p) induction of diarrhea in mice by 5-HT. Evaluation of diarrhea was made within 15 min administration of 5-HT

significance of differences between groups with or without diarrhea. Small intestinal transit were expressed as mean ± S.E. and Dunnets multiple range test had been used for coming to conclusion, *p* value less than 0.05 was considered as significant .

RESULTS

5-HT-induced diarrhea:

In preliminary experiments we have found that 5-HT, 0.25 mg/kg i.p. induced diarrhea in 20% of mice. Incidence of diarrhea was increased in dose related manner as 0.5 mg/kg and 1.0 mg/kg produced 50% and 80% incidence of diarrhea (Fig.1).

Pretreatment with L-NNA does-dependently inhibited 5-HT-induced diarrhea. L-NNA at low dose (1 mg/kg) had no effect but 5 mg/kg, had reduced the percentage incidence of diarrhea but not significantly. At a dose of 10 mg/kg., L-NNA had significantly inhibited the diarrhea induced by 5-HT. Maximum effect of L-NNA was observed at 30 mg/kg in which percentage incidence of diarrhea was 20%. (Fig. 2).

L-arginnie did not modify 5-HT-induced diarrhea (data not shown), but it counteracted the effect of 30 mg/kg of L-NNA in a dose related manner. 50 mg/kg of L-arginine significantly reversed L-NNA inhibitory effect and at a

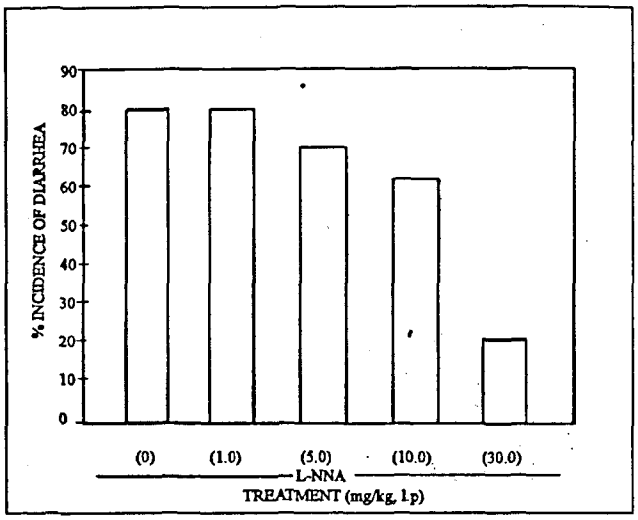

Fig. 2: Inhibitory effect of L-NNA on 5HT-induced diarrhea in fasted mice

Dose dependent effect of graded doses (1,5,10 and 30 mg/kg I.p.) of L-NNA on 5-HT (1.0 mg/kg I.p.) induced diarrhea in fasted mice. Evaluation of diarrhea was made within 15 min administration of 5-HT. L-NNA was administered 30 min prior to 5-HT administration. L-NNA stands for NG - Nitro - L -Arginine.

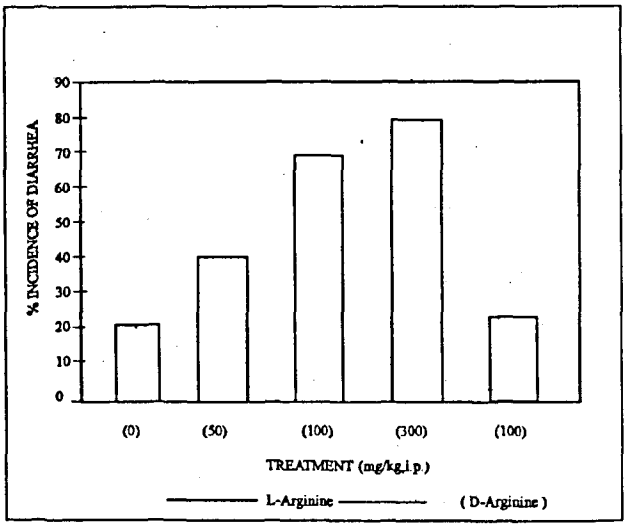

Fig. 3: Effect of L-Arginine and D-arginine on L-NNA-inhibited 5HT-induced diarrhea in fasted mice.
Dose dependent (50, 100 and 300 mg/kg I.p.) effect of L-Arginine and D-Arginine (100 mg/kg, I.p.) on L-NNA was administered 30 min prior to 5-HT, where as L-Arginine and D-Arginine were administered 15 min after administration of L-NNA Dirrhea was evaluated within 15 min administered of 5-HT

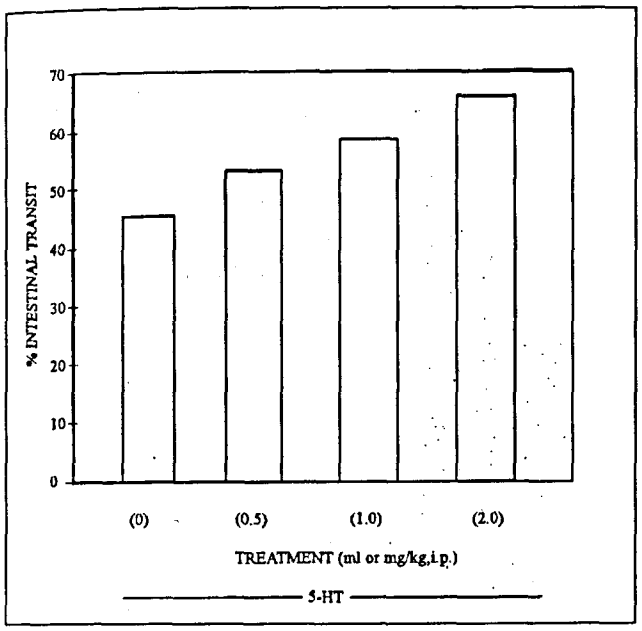

Fig. 4: 5HT-induced intestinal transit of charcoal meal in rats

Dose dependent effect of 5-HT (0.5, 1.0 and 2.0 mg/kg s.c.) on intestinal transit of charcoal meal in rats. The results were analysed and confirmed by Dunnets multiple range test by comparing with control (Saline treated) and expressed as mean ± S.E. *Dunnets multiple range test suggests that all the 3 groups of 5-HT treated have significantly (P<0.05) enhanced the Intestinal transit of Charcoal meal as compare to control

dose of 300 mg/kg, completely reversed L-NNA inhibitory effect on 5-HT- induced diarrhea (Fig. 3).

Small intestinal transit:

5-HT significantly (P<0.05) increased intestinal transit and it was dose dependent 5-HT, at a dose of 2 mg/kg administrated subcutaneously, produced 63.7% increase in intestinal transit which was the maximal effect. 5-HT at a dose of 1 mg/kg was used in the subsequent experiments to study the effect of nitric oxide as it produced an increased intestinal propulsion of charcoal meal. (Fig. 4).

L-arginine dose- dependently reduced 5-HT-induced intestinal transit. L-arginine of dose 50 mg/kg significantly reduced 5-HT-induced intestinal transit whereas L-arginine at a dose of 100 mg/kg completely reversed 5-HT induced intestinal transit. Administration of L-arginine at very high dose (300 mg/kg) reduced intestinal transit and

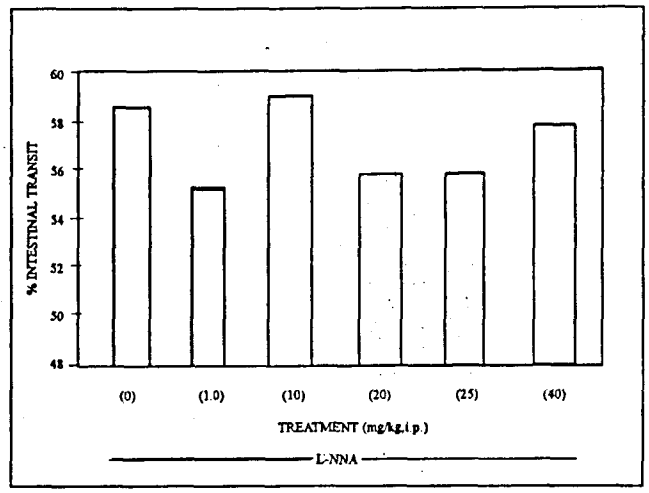

Fig. 5: Effect of L-NNA on 5HT-induced intestinal transit of charcoal meal in rats

Responses of graded doses (mg/kg) of N^G-Nitro-L-Arginine (1, 10, 20, 25 and 40) on 5-HT (1.0 mg/kg, s.c.) induced intestinal transit, administered 30 min prior to 5-HT. Results were analysed and confirmed by Dunnets multiple Range test by comparing with control (5-HT-treated) and expressed as mean± S.E. *Dunnets multiple test suggests that Means of L-NNA pretreated groups does not differ significantly (P<0.05) from control

this was less than the control but D-arginine (100 mg/kg) had no significant effect (Fig. 6).

L-NNA did not modify 5-HT- induced intestinal transit significantly but, it counteracted the effect of L-arginine. Pretreatment with L-NNA upto the dose of 40 mg/kg had no effect on 5-HT induced (1.0 mg/kg, s.c.) intestinal transit of charcoal meal significantly (Fig. 5). L-NNA of dose (0.5, 1.0 and 10 mg/kg0 administered intraperitoneally had dose dependently reversed L-arginine (100 mg/kg) inhibitory effect on 5-HT- induced intestinal transit. L-NNA of dose (10 mg/kg,i.p.) completely reversed L-arginine inhibitory effects on 5-HT-induced intestinal transit (Fig. 7).

DISCUSSION

Diarrhea is a well known effect of drugs that increase intestinal secretion and motility 5-HT induced diarrhea in fasted mice which was probably caused by net secretion in the intestine and a rapid orocecal transit[27]. 5-HT evokes an increase in intestinal short circuit current due to chloride secretion, the guinea pig ilume [28,29] and distal colon [30,31]. The present study confirms that NO is involved

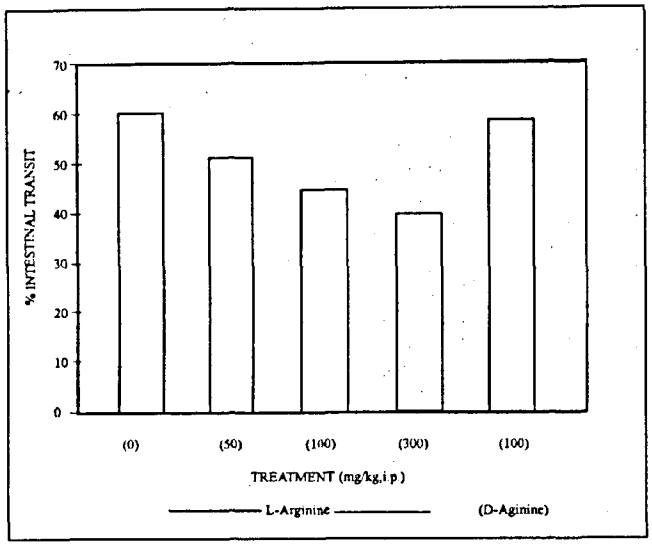

Fig. 6: Effect of L- and D- arginine on 5HT-induced intestinal transit of charcoal meal in rats

Graded dose responses of L-Arginine (50, 100 and 300 mg/kg, i.p.) and D-Arginine (100 mg/kg, i.p.) on 5-HT-induced intestinal transit of charcoal meal, administered 30 min prior to 5-HT. Results were analysed and confirmed by Dunnets multiple range Test, by considering 5-HT (1.0 mg/kg, s.c.) treated group as control. The results were expressed as mean±S.E. *Dunnets multiple range test suggests that means of L-Arginine pretreated groups has inhibited 5-HT effect significantly (P<0.05). **Dunnets multiple range test have suggested that D-Arginine (100 mg/kg, i.p.) had no effect on 5-HT (1.0 mg/kg, s.c.)-induced intestinal transit of charcoal meal.

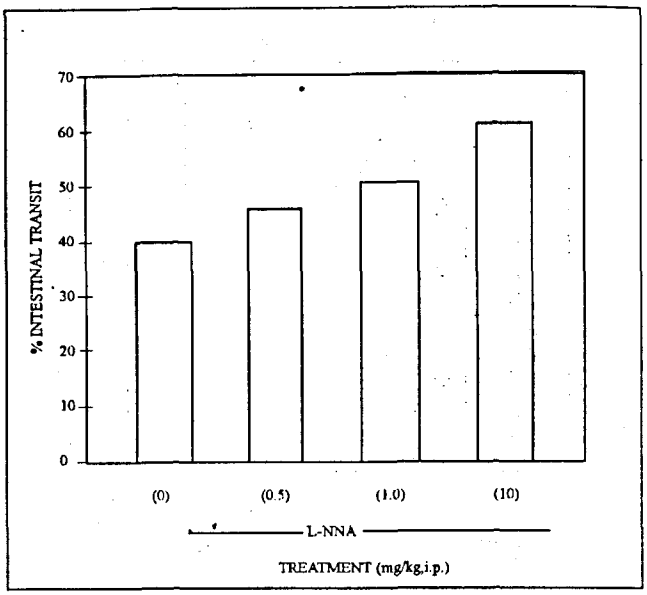

Fig. 7: Reversal by L-NNA of the effect of 1-Arginina of the 5HT-induced intestinal transist of charcoal in rats.

L-NNA (0.5, 1.0 and 10 mg/kg, i.p.) was administered 30 min prior to 5-HT (1 mg/kg, s.c.) administration and L-Arginine (100 mg/kg) was administered 15 minutes after L-NNA.The results were analysed and confirmed by Dunnets multiple range Test and were expressed as mean ± S.E. *Dunnets multiple range test has suggests that L-NNA (10 mg/kg, i.p.) significantly (P<0.05) inhibited the effect of L-Arginine (100 mg/kg) on 5-HT (1.0 mg/kg s.c.) -induced intestinal transit of charcoal meal in rats.

in 5-HT induced diarrhea[25], 5-HT-induced diarrhea was reduced by NOS inhibitor, L-NNA. In addition, the inhibitory effect on the diarrhea was reversed by L-arginine, this effect was enantiomer specific because D-argininewas without an effect. These findings imply that during 5-HT-induced diarrhea, NO is generated within the lumen, which could explain the antidiarrheal effect by L-NNA. Thus results support these findings of previous studies that the cathartic effect of 5-HT in mice involves the NO pathways[24-25].

The increase in intestinal transit is believed to be due to reduced resistance to flow, because of relaxation of the circular muscle of the intestine. The mechanism has been implicated in the action of other laxatives [32]. NO may be involved in this inhibitory effect because NO inhibits gut motor activity through an indirect (neural) and/or direct action on the gut [33-35]. In the light of the accepted hypothesis that NO may mediate the relaxaiton of intestinal muscle [36-33], it was expected that transit might be modified in rats given L-NNA. In the present results L-NNA did not modify the effect of the 5-HT and does not influence the intestinal transit in rats. The ability of L-NNA given i.p. to inhibit diarrhea at doses which had no significant effect on the transit rate strongly suggests that the antidiarrheal effect of L-NNA is related to antisecretory mechanism and not to inhibition of transit. The mechanism underlying the antisecretory effect of L-NNA is however unclear[24]. The mechanism of diarrhea induced by 5-HT includes an increase in intesetinal motility by increased consequences of longitudinal muscle contraction[39] and inhibition of the circular muscle contraction[40,41]. The relaxation of circular muscle may involve the release of NO by the action of 5-HT on the myenteric and submucosal plexi[24,25].

In the present study, L-arginine did not modify 5-HT-induced diarrhea but influences intestinal transit. The

maximum dose of L-arginine administrated intraperitioneally has reduced the intestinal transit less than the control indicating the presence of other mechanisms that may act synergestically action with L-arginine. We hyptothesize that the dose of 5-HT selected for this study induced diarrhea mainly by secretory mechanisms but not by intestinal propulsion[24-25]. Higher doses of L-NNA have not been selected to avoid other complications that way arise due to over dosage.

Our results support the previous reports that NO is involved in 5-HT-induced diarrhea. The failure of L-NNA to affect intestinal motility changes induced by 5-HT, probably implies that NO is not produced by the intestinal mucosa in amounts sufficient to affect above function.

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