

SHORT COMMUNICATION

AUTOINHIBITION AND DESENSITIZATION OF SEROTONERGIC
RESPONSES IN GUINEA PIG ILEUM

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Summary : The present study was undertaken to investigate the autoinhibition and desensitization of 5-hydroxytryptamine (5-HT) using another agonist MK-212 on guinea pig ileum. 5-HT and MK-212 produced dose dependent contractions of guinea pig ileum. The responses to MK-212 were reduced in the presence of 5-HT and vice versa. Neither 5-HT nor MK-212 produced any change in the responses to histamine, acetylcholine or KCl. Increase in Ca^{++} in bathing fluid reversed the desensitization produced by MK-212 or 5-HT. Our data suggest that 5-HT and MK-212 produce desensitization which is specific for serotonergic receptors and possibly involves Ca^{++} ions.

Key words : 5-hydroxytryptamine

MK-212

guinea pig ileum

INTRODUCTION

5-Hydroxytryptamine (5-HT) is known to produce contraction of various smooth muscles. In the gastrointestinal tract 5-HT produces a mixture of direct and indirect nerve mediated effects (7, 8). Repeated administration of 5-HT shows less intense and erratic response showing tachyphylaxis and fade (6, 7, 10). Huidobro-Toro and Foree (8) have demonstrated dual effects, agonistic and antagonistic, of 5-HT and related structural analogues in guinea pig ileum.

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MK-212 (6-chloro-2 (1-piperazinyl) pyrazine) is reported to exert 5-HT like actions in the central nervous system (1, 2, 3) as well as smooth muscles (11). The present work is a study on autoblockade, and dual (agonistic and antagonistic) actions of 5-HT and MK-212 in guinea pig ileum.

MATERIAL AND METHODS

Pieces of terminal part of ileum (3-4 cm away from ileo-caecal junction) were obtained from freshly sacrificed animals (350-400 g) and suspended in Tyrode solution maintained at 37°C (composition, g/l NaCl, 8.0; KCl, 0.2; CaCl₂, 0.18; NaH₂PO₄, 0.05; MgCl₂, 0.1; NaHCO₃, 1.0 and glucose, 1.0) and continuously bubbled with air. The isotonic contractile responses were recorded under seven fold magnification and 0.5g tension. Tissues were equilibrated for 45 min and the bathing fluid changed every 10 min.

In the first set of experiments, the ileum was exposed to graded concentrations (8.6×10^{-8} to 8.6×10^{-5} M) of 5-HT (5-hydroxytryptamine creatinine sulfate, Sigma, U. S. A.) to elicit a full dose response curve. The curves of 5-HT were reelicited after 15 min exposure to 5-HT (2.6×10^{-8} and 8.6×10^{-8} M) or MK-212 (7.3×10^{-8} M and 1.0×10^{-7} M). The preparations were exposed to 5-HT or MK-212 after 20 min of completion of previous dose response curves.

Similar experiments were carried out with MK-212. The dose response curve of MK-212 (M. S. and D., U. S. A.; 1.0×10^{-7} to 7.3×10^{-5} M) was first elicited in control preparations. The curves were re-elicited after 15 min exposure to MK-212 (7.3×10^{-8} and 1.0×10^{-7} M) or 5-HT (2.6×10^{-8} and 8.6×10^{-8} M).

The effects of MK-212 (1×10^{-7} M) and 5-HT (2.6×10^{-8} M) were also studied on the responses to acetylcholine (1.6×10^{-6} to 4.8×10^{-4} M), histamine (2.6×10^{-7} to 7.8×10^{-5} M) and nicotine (2.6×10^{-6} to 7.8×10^{-5} M).

The interaction of 5-HT and MK-212 with MK-212 (7.3×10^{-8} M) and 5-HT (2.6×10^{-8} M) respectively was also studied in bath fluid containing double CaCl₂ (0.38 mM).

RESULTS

5-HT as well as MK-212 produced dose dependent contractions of guinea pig ileum (Fig. 1). 5-HT (2.6×10^{-8} M and 8.6×10^{-8} M) inhibited the responses to 5-HT in a dose dependent manner. The maximum response was significantly ($P < 0.05$) reduced and there

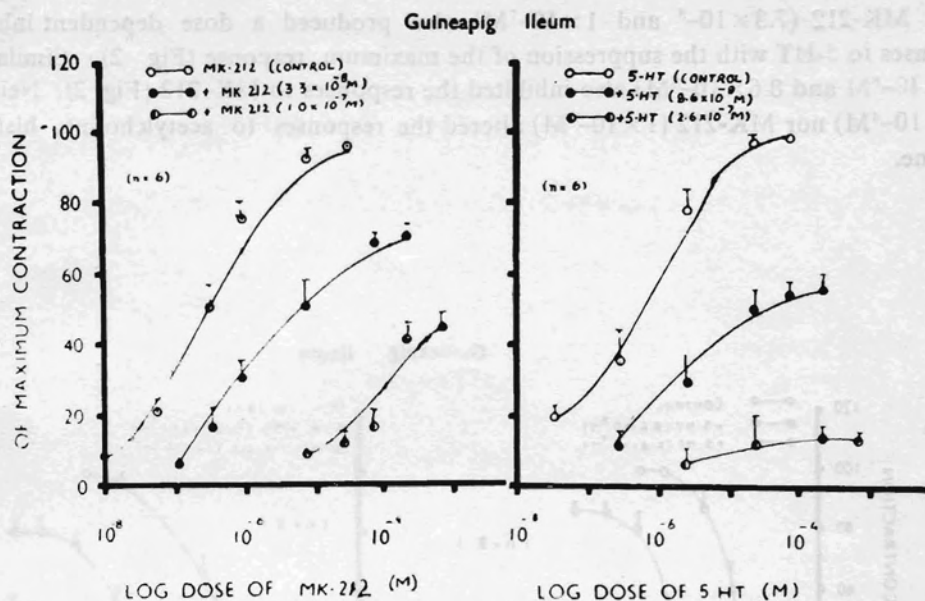


Fig. 1 : Effects of MK-212 and 5-HT and their interaction with MK-212 and 5-HT respectively in guinea-pig ileum. Each point depicts the mean and the vertical bar represents \pm SEM of 6 experiments.

was an increase in EC_{50} (Table I). Similarly, MK-212 ($7.3 \times 10^{-8}M$ and $1 \times 10^{-7}M$) inhibited its own responses with suppression of the maximum response and increase in EC_{50} value (Table I).

Table I : EC_{50} and Maxima of 5-HT and MK-212 under various drug treatments on guinea pig ileum.

Set I	Treatments	$EC_{50}(uM)$	Maxima (%)
I	5HT (Control)	6.61 ± 2.12	100.0
	5HT + 5-HT ($8.6 \times 10^{-8}M$)	13.49 ± 0.82	$56.0 \pm 4.5^{**}$
	5HT + 5-HT ($2.6 \times 10^{-7}M$)		$13.5 \pm 2.0^{**}$
II	MK-212 (Control)	3.16 ± 0.32	100.0
	MK-212 + MK-212 ($3.3 \times 10^{-8}M$)	$14.13 \pm 1.15^*$	$69.5 \pm 4.00^{**}$
	MK-212 + MK-212 ($1.0 \times 10^{-7}M$)	$724.40 \pm 8.26^{**}$	$44.0 \pm 5.0^{**}$
III	5HT (Control)	12.59 ± 2.32	100.0
	5HT + MK-212 ($3.3 \times 10^{-8}M$)	$134.90 \pm 6.31^{**}$	$82.3 \pm 4.3^*$
	5HT + MK-212 ($1.0 \times 10^{-7}M$)	$302.20 \pm 3.62^{**}$	$57.4 \pm 3.2^{**}$
IV	MK-212 (Control)	3.55 ± 0.81	100.0
	MK-212 + 5HT ($8.6 \times 10^{-8}M$)	$17.38 \pm 1.45^{**}$	$84.0 \pm 2.5^*$
	MK-212 + 5HT ($2.6 \times 10^{-7}M$)	$186.20 \pm 6.32^{**}$	$47.0 \pm 8.0^{**}$
V	5HT (Control)	8.32 ± 1.38	100.0
	5HT (in 0.38 mM $CaCl_2$)	$19.95 \pm 3.21^{**}$	100.0
	5HT + MK-212 ($1.0 \times 10^{-7}M$ in 0.38 mM $CaCl_2$)	$21.62 \pm 3.93^+$	98.3 ± 1.1
VI	MK-212 (Control)	3.80 ± 0.54	100.0
	MK-212 (in 0.38 mM $CaCl_2$)	$12.59 \pm 1.81^{**}$	100.0
	MK-212 + 5HT ($8.6 \times 10^{-8}M$ in 0.38 mM $CaCl_2$)	$12.68 \pm 1.62^+$	97.8 ± 2.1

*Significantly different from control ($P < 0.05$), ($P < 0.01$)**

+significantly different from control ($P < 0.01$) but not from the other group ($P > 0.05$).

MK-212 (7.3×10^{-8} and 1×10^{-7} M) also produced a dose dependent inhibition of responses to 5-HT with the suppression of the maximum response (Fig. 2). Similarly 5-HT (2.6×10^{-8} M and 8.6×10^{-8} M) also inhibited the responses to MK-212 (Fig. 2). Neither 5-HT (2.6×10^{-4} M) nor MK-212 (1×10^{-5} M) altered the responses to acetylcholine, histamine or nicotine.

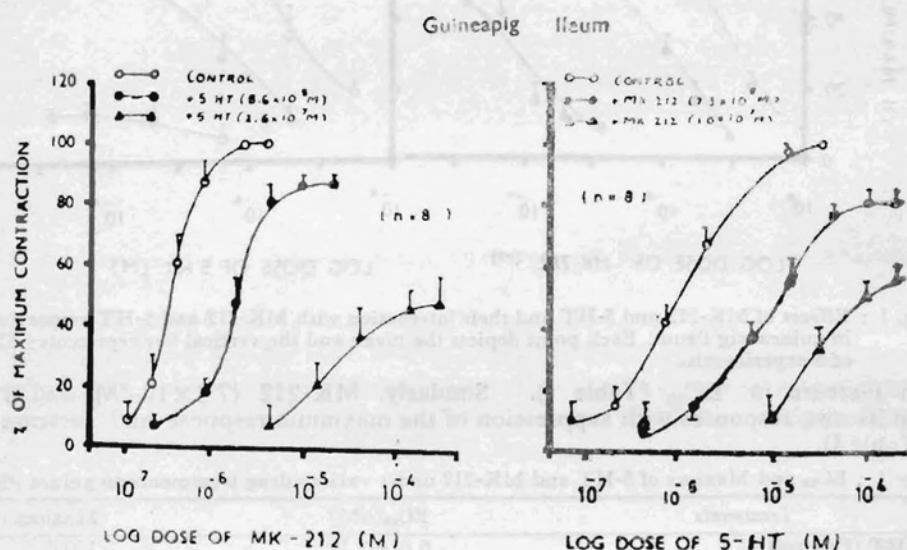


Fig. 2 : Effect of 5HT and MK-212 and their interaction with MK-212 and 5-HT respectively in guinea pig ileum. Each point indicates the mean and the vertical bar depicts \pm SEM of 8 experiments.

Increase in CaCl_2 in PSS did not affect the maximum responses to 5-HT and MK-212 significantly. There was significant increase in the EC_{50} value (Table I). MK-212 or 5-HT-

induced inhibition of 5-HT or MK-212 respectively, was however, abolished when the preparations were bathed in fluid with high CaCl_2 (Fig. 3).

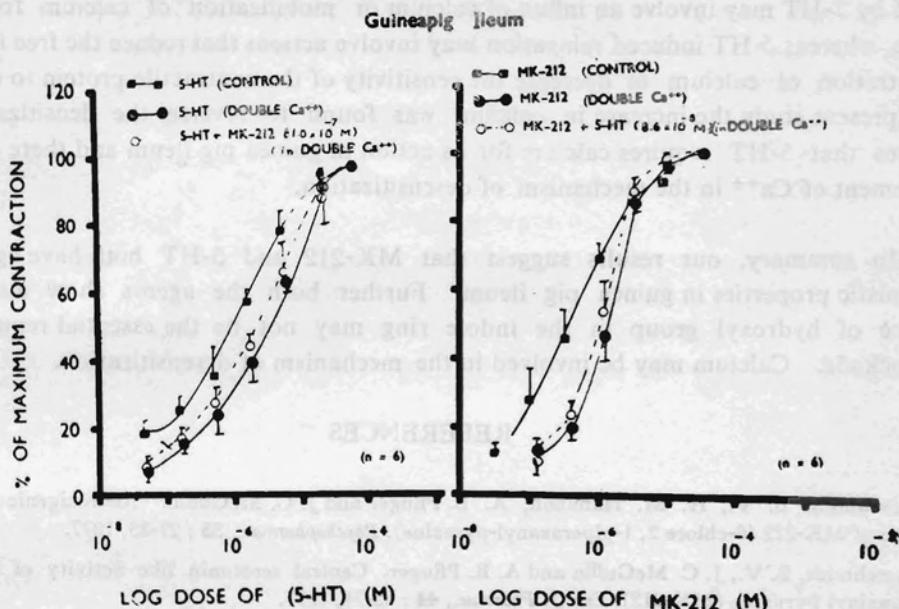


Fig. 3 : Interaction of 5-HT and MK-212 with MK-212 and 5-HT respectively in guinea pig ileum bathed in double Ca^{++} . Each point depicts the mean and the vertical bar represents \pm SEM of 6 experiments.

DISCUSSION

Repeated administration of 5-HT to intestinal strips decreases the responses to 5-HT upto the point of a complete obliteration of its effects (5, 6, 7). These reports are confirmed by our results. Furthermore, present results also suggest that both 5-HT and MK-212 have agonist and antagonist properties in guinea pig ileum.

Huidobro-Toro and Foree (8) have shown that 5-HT induced autblockade and desensitization is very specific to only 5-HT. Huidobro-Toro and Foree (8) have suggested that 5-HT induced autblockade is very specific and selective to drugs chemically related to hydroxyindolamines. Further, the essential requirement for autblockade is the presence of a hydroxyl group in the position five of the indole group. However, our results show that MK-212 which is devoid of indole group and does not contain hydroxyl group in its structure also showed autblockade of its own and of 5-HT responses. This shows that the presence of hydroxyl group at position five in the indole ring may not be the only criterion for a drug to show autblockade.

Drugs stimulate the smooth muscles by interacting with receptors directly or indirectly and eventually raising the levels of free intracellular calcium (9). Smooth muscles contraction elicited by 5-HT may involve an influx of calcium or mobilization of calcium from cellular sources, whereas 5-HT induced relaxation may involve actions that reduce the free intracellular concentration of calcium or decrease the sensitivity of the contractile protein to calcium (4). In the present study the increase in calcium was found to reverse the desensitization. This indicates that 5-HT requires calcium for its action in guinea pig ileum and there seems to be involvement of Ca^{++} in the mechanism of desensitization.

In summary, our results suggest that MK-212 and 5-HT both have agonistic and antagonistic properties in guinea pig ileum. Further both the agents show autoblockade. Presence of hydroxyl group in the indole ring may not be the essential requirement for autoblockade. Calcium may be involved in the mechanism of desensitization.

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