

EVIDENCE FOR CATECHOLAMINE-DEPLETING ACTION OF FLUOXETINE

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(Received on July 7, 1993)

Abstract: The present investigation was undertaken to study the interaction of fluoxetine with 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in rat anococcygeus muscle and vas-deferens. In rat anococcygeus muscle responses to NA were significantly potentiated after 30 min and 60 min incubation with fluoxetine (2.9×10^{-9} M). The responses to 5-HT were however, inhibited after 30 min incubation with fluoxetine in this preparation. On rat vas-deferens also, the responses to NA were potentiated after 30 min incubation with fluoxetine. The response to 5-HT were not altered significantly.

In rats pretreated with fluoxetine (5 mg/kg, ip) for seven days, the responses to NA were significantly potentiated in rat anococcygeus muscle. Whereas the responses to 5-HT and tyramine were significantly inhibited. The inhibited responses to 5-HT restored back to normal when the anococcygeus muscle was pre-incubated with NA for 30 min.

Our data provides an evidence for catecholamine depleting action of fluoxetine.

Key words: fluoxetine rat anococcygeus muscle rat vas-deferens

INTRODUCTION

Fluoxetine (3-(p-trifluoromethylphenoxy)-N-methyl- 3-phenyl propylamine] belongs to class of straight chain compounds and was developed as an antidepressant candidates based on its ability to inhibit selectively the neuronal uptake of serotonin (1, 2). Unlike many other antidepressant drugs fluoxetine has little affinity for muscarinic, histaminic, serotonergic 5-HT₁ and 5-HT₂ and noradrenergic alpha-1 and alpha-2 receptors on rat brain membranes (3). However, fluoxetine is associated with number of side effects like nausea, nervousness, insomnia, tremors, sweating and dry mouth. The mechanisms of which are unknown (4). Further, it was found that in patients taking fluoxetine there is a decrease in mean heart rate by some unknown mechanism (5).

The present investigation was undertaken to study

the autonomic effects of fluoxetine using rat anococcygeus muscle and rat vas-deferens.

METHODS

Healthy albino male rats of Wistar strain weighing 200-250 g were stunned by a sharp blow on the head and sacrificed by cutting the neck blood vessels. The abdominal wall was quickly opened and both vas-deferens and anococcygeus muscles were promptly isolated from the animal. Semen from each vas-deferens was removed by applying gentle pressure from one end to another. The sheath and connective tissue were removed from vas-deferens and anococcygeus muscle were then suspended in organ baths containing 20 ml Krebs' bicarbonate solution. The composition of Krebs' bicarbonate solution (PSS) in mM was NaCl, 94.01; KCl, 4.69; CaCl₂, 2.52; MgSO₄, 7H₂O, 0.46; KH₂PO₄, 1.7; NaHCO₃, 25.0 and

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glucose, 10-75. The PSS was maintained at $37 \pm 1^\circ\text{C}$ and continuously bubbled with air. The stabilisation period was 30 mins for both the tissues and during which PSS was changed at every 10 min interval.

The responses to various drugs were recorded using force displacement transducer coupled to student's physiograph (Bio device, India). The external tension exerted on rat anococcygeus muscle was 1 g and on rat vas-deferens 500 mg.

In the first set of experiments the preparations were exposed to graded doses of noradrenaline (2.96×10^{-8} M to 8.89×10^{-4} M) added in cumulative fashion. Then the preparations were exposed to fluoxetine (2.90×10^{-9} M) for either 30 min and/or 60 min. The dose response curves of NA and 5HT were then re-elicited after giving wash to the preparation.

In another set of experiments the rats were pretreated with fluoxetine (5 mg/kg, i.p.) for seven days. On the eighth day the rats were sacrificed and

anococcygeus muscle were dissected out and mounted as described earlier. The dose response curves of NA (2.96×10^{-8} M to 8.89×10^{-4} M), 5-HT (2.58×10^{-7} M to 7.74×10^{-4} M) and tyramine (7.29×10^{-8} M to 2.18×10^{-4} M) were elicited. The tissues were then incubated with NA (1.48×10^{-7} M) for 30 min and the dose response curves of 5-HT and tyramine were reelicited.

RESULTS

Noradrenaline (2.96×10^{-8} M to 8.89×10^{-4} M) and 5-hydroxytryptamine (2.96×10^{-7} M to 7.70×10^{-4} M) produced a dose dependent contraction on rat anococcygeus muscle and rat vas-deferens. The responses to noradrenaline were found to be significantly potentiated in the presence of fluoxetine after 30 min or 60 min (Fig. 1). However, the responses to 5-HT were significantly inhibited on rat anococcygeus muscle (Fig. 2). The maxima was significantly depressed by fluoxetine.

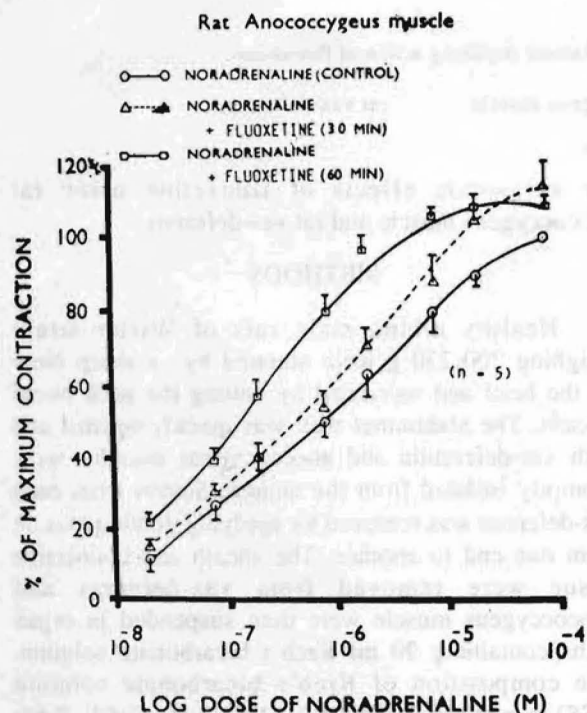


Fig. 1 : The effect of fluoxetine (2.9×10^{-9} M) on noradrenaline induced responses on rat anococcygeus muscle. Each point and bar represent the mean \pm SEM of five experiments.

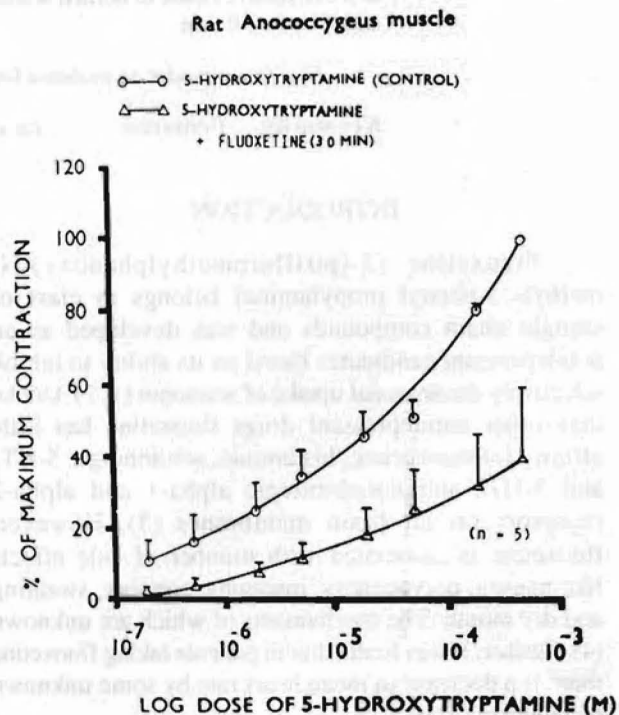


Fig. 2 : The effect of fluoxetine (2.9×10^{-9} M) on 5-hydroxytryptamine induced responses on rat anococcygeus muscle. Each point and bar represent the mean \pm SEM of five experiments.

On rat vas-deferens fluoxetine (2.9×10^{-9} M) on 30 min interaction did not alter significantly the responses to 5-HT, however, the responses to NA were slightly potentiated (Fig. 3).

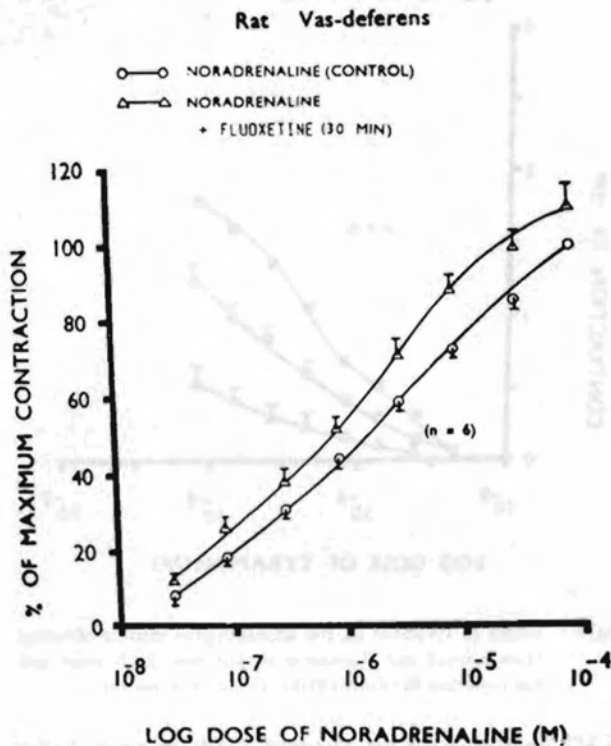


Fig. 3: The effect of fluoxetine (2.9×10^{-9} M) on noradrenaline induced responses on rat vas-deferens. Each point and bar represent mean \pm SEM of six experiments.

The NA, 5-HT and tyramine produced a dose-dependent contraction of rat anococcygeus muscle in the preparation obtained from fluoxetine treatment. The responses to NA were significantly potentiated in preparation obtained from fluoxetine pretreated rats. There was a leftward shift of the dose response curve of NA with an increase in maxima (Fig. 4). However, the responses to 5-HT (Fig. 5) and tyramine (Fig. 6) were significantly inhibited in anococcygeus muscle obtained from fluoxetine pretreated rats. These responses were partially restored back after 30 min incubation with NA (1.48×10^{-7} M).

DISCUSSION

In rat anococcygeus muscle, 5-HT induced contractile effects are mediated through an indirect

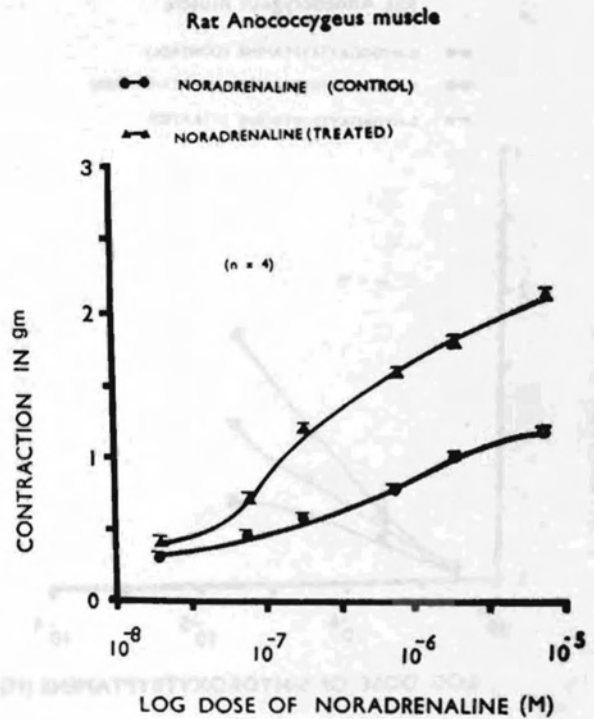


Fig. 4: Effect of noradrenaline on the anococcygeus muscle obtained from control and fluoxetine treated rats. Each point and bar represent the mean \pm SEM of four experiments.

sympathomimetic effect involving release of noradrenaline (9, 10). It was proposed that 5-HT, like tyramine first taken up by adrenergic neurone and by mole to mole displacement causes release of NA (11). Therefore, agent that blocks 5-HT uptake inhibits the responses to 5-HT and may potentiate the responses to NA.

In the present investigation, it was found that after 30 min and 60 min interaction of fluoxetine with anococcygeus muscle the responses of NA were significantly potentiated (Fig 1) and that of 5-HT were significantly inhibited (Fig. 2). Guanethidine is reported to produce reversible adrenergic neurone blockade in various smooth muscle preparations (12,13). It is also reported for guanethidine that, while it is ineffective within 10 min, it produces inhibition of noradrenaline release after 30 min. The reason for the delayed effect is quarternary ammonium group in guanethidine making the compound less diffusible. However, fluoxetine chemically does not contain any of such characteristic

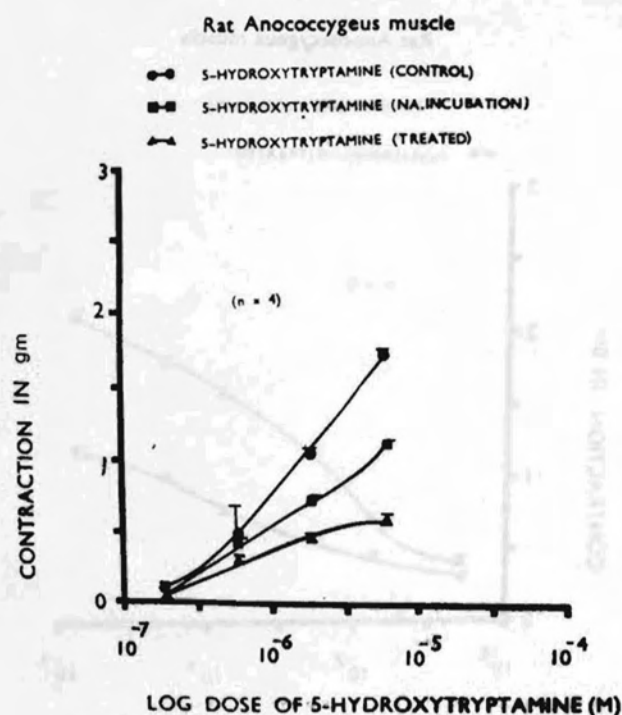


Fig. 5: Effect of 5-hydroxytryptamine on the anococcygeus muscle obtained from control and fluoxetine treated rats. Each point and bar represent the mean \pm SEM of four experiments.

that could explain delayed onset of action of fluoxetine, like guanethidine in this preparation. Another possibility is that fluoxetine cause depletion of NA like 6-hydroxydopamine (6-OHDA). 6-OHDA is reported to cause depletion of NA content from rat anococcygeus muscle (14). It is reported that 6-OHDA causes inhibition of 5-HT responses and potentiation of NA responses in rat anococcygeus muscle. Further in rat vas-deferens incubation of the preparation with fluoxetine for 30 min caused slight potentiation of the responses of NA but failed to alter 5-HT responses which can be attributed to development of supersensitivity for NA. Results of *in vivo* experiments also support this findings. In rat treated with fluoxetine for seven days, it was found that responses to 5-HT and tyramine are significantly inhibited and that of NA are significantly potentiated in rat anococcygeus muscle. This inhibition of 5-HT and tyramine induced responses can be attributed to the depletion of catecholamines from the neuronal stores of treated animals. It was also found that responses of

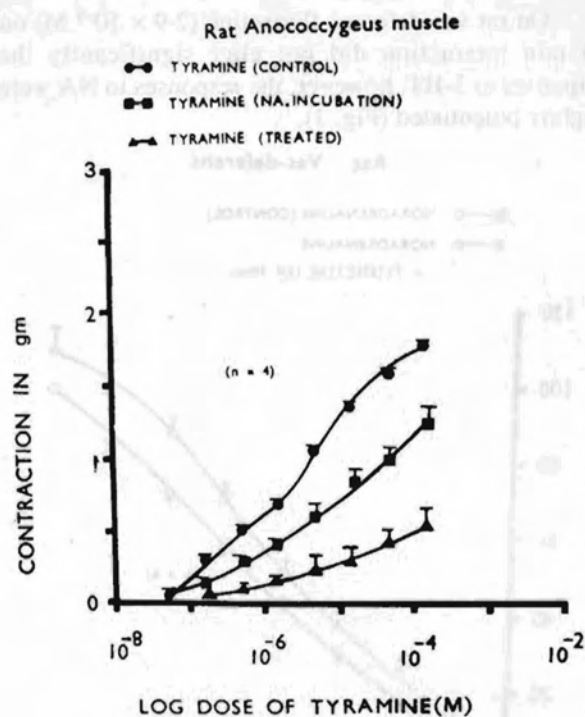


Fig. 6: Effect of tyramine on the anococcygeus muscle obtained from control and fluoxetine treated rats. Each point and bar represent the mean \pm SEM of four experiments.

5-HT and tyramine are restored nearly to normal after incubation of the preparation with NA for 30 min. This further supports the possibility of depletion of catecholamines by fluoxetine. In rat anococcygeus muscle reserpine is reported to cause an inhibition of 5-HT and tyramine reduced contractions and also cause potentiation of NA induced contractions (15). This has been explained on the basis of depletion of NA caused by reserpine. Results of the present investigation with Fluoxetine are identical to those of reserpine. Various adverse effects of reserpine such as drowsiness, nightmares, bradycardia, nausea, diarrhoea have been attributed to its catecholamine depleting action. Some of these side effects like nausea, drowsiness, insomnia and diarrhoea have been reported for fluoxetine (16). Fluoxetine is also reported to produced bradycardia in patients (5).

In conclusion we suggest that, fluoxetine causes an inhibition of 5-HT and tyramine and potentiation of

NA induced responses in rat anococcygeus muscle which might be due to catecholamine depletion as seen with

reserpine. Some of the side effects of fluoxetine might be explained on the basis of this action of fluoxetine.

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