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EFFECT OF ACUTE AND CHRONIC ADMINISTRATION OF L-ARGININE ON MORPHINE INDUCED INHIBITION OF GASTROINTESTINAL MOTILITY

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Abstract : Effect of acute and chronic administration of L-arginine on morphine induced gastrointestinal inhibition was tested in rats. In the test for acute effect, L-arginine (200 mg/kg, i.v.) was given 10 minutes before the charcoal meal test. In the test for chronic effects, L-arginine (200 mg/kg, i.v.) was given twice a day for 4 days. Charcoal meal test was done on the fifth day. Morphine was administered 45 minutes before the charcoal meal test . Results showed that acute administration of L-arginine did not affect the morphine's action on the GIT. In contrast, chronic administration of L-arginine reversed the morphine induced decrease in gastrointestinal motility. The reversal was however, not complete. This data suggests that inhibition of NO may be one of the mechanism of morphine induced constipation.

Key words : morphine, L-arginine, Nitric oxide, GIT motility

INTRODUCTION

Morphine has a dual action (excitatory and inhibitory) on the gastrointestinal tract (1,2). It produces increased resting tone and spasm of the intestine with apparent loss of relaxation. Segmental contractions usually are enhanced but propulsive contractions are markedly diminished. No satisfactory explanation has been put forward for this dual action of morphine.

Presynaptic inhibition of acetylcholine release and postsynaptic inhibition of the action of acetylcholine have been postulated as possible modes of opioid action (3,4) Aside from acetylcholine, a number of additional neurotransmitters have been implicated including vasoactive intestinal peptide (VIP), cholecystokinin (CCK), substance P, prostaglandins, bradykinin, neurotensin, bombesin, motilin, catecholamines and serotonin (5) However, none of the hypothesis satisfactorily explain the excitatory and inhibitory action of the opioids.

Nitric oxide (NO) is one of the main neurotransmitter of the inhibitory

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NANC nerves in the GIT (6). Evidence shows that NO may be involved in the pathogenesis of postoperative ileus (7). Considerable evidence suggests that nitrergic pathways may be involved in the acute and chronic actions of the opioids (8). Moreover nitrates are known to inhibit opioid induced spasm of the sphincter of oddi.

We hypothesize that opioids may be causing tonic spasms of the intestines by inhibiting the generation of NO, an important inhibitory neurotransmitter. To test the hypothesis, effects of acute and chronic administration of L-arginine, a precursor of NO, on morphine induced decrease in gastrointestinal motility were investigated.

METHODS

Wistar rats (150-200 g) housed under standard environmental conditions were used. Food and water was provided ad libitum. Charcoal meal test was carried out between 0800 and 1200 hours in all animal groups.

Charcoal meal test (9)

Rats (fasted overnight) were given a charcoal meal (0.5 ml of aqueous suspension of 10% charcoal and 5% gum acacia) by gavage. Animals were sacrificed by anaesthetic ether 20 minutes later. The small intestine (from pylorus to ileo-caecal junction) was removed and stretched by hanging a 3 g weight from one end for 20 seconds. The total length of intestine was measured by laying the stretched intestine on a scale engraved on a plain surface. The length traversed by charcoal was confirmed by making incisions at different points of the stretched intestine and identifying charcoal particles. The percentage length of small intestine traversed by charcoal was recorded.

Experimental protocol

Acute effect of L-arginine (n = 10): Morphine (2 mg/kg s.c) was administered 45 minutes before the charcoal meal. L-arginine (200mg/kg IV) or Saline (1 ml/kg, IV) were given 10 minutes before the meal.

Chronic effect of L-arginine (n = 10) : L-arginine (200 mg/kg, IV) or Saline (1 ml/kg, IV) were given twice a day for four days. Effect of morphine on charcoal meal was tested on the fifth day.

To confirm the role of NO, another group (n = 10) of rats was administered L-arginine (200 mg/kg, IV) plus a nitric oxide synthetase inhibitor, N-nitro-L-arginine [L-NOARG] (5 mg/kg, IV) twice a day for four days. Charcoal meal test under morphine was done on the fifth day.

To study the action of L-arginine alone and L-NOARG alone on GIT, the effect of single administration of L-arginine and chronic administration of L-arginine and L-NOARG on charcoal transit was tested in three separate groups of 10 rats each. The doses and regimens used were same as mentioned above.

Statistical Analysis

Group differences were assessed by one-way ANOVA followed by post-hoc Scheffe's test. A "p" value of less than 0.05 was considered significant and 0.01 was considered as highly significant.

RESULTS

Figure 1 shows the effect of acute administration of L-arginine on morphine induced decrease in GIT motility. Morphine significantly (21.40 + 5.3 vs 96 + 4.8 P<0.01) reduced the percent length of intestine traversed by charcoal (as compared to the saline group). Acute administration of L-arginine had no effect on the morphine induced inhibition of GIT motility (22.6 + 6.1 vs 21.40 + 5.3 P>0.05).

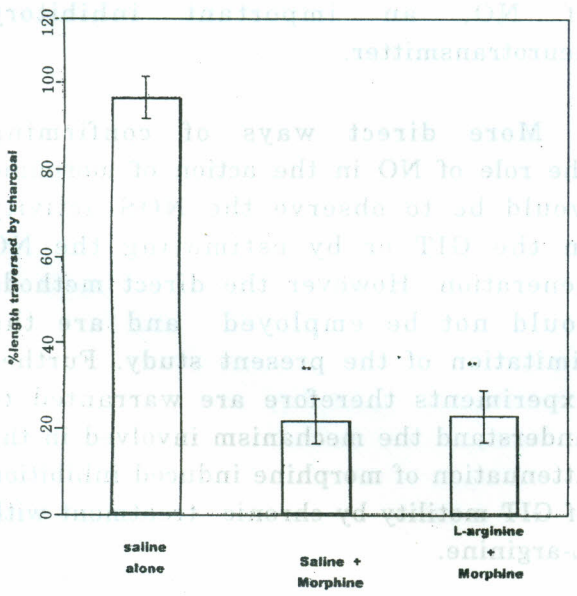

Fig. 1

Figure 2 shows the effect of chronic administration of L-arginine on morphine induced decrease in gastrointestinal transit. Chronic administration significantly reversed the morphine induced inhibition (71.4 + 7.3 vs 23.45 + 4.9 P<0.01). The reversal however was not complete [71.4 + 7.3 vs 96.6 + 4.8; P<0.05 (as compared to saline group)]. Further, the administration of L-NOARG along with L-arginine prevented the action of L-arginine (37.7 + 6.5 vs 71.4 + 7.3 P<0.05)

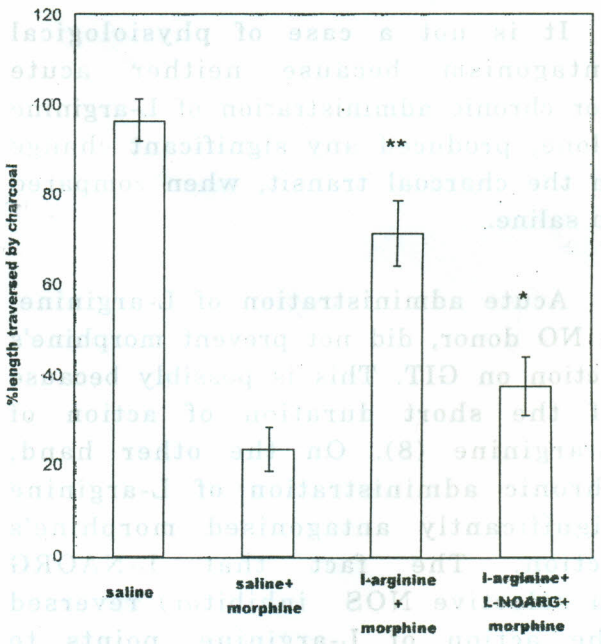

Fig. 2

Neither acute administration of L-arginine alone (94.7 + 8.2; vs 96.6 + 4.8; P>0.05) nor chronic administration (93.1 + 6.7 vs 96.6 + 4.8; P>0.05) had any significant effect on the charcoal transit as compared to saline group. Similarly, L-NOARG did not have any effect of its own on the GIT motility (94.3 + 7.2 vs 96.6 + 4.8; P>0.05).

DISCUSSION

The present study clearly demonstrates that chronic administration of L-arginine causes reduction in the inhibitory action of morphine on GIT. Acute L-arginine administration did not alter morphine's action in rats. It is clear that chronic L-arginine treatment induces changes that result in decreased morphine action on GIT.

It is not a case of physiological antagonism because neither acute nor chronic administration of L-arginine alone, produced any significant change in the charcoal transit, when compared to saline.

Acute administration of L-arginine, a NO donor, did not prevent morphine's action on GIT. This is possibly because of the short duration of action of L-arginine (8). On the other hand, chronic administration of L-arginine significantly antagonised morphine's action. The fact that L-NAORG (a selective NOS inhibitor) reversed the action of L-arginine, points to the fact that NOS activity and therefore NO was involved in some manner.

This differential effect of acute and chronic administration of L-arginine is also seen in its ability to attenuate morphine induced antinociception (8). The explanations for this differential effect, that have been postulated so far, are that, L-arginine on chronic administration upregulates the nitric oxide synthetase (NOS) activity and also displaces morphine from its binding sites in the CNS. Similar reasons may apply for its effect of reversing morphine's action on the GIT in this study.

The findings that L-arginine or L-NOARG, do not have any significant action of their own on the GIT motility, the reversal of the action of morphine by L-arginine, a NO donor; and a rereversal of morphine's action by L-NOARG, NOS inhibitor, is a clear indicator of the involvement of NO. Although indirect, these evidences do conform to our original hypothesis that morphine causes tonic spasms of intestine by inhibiting the generation of NO, an important inhibitory neurotransmitter.

More direct ways of confirming the role of NO in the action of morphine would be to observe the NOS activity in the GIT or by estimating the NO generation. However the direct methods could not be employed and are the limitation of the present study. Further experiments therefore are warranted to understand the mechanism involved in the attenuation of morphine induced inhibition of GIT motility by chronic treatment with L-arginine.

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