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EFFECT OF 5–HT_3 ANTAGONIST ONDANSETRON ON WRAP–RESTRAINT AND CONDITIONED EMOTIONAL STRESS INDUCED DEFECATION

RAJESH S. KARAN, LEKHA SAHA, S. S. HANDU AND VINOD K. BHARGAVA*

Department of Pharmacology,
Postgraduate Institute of Medical Education and Research,
Chandigarh – 160 012

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Abstract : The effect of ondansetron was investigated in wrap–restraint and conditioned emotional (footshock) stress induced colonic hypermotility in rat. Ondansetron was administered in doses of 0.1, 0.5 and 1 mg/kg, sc. In wrap–restraint group, ondansetron dose–dependently reduced the fecal output to baseline levels (0.51±0.20gm/rat, 0.48±0.38gm/rat, 0.38±0.16 gm/rat with 0.1, 0.5 and 1 mg/kg respectively). Diazepam, 5 mg/kg and atropine, 1 mg/kg also decreased the fecal output in this model. In footshock group, ordansetron did not affect the emotional stress induced increase in fecal pellet output. Diazepam and atropine however, decreased the fecal output in this model also.

Key words : 5–HT_3–receptor antagonist, ondansetron, conditioned emotional stress, colonic motility, wrap–restraint, defecation

INTRODUCTION

Stress–related functional bowel disease including irritable bowel syndrome (IBS) is a common, often incapacitating gastrointestinal disorder in humans (1). It has been suggested that serotonin (5–HT) acts as a mediator of gut dysfunction caused by stress, presumably acting through 5–HT_3 receptors (2). The actions attributable to the 5–HT_3 receptor could be of both central or peripheral origin. This has led to the study of several selective 5–HT_3 receptor antagonists in different animal models of gut dysfunction, however, the results are conflicting (2, 4, 5, 6). In the present study, the effect of selective 5–HT_3 receptor antagonist ondansetron in two rat models of stress–induced colonic hypermotility was investigated. The aim was to examine whether 5–HT_3 receptors are involved in these models and whether ondansetron can prevent the stress–induced colonic hypermotility.

METHODS

Male wistar rats weighing 150 to 200 gm. were used. The rats were housed under standard environmental conditions with food

*Corresponding Author

and water provided ad libitum. All experiments were carried out between 0800 and 1400 hours.

Wrap-restraint stress-induced defecation in rats :

The procedure followed was that of Williams et al., 1988 (3). Rats were anaesthetized lightly with ether, and their foreshoulders, upper forelimbs and thoracic trunks were wrapped in cloth tape to restrict, but not prevent, their movements. The animals recovered from anaesthesia within 2 to 5 minutes and immediately moved around in the cages, but the mobility of forelimbs was restricted, which prevented them from grooming the face, the upper head and the neck. The animals were placed individually in separate cages of size 40×20×15 cm which had a sheet of paper spread to collect all the fecal pellets excreted for 1 hour after wrap-restraint. The difference in the dry weight of paper before and after collection gave the weight of fecal pellets excreted.

Emotional stress-induced defecation in rats

This involves a conditioned emotional response which leads to an increased colonic motility and defecation (4). Briefly, each rat was placed in a closed box for 5 minutes and received a series of 5 footshocks (50V, 1 sec each) at intervals of 1 minute, through the grid floor of the box. The animals were placed individually in the same box, 24 hours after the shock sessions and the excreted fecal pellets were collected in the collection tray placed under the grid floor. The fecal output was recorded for 1 hour.

Drugs

All the drugs were dissolved in normal saline and administered 30 minutes before the exposure to stress. The drugs and their doses used were, ondansetron (Natco Pharmaceuticals, Hyderabad) in doses of 0.1, 0.5 and 1 mg/kg, sc, diazepam (Ranbaxy Laboratories, New Delhi) and atropine sulphate (Sigma Chemicals, USA) in doses of 5 mg/kg, ip and 1 mg/kg, sc respectively.

Statistical analysis

The fecal pellet output is expressed as mean±SEM for each group. The mean values before and after stress, after each treatment were tested by analysis of variance (ANOVA) for any significant differences, which was followed by post-hoc t-test. A difference was considered significant at $P<0.05$.

RESULTS

Wrap-restraint significantly increased the fecal pellet output of rats (2.01±0.55 gm/rat vs 0.49±0.17 gm/rat). Ondansetron, when administered 30 minute prior to wrap-restraint, dose dependently reversed the increase in fecal pellet output (0.51±0.20 gm/rat, 0.48±0.38 gm/rat, 0.38±0.16 gm/rat with 0.1, 0.5 and 1 mg/kg respectively). Diazepam 5 mg/kg and atropine 1 mg/kg also reversed the stress induced increase in fecal pellet output (0.53±0.21 gm/rat and 0.60±0.22 gm/rat respectively).

In the conditioned emotional response, the emotional stress 24 hours post-shock

TABLE I : Effect of ondansetron 0.1, 0.5 and 1 mg/kg on fecal pellet output in wrap-restraint and shock induced stress.

Groups	*Fecal Pellet Output (gm/rat)*	
	Wrap-restraint model	*Post-shock model*
Vehicle		
(Without restraint/shock	0.49 ± 0.17	1.27 ± 0.21#
(With restraint/shock)	2.01 ± 0.55**	2.05 ± 0.33**
Ondansetron		
0.1 mg/kg	0.51 ± 0.20*	1.63 ± 0.33
0.5 mg/kg	0.48 ± 0.38**	1.54 ± 0.32
1.0 mg/kg	0.38 ± 0.16**	1.52 ± 0.45
Diazepam		
5.0 mg/kg	0.53 ± 0.21*	0.37 ± 0.17**
Atropine		
1.0 mg/kg	0.60 ± 0.22*	0.77 ± 0.39*

Values are as mean±SE
*P<0.01, **P<0.001 compared to respective control groups
n=6 per group. #P<0.05 compared to the other model.

produced a significant increase in the fecal pellet output of rats (2.05±0.33 gm/rat vs 1.27±0.21 gm/rat). Placing the animals individually in the closed box without applying shock previously also increased pellet output when compared to the base line value of wrap-restraint stress (1.27±0.21 gm/rat vs 0.49±0.17 gm/rat). Ondansetron, 30 minute prior to test did not affect the emotional-stress induced increase in fecal pellet output significantly (1.63±0.33 gm/rat, 1.54±0.32 gm/rat, 1.52±0.45 gm/rat). Diazepam, 5 mg/kg and atropine 1 mg/kg however reversed the increase in the fecal output (0.37±0.17 gm/rat and 0.77±0.39 gm/rat).

DISCUSSION

In the wrap-restraint stress model, ondansetron dose dependently inhibited the fecal pellet output. However, in the emotional stress model, ondansetron with the same doses did not inhibit fecal pellet output. This differential effect of ondansetron in the two models is difficult to explain. Moreso, when the hypothesized mechanism of increased colonic motility by the two types of stress is the same (4, 5).

It is believed that stress results in the release of corticotropin releasing factor [CRF] in the CNS, which in turn promotes the release of thyrotropin releasing hormone [TRH] (7, 8). TRH acts on the myentric plexus in the GIT, to release endogenous 5HT. 5HT acts on the $5HT_3$ or other receptor subtype to modulate cholinergic neurons. Ultimately it is the cholinergic receptors which mediate colonic dysfunction (5). The efficacy of diazepam and atropine in both the models is well explained by this hypothesis (6, 9).

$5HT_3$–receptor antagonists usually do not show antistress or antianxiety effects especially in the models of aversive conditioning or conflict (10, 11). Thus, it is not surprising that ondansetron failed to prevent increase in colonic hypermotility in the shock-stress model by its central component of activity.

It has been recently shown that, along with $5HT_3$, $5HT_4$ receptor subtype are also involved in modulating colonic motility(12). Combined antagonism of $5HT_3$ and $5HT_4$ receptors inhibit the 5HT induced colonic motility more completely than antagonism of either receptor alone (12). Thus activity of ondansetron at $5HT_3$-receptors in gut could not significantly reverse the increase in fecal output in the emotional stress model.

In wrap restraint, stress is of mild intensity (3). It is presumed that the antagonism of $5HT_3$ receptors in the gut along with the antianxiety action of ondansetron was sufficient to inhibit colonic hypermotility in this model.

To conclude, although $5HT_3$ receptors are involved in the stress induced colonic hypermotility, other subtypes of 5HT receptors may also be involved. The differential effect of ondansetron on the two rat models suggest that $5HT_3$ receptor antagonism alone will not be adequate treatment for stress related colonic dysfunction.

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