SHORT COMMUNICATION

EVIDENCE FOR HISTAMINE AND 5-HYDROXYTRYPTAMINE LIKE EFFECTS OF MK-212 ON GUINEAPIG ILEUM, TAENIA COLI AND RAT FUNDUS STRIP

UMESH P. PATEL AND SUBHASH C. VERMA*

Department of Pharmacology,
L. M. College of Pharmacy, Ahmedabad – 380 009

(Received on December 17, 1980)

Summary : MK-212 ($1x10^{-7}M - 1x10^{-5}M$) produced dose-dependent contractions of guineapig ileum, taenia coli and rat fundus strip. The responses to MK-212 in all three preparations were blocked competitively by cyproheptadine ($1x10^{-8}M$) a 5-HT receptor antagonist. Mepyramine ($1x10^{-8}M$)–H_1 receptor antagonist also inhibited competitively the responses of guineapig ileum and taenia coli to MK-212. However, it failed to block significantly the responses of rat fundus strip to MK-212. Metiamide ($1x10^{-6}M$), propranolol ($1x10^{-6}M$) or atropine ($1x10^{-6}M$) did not produce any significant effects on MK-212 induced contractile responses of guineapig ileum, taenia coli and rat fundus strip. Our findings suggest that MK-212 produces both 5-HT as well as histamine like effects on the guinea-pig ileum, taenia coli and rat fundus strip.

Key words : MK-212 (6–chloro–2–(1-piperazinyl)–pyrazine)
guinea pig ileum
guinea pig taenia coli
histamine receptor
5-HT receptor
rat fundus strip

INTRODUCTION

6-Chloro-2-(1-piperazinyl)-pyrazine (MK-212) was synthesized by Lumma (15) and is reported to produce several 5-hydroxytryptamine (5–HT)-like effects in the central nervous system. It causes increase in the frequency of twtiching of head in mouse and strength of crossed extensor reflex in spinalized rat (7, 11). It is also reported to produce anorexigenic and ancillary effects in rat (6, 8, 10). The anorectic action of MK-212 depends upon the integrity of 5-HT containing neurons in the CNS (10). The effect of MK-212 resembles fenfluramine, which mimics the action of 5-HT (4, 5, 13). Pretreatment with

*Demised, July 18th, 1981.

methergoline (3) abolishes CNS effects of MK-212 (6, 7, 9). Biochemical studies also show that MK-212 inhibits the uptake of ^{3}H-5-HT in cerebral cortical tissue (9). Although the 5-HT-like actions of MK-212 have been widely studied in CNS, its effects on peripheral tissues have not been studied. The present study was undertaken to investigate the actions of MK-212 on specific receptors of suitable smooth muscle preparations such as guineapig ileum and taenia coli and rat fundus strip.

MATERIALS AND METHODS

Adult guineapigs of either sex (400–500 g) were sacrificed by a sharp blow on the head. The guineapig terminal ileum and taenia coli 4–5 cm long were quickly dissected and mounted in 20 *ml* organ bath containing Tyrode solution. The composition of the Tyrode solution (mili-equivalents) was : NaCl 138, $CaCl_2$ 1.64, KCl 2.6, glucose 5, $NaHCO_3$ 12, $MgSO_4$ 1.06, NaH_2PO_4 0.4. The physiological solution was maintained at 37°± 1°C and bubbled with air. The responses were recorded on a smoked drum using isotonic frontal writing lever which exerted 0.5 g tension on the tissue and gave 10–fold magnification. The tissue was allowed to equilibrate for 30 min. The bathing solution was changed every 10 min. The agonist contact time with the tissue was 30 sec and antagonist contact time was 10 min.

Similarly adult albino rats of either sex (200–300 g) fasted for 48 hr were sacrificed. The fundus strip of 4–5 cm was dissected and suspended in 20 *ml* organ bath containing Kreb's bicarbonate solution maintained at 37°C and bubbled with carbogen gas. The composition of Kreb's bicarbonate solution (miliequivalents) was : NaCl 121, $NaHCO_3$ 25.2, glucose 10.5, KCl 45.5, $CaCl_2$ 25.5, $MgSO_4$ 2.5, KH_2PO_4 1.17. The responses were recorded in a cumulative manner using isotonic frontal writing lever which exerted 1.5 g of tension on the tissue and gave 10-fold magnification. The agonist contact time was 90 sec and antagonist contact time with the tissue was 10 min.

Drugs used :

MK-212 (Merck Institute for Therapeutic Research, U.S.A.), mepyramine maleate (May and Baker, India), cyproheptadine (Merck, Sharp and Dohme of India Ltd.), metiamide (Smith Kline & French Lab., England), atropine sulphate (C.H. Boehringer Sohn, W. Germany) and propranolol (Imperial Chemical Industries, London).

RESULTS

Fig. 1 illustrates dose-dependent contractions produced by MK-212 (1×10^{-7} M to 1×10^{-5} M) on guineapig ileum. The dose response curves of contractile responses to

MK-212 were shifted to the right in a parallel manner by the specific H_1-receptor antagonist-mepyramine maleate ($1x10^{-8}$ M) and by 5-HT receptor antagonist-cyproheptadine ($1x10^{-8}$ M) (Fig. 1). Similarly MK-212 ($1x10^{-7}$ M to $1x10^{-5}$ M) also produced dose-

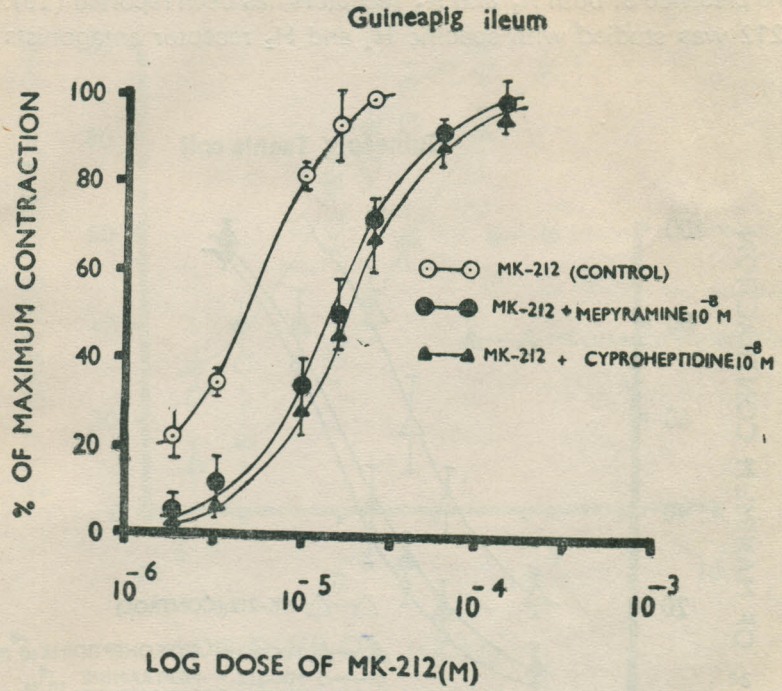

Fig. 1 : The effect on isolated guineapig ileum of MK-212 and its interaction with mepyramine $1x10^{-8}$M and cyproheptadine $1x10^{-8}$M. Each point represents the mean ±S.E.M. of 5 experiments.

dependent contractions of guineapig taenia coli (Fig. 2). The dose response curves produced by MK-212 were displaced to the right in a parallel fashion by mepyramine maleate ($1x10^{-8}$ M) and by cyproheptadine ($1x10^{-8}$ M) (Fig 2). The dose-response curve of contractile responses of rat fundus strip to MK-212 was shifted to the right in a parallel manner by cyproheptadine ($1x10^{-8}$M) (Fig. 3). However, mepyramine maleate ($1x10^{-6}$ M) failed to inhibit significantly the responses to MK-212 (Fig. 3). Metiamide ($1x10^{-6}$ M), propranolol ($1x10^{-6}$ M) and atropine sulphate ($1 x 10^{-6}$ M) did not produce any change in MK-212 induced contractions of guineapig ileum, teania coli, rat fundus strip.

DISCUSSION

Guineapig ileum and taenia coli contract to histamine (1,16). Histamine also produces contraction of fundus strip in high concentractions (19). Histamine receptors involved in guineapig ileum have been classified as H_1 - receptors (1). In the guineapig taenia coli, the presence of both H_1 and H_2 receptors has been reported (16). The interaction of MK-212 was studied with specific H_1 and H_2 receptor antagonists namely mepy-

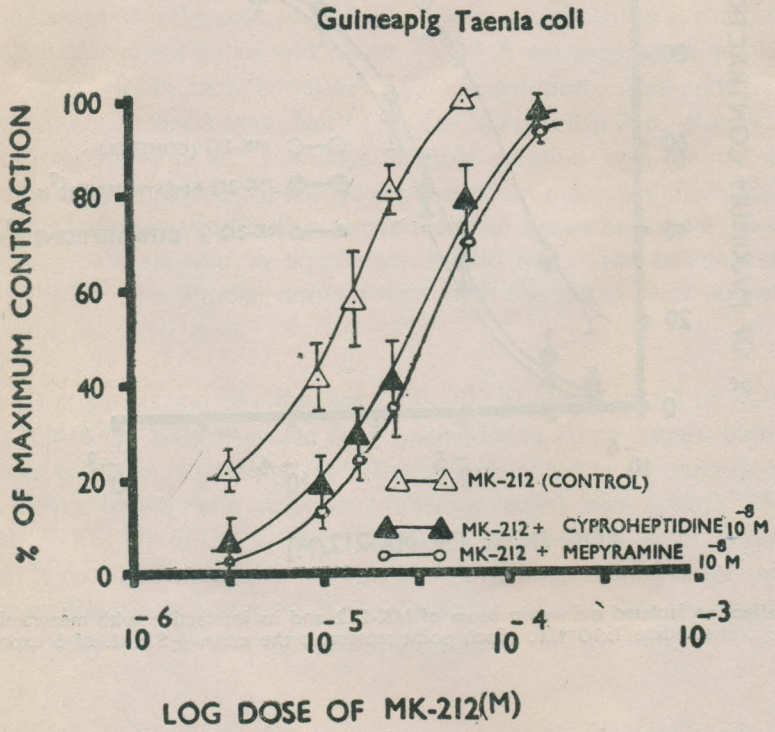

Fig. 2 : The effect on isolated guineapig taenia coli of MK-212 and its interaction with mepyramine maleate 1x10-^{8}M and cyproheptadine 1x10-^{8}M. Each point represents the mean ±SEM of 5 experiments.

ramine maleate and metiamide respectively. The effects of MK-212 were blocked by mepyramine maleate (1x10^{-8} M) on guinea pig ileum (Fig. 1) and taenia coli (Fig. 2). However, metiamide (1x10^{-6} M) failed to inhibit responses to MK-212 in guineapig ileum and taenia coli. This suggests the possible involvement of H_1 receptors in MK-212 induced responses. The failure of mepyramine maleate to inhibit responses of rat fundus strip (Fig. 3) suggest the presence of receptors other than those of H_1 type.

5-HT-like histamine has also been reported to produce contraction of various smooth muscles such as rat fundus (19), rat uterus (2), guineapig ileum (12,17) and guineapig

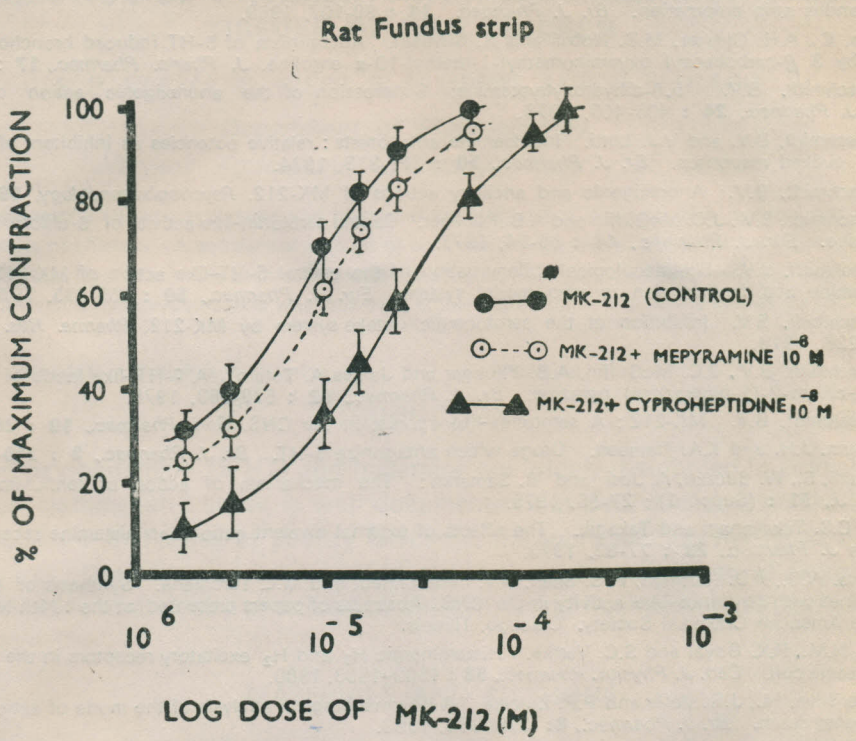

Fig. 3 : The effect on isolated rat fundus strip of MK-212 and its interaction with mepyramine maleate 1x10-6 M and cyproheptadine 1x10-8 M. Each point represents the mean ±SEM of 6 experiments.

taenia coli (14). The responses in these tissues are mediated through specific 5-HT receptors and are blocked by cyproheptadine and lysergic acid diethylamide (18). That MK-212-induced contractions in the guineapig ileum (Fig. 1), taenia coli (Fig. 2) and rat fundus strip (Fig. 3) could also be due to 5-HT-like actions of MK-212, was suggested by the observations that the effects of MK-212 were blocked by cyproheptadine (1x10-8 M) (Fig. 1, 2 3). The parallel shift produced by antagonists used indicates a competitive block. This conclusion is in accord with the reported 5-HT effects of MK-212 in the CNS (6, 7, 8). Metiamide, propranolol or atropine had no effect on the MK-212 induced contractions in guineapig ileum, taenia coli and rat fundus strip, which rules out the involvement of H_2 or adrenoceptive or cholinoceptive mechanisms respectively in MK-212-induced responses.

REFERENCES

1. Ash, A.S.F. and H.O. Schild. Receptors mediating some activities of histamine. *Br. J. Pharmac.*, **27** : 427-439, 1966.
2. Barlow, R.B. and I. Khan. Actions of some analogues of tryptamine on isolated rat uterus and on the isolated rat fundus strip preparation. *Br. J. Pharmac.* **14** : 99-107, 1959.
3. Bretta, C., A.H. Glasser, M.B. Nobili and R. Silvestri. Antagonism of 5-HT induced bronchospasm in guinea pig by 8 β-carbobenzyl oxyaminomethyl-1-methyl-10-α ergoline. *J. Pharm. Pharmac.*, **17** : 423-428, 1965.
4. Clineschmidt, B.V. 5,6-dihydroxytryptamine: Suppression of the anorexigenic action of fenfluramine. *Eur. J. Pharmac.*, **24** : 405-409, 1973.
5. Clineschmidt, B.V. and V.J. Lotti. Indoleamine antagonists : relative potencies as inhibitors of tryptamine and 5-HT evoked responses. *Br. J. Pharmac.*, **50** : 311-313, 1974.
6. Clineschmidt, B.V. Anorexigenic and ancillary actions of MK-212. *Psychopharmacology*, **55** : 27-33, 1977.
7. Clineschmidt, B.V., J.C. McGuffin and A.B. Pflueger. Central serotonin-like activity of 6-chloro-2-(1-piperazinyl) pyrazine. *Eur. J. Pharmac.*, **44** : 65-74, 1977.
8. Cineschmidt, B.V. Pharmacological differentiation of the central 5-HT–like action of MK-212, P-methoxyamphetamine and fenfluramine *in vivo* model system. *Eur. J. Pharmac.*, **50** : 369-375, 1978.
9. Clineschmidt, B.V. Inhibition of the serotonergic uptake system by MK-212. *Pharma. Res. Comm.* **10**(**3**) : 219-228, 1978.
10. Clineschmidt, B.V., J.C. McGuffin, A.B. Pflueger and James A. Totaro. A 5-HT-like mode of anorectic action for 6-chloro-2-(1-piperazinyl) pyrazine. *Br. J. Pharmac.*, **62** : 579-589, 1978.
11. Clineschmidt, B.V. MK-212 : A serotonin–like agonist in the CNS. *Gen. Pharmac.*, **10** : 287-290, 1979.
12. Gaddum, J.H. and K.A. Hameed. Drugs which antagonize 5-HT. *Br. J. Pharmac.*, **9** : 240-248, 1954.
13. Garattini, S., W. Buczko, A. Jori and R. Samanin : The mechanism of action of fenfluramine. *Post–Grad. Med. J.*, **51** : (Suppl. 1) : 27-35, 1975.
14. Liao, C.S. Takayanagi and Takagik. The effects of external divalent cations on histamine receptor interaction. *Japan J. Pharmac.*, **23** : 77-82, 1973.
15. Lumma, W.C., R.D. Hartman, W.S. Saari, B.V. Clineschmidt and M.L. Torchiana. Synthesis of 2-(1-piperazinyl) pyrazines with serotonin–like activity in the CNS. Abstracts of papers presented at the 174th National Meeting of the American Chemical Society, Chicago, Illinois.
16. Patel, N.M., R.K. Goyal and S.C. Verma. Histaminergic H_1 and H_2 excitatory receptors in the guineapig uterus and taenia coli. *Can. J. Physiol. Pharmac.*, **58** : 1500–1503, 1980.
17. Roche Silva, M., J.R. Valle and P.P. Zuleika. A pharmacological analysis of the mode of action of 5–HT upon guineapig ileum. *Br. J. Pharmac.*, **8**: 378–388, 1953.
18. Stone, C.A., H.C. Wanger, C.T. Lodden, I.M. Stavorski and C.A. Ross. Antiserotonin-antihistaminic properties of cyproheptadine. *J. Pharmac. Exp. Ther.*, **131** : 73–84, 1961.
19. Vane, J.R. A sensitive method for the assay of 5–HT. *Br. J. Pharmac.*, **12** : 344–349, 1957.