Biomedical & Pharmacology Journal, September 2019. Vol. **12**(3), p. 1339-1343

Effect of Duloxetine Pretreatment on 5-HTP and Dexfenfluramine Induced Behaviours in Albino Rats

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http://dx.doi.org/10.13005/bpj/1762

(Received: 10 June 2019; accepted: 29 August 2019)

Activation of central brain serotonergic receptors viz 5-HT_{1A} and 5-HT_{2A} by serotonin (5-HT) induces the 5-HT behavioural syndrome in rats. 5-HTP and dexfenfluramine produce 5-HT mediated behaviours. We have carried out the experiment with the aim to study the effect of duloxetine pretreatment on 5-hydroxytryptophan and dexfenfluramine induced behaviours in albino rats. Pre-treatment with 20 mg/kg duloxetine, a SNRI was found to potentiate 75 mg/kg 5-HTP mediated behavioural syndrome. However, 5, 10 and 20 mg/kg duloxetine had decreased the intensity of the behavioral syndrome produced by 10 mg/kg dexfenfluramine significantly. Duloxetine at 5, 10 and 20 mg/kg had produced inhibition of serotonin transporter (SERT) and inhibited dexfenfluramine uptake which had significantly antagonised its behaviouralsyndrome. Duloxetine at 5 and 10 mg/kg did not affect 5-HTP induced behavioral syndrome significantlywhere as 20 mg/kg duloxetine did significantly potentiate 5-HTP induced behavioral syndrome. This indicates 20 mg/kg dose of duloxetine blocks neuronal reuptake of 5-HT by blocking SERT and effectively increase its concentration to greater level in the synaptic gap which causessynergistic stimulation of the central postsynaptic 5-HT_{1A} and 5-HT_{2A} receptors and potentiation of 5-HTP behavioral syndrome.

Keywords: Duloxetine, 5-HTP, Dexfenfluramine, Behavioural Syndrome**.**

The stimulation of central brain serotonergic receptors viz 5-HT_{1A} and 5-HT_{2A} , induces the serotonergic behaviours in rats characterized by lateral head weaving, forepaw treading, hind limb abduction, flat body posture, straub tail and wet-dog shakes[1,2,3]. This serotonergic behaviours can be induced by drugs like 5-HT precursors viztryptophan, 5-hydroxytryptophan [4,5,6], 5-HT releasers like dexfenfluramine, p-chloroamphetamine (PCA) which selectively release neuronal 5-HT[7,8,9] and 5-HT receptor agonists[10,11,12] which stimulate 5-HT_{1A} and 5-HT_{2A} receptors. This behavioural syndrome induced by 5-HT has been scored by different methods on the basis of rating scales for its components[3,11,12,13].

This studyhas investigated the behavioural effects of 5-HTP and dexfenfluramine in rats pretreated with different doses of duloxetine and scored by scoring method of Sloviteret al[3].

MATERIALS AND METHODS

Animals

Male and female albino rats weighing 100-200 gm were used for experiments. Animals used for the experiments were bred in Central

Animal House of the Institute. The animals were kept under standard conditions, maintained on a 12 hr light/dark cycle and theywere given food and water ad libitum till the time of experimentation. The rats were brought to the experimental area at least 1 hr before the experimentation. Experimental design consisted of 8 groups of fresh rats (n = 6 in each group). All observations were made blind with respect to the treatments used. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee and carried according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Drugs

Duloxetine (Macleod), dexfenfluramine hydrochloride (Wockhardt), 5 - hydroxytryptophan (Sigma) were used in pure powder form. Fresh drug solutions were prepared by dissolving drug in distilled water (DW). Drugs were injected intraperitoneally and injection volume was 2 ml/kg body weight. Doseselection was done on the basis of literature and past studies carried out in our department.

Effect of Duloxetine pretreatment on behavioral syndrome induced by 5-HTP and Dexfenfluramine (DEX) in Rats

Animals were placed separately in perspex cage. They were individually observed as per the method of Sloviteret al[3]for 1 min, once after every 5 min, from 10 to 125 min timing after injection of 5-HTP and DEX for 20 scoring periods. Serotonergicbehavioursobserved were head and whole body shakes, reciprocal forepaw treading, lateral head weaving, flat body posture and hind limb abduction. Animals were scored separately (0 or 1) in each of the twenty intervals for 1 min observation period. Total score of each animal in the group for every scoring period was taken and the mean value of the group calculated. Control group (DW 2 ml/kg i.p.) and test group (duloxetine 5,10,20 mg/kg) received the drug one hour before receiving 5-HTP/DEX.

Effect of Duloxetine pretreatment on 5-Hydroxytryptophan Induced Behaviour in Rats

Groups	Treatment usedi.p mg/kg
1.	DW (2 ml/kg) + 5-HTP 75
2.	Duloxetine 5+ 5-HTP 75
3.	Duloxetine 10 + 5-HTP 75
4.	Duloxetine 20 + 5-HTP 75

Effect of Duloxetine pretreatment on Dexfenfluramine Induced Behaviour in Rats

Groups	Treatment usedi.p mg/kg
1.	DW (2 ml/kg) + Dexfenfluramine 10
2.	Duloxetine 5 + Dexfenfluramine 10
3.	Duloxetine 10+ Dexfenfluramine 10
4.	Duloxetine 20 + Dexfenfluramine 10

Data Analysis

Data was analysedby using Graph pad instat software. Non-parametricANOVA, Kruskal Wallis test followed by post hoc Dunn's multiple comparison test was used . $p< 0.05$ was considered statistically significant.

Effect of Duloxetine pretreatment on 5-HTP induced Behavioral Syndrome in Rats

As per table 1, 5-HTP treated control group rats showed the $5HT_{1A}$ and $5HT_{2A}$ receptor mediated behavioral syndrome. Pretreatment with 20 mg/kg duloxetine potentiated the behavioral syndrome intensity induced by 5-HTP (75 mg/kg) which is statistically significant. Pretreatment with 5 & 10 mg/kg did not influence behavioural syndrome inducedintensity by 5-HTP (75 mg/kg).

Effect of Duloxetine pretreatment on Dexfenfluramine induced Behavioral Syndrome in Rats

As per table 2, pretreatment with all doses ofduloxetine(5,10,20 mg/kg) used in study showed statistically significant decrease in intensity of the behavioral syndrome induced by 10 mg/kg DEX.

DISCUSSION

In the serotonergic neurons 5-HTP enters into neurons via the amino acid transport system which is different from the 5-HT uptake carrier protein transport system SERT, responsible for the neuronal uptake of 5-HT and drugs like parachloramphetamine (PCA), dexfenfluramine

Table 1. Effect of Duloxetine pretreatment on 5-HTP Induced Behaviour in Rats

Group Testing Time Interval in (min)	Control+5 – HTP75 Mean ± SD	Study Groups (mg/kg) DUL5+ 5-HTP75 Mean ± SD	DUL10+ 5-HTP75 Mean ± SD	DUL20+ 5-HTP75 Mean ± SD	KW Value	P Value
10	2.33 ± 0.51	2.16 ± 0.40	1.66 ± 0.81	3.33 ± 0.51	19.363	0.0002
16	2.83 ± 0.40	3 ± 0 (3)	2.16 ± 0.40	4 ± 0 *	19.806	0.0002
22	3.16 ± 0.40	2.66 ± 0.51	2.66 ± 0.51	4 ± 0	16.211	0.0010
28	3.33 ± 0.51	2.66 ± 0.51	2.83 ± 0.40	4 ± 0	15.889	0.0012
34	3.33 ± 0.51	2.83 ± 0.40	3.5 ± 0.54	4.16 ± 0.40	12.968	0.0047
40	3.16 ± 0.40	2.66 ± 0.51	3.66 ± 0.51	4.5 ± 0.54*	16.132	0.0011
46	3 ± 0	2.83 ± 0.40	3.83 ± 0.40	4.5 ±0.54**	19.085	0.0003
52	3 ± 0	2.83 ± 0.40	3.83 ± 0.40	4.5 ±0.54**	19.085	0.0003
58	2.83 ± 0.40	2.66 ± 0.51	3.66 ± 0.51	4.66 ± 0.51**	17.659	0.0005
64	3 ± 0	2.5 ± 0.54	3.66 ± 0.51	4.5 ± 0.54*	18.003	0.0004
70	3 ± 0	2.16 ± 0.40	3.66 ± 0.51	4.83 ± 0.40*	20.244	0.0002
76	3 ± 0	2 ± 0	3.66 ± 0.51	4.66 ± 0.51	20.878	0.0001
82	2.66 ± 0.51	2 ± 0	3.66 ± 0.51	4.5 ± 0.54*	19.548	0.0002
88	2.66 ± 0.51	1.83 ± 0.40	3.66 ± 0.51	4 ± 0*	19.512	0.0002
94	3 ± 0	2 ± 0	3.33 ± 0.81	4 ± 0	18.062	0.0004
100	3 ± 0	2 ± 0	3.16 ± 0.75	4 ± 0	18.603	0.0003
106	2.66 ± 0.51	1.83 ± 0.40	3 ± 0.89	4.16 ± 0.40	16.273	0.0010
112	2.66 ± 0.51	1 ± 0	2.33 ± 0.81	4 ± 0	19.970	0.0002
118	3 ± 0	0.5 ± 0.54*	2 ± 0.63	4 ± 0	21.647	< 0.0001
124	3 ± 0	0.33 ± 0.51**	1.5 ± 0.54	3.33 ± 0.51	20.831	0.0001

Table 2. Effect of Duloxetine pretreatment on DEX Induced Behaviour in Rat

Group Testing Time Interval in (min)	Control+ DEX10 Mean ± SD	Study Groups(mg/kg) DUL5+ DEX10 Mean ± SD	DUL10+ DEX10 Mean ± SD	DUL20+ DEX10 Mean ± SD	KWValue	P Value
10	1.33 ± 0.51	1 ± 0	0.66 ± 0.81	0 ± 0 (0) **	14.092	0.0028
16	2.33 ± 0.51	1.5 ± 0.54	1 ± 0.89	0.16 ± 0.40***	15.152	0.0017
22	2.66 ± 0.81	1.5 ± 0.54	1.16 ± 0.75	0.16 ± 0.40***	16.304	0.0010
28	2.66 ± 0.81	1.33 ± 0.51	1.16 ± 0.75	0.66 ± 0.51	13.814	0.0032
34	3.16 ± 0.40	1 ± 0 *	1.33 ± 0.51	0.16 ± 0.40	20.357	0.0001
40	3 ± 0.63	1 ± 0	0.83 ± 0.75*	0.16 ± 0.40***	17.756	0.0005
46	3 ± 0.63	1.5 ± 0.54	0.83 ± 0.75*	0.16 ± 0.40***	17.779	0.0005
52	3 ± 0.63	1 ± 0	0.5 ± 0.54**	0.33 ± 0.51 ***	17.473	0.0006
58	3 ± 0.63	0.66 ± 0.51	0.33 ± 0.51**	0.33 ± 0.51 **	15.416	0.0015
64	3 ± 0.63	0.66 ± 0.51	0.33 ± 0.51**	0.33 ± 0.51**	15.416	0.0015
70	3 ± 0.63	0.66 ± 0.51*	0.33 ± 0.51**	0.5 ± 0.54*	15.146	0.0017
76	3 ± 0.63	0.5 ± 0.54 *	0.5 ± 0.54*	0.5 ± 0.54 *	14.540	0.0023
82	2.83 ± 0.75	0.66 ± 0.81*	0.33 ± 0.51**	0.33 ± 0.51 **	14.520	0.0023
88	2.83 ± 0.75	0.16 ± 0.40**	0.5 ± 0.54*	0.33 ± 0.51 **	15.729	0.0013
94	2.83 ± 0.40	0.16 ± 0.40**	0.33 ± 0.51 *	0.33 ± 0.51 *	15.940	0.0012
100	2.5 ± 0.54	0.16 ± 0.40**	0.33 ± 0.51*	0.33 ± 0.51 *	15.840	0.0012
106	2.83 ± 0.40	0 ± 0***	0.33 ± 0.51*	0.16 ± 0.40**	18.094	0.0004
112	3 ± 0	0.16 ± 0.40**	0.33 ± 0.51 *	0.16 ± 0.40**	16.790	0.0008
118	2.5 ± 0.54	0 ± 0 ***	0.33 ± 0.51 *	0.33 ± 0.51*	17.222	0.0006
124	2.5 ± 0.54	0 ± 0***	0.33 ± 0.51*	0.16 ± 0.40 **	17.969	0.0004

and 3,4-dioxymethylene-methamphetamine (MDMA)[14,15].5-HTP is decarboxylated in the cytoplasm to 5-HT by the 5-HTP decarboxylase enzyme in the neurons. Once the storing or metabolising capacity of the presynaptic neuron exceeds, then the newly synthesised5-HT from 5-HTP spills over into the synaptic cleft.The 5-HT which spills over into the synaptic cleft stimulates the central postsynaptic $5HT_{1A}$ and $5HT_{2A}$ receptors and induces the behavioral syndrome.

There was no statistically significant effect of duloxetine at doses 5 and 10 mg/kg on 75 mg/kg 5-HTP induced behavioral syndromewhere as 20 mg/kg duloxetine did significantly potentiate 5-HTP induced behavioral syndrome. This indicates 20 mg/kg dose of duloxetine inhibits neuronal reuptake of spilt 5-HT effectively and increases 5-HT concentration to a greater extent in the synapse with greater stimulation of the central postsynaptic $5HT_{1A}$ and $5HT_{2A}$ receptors and potentiation of 5-HTP behavioral syndrome.

Dexfenfluramine is taken up by the 5-HT uptake carrier protein transport system SERT, which is responsible for the uptake of 5-HT into serotonergic neurons. Dexfenfluramine after gaining entry into the serotonergic neurons, causes brisk release of 5-HT from serotonergic neurons with resultant stimulation of central postsynaptic serotonergic receptors viz $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A.}$ This released 5-HT produces behavioural syndrome in rats[7,9,14,15]. Further all doses of duloxetinehad significantly antagonised the intensity of 10 mg/kg dexfenfluramine induced behavioral syndrome. This indicates that these doses of duloxetine exert 5-HT reuptake (SERT) inhibiting activity which is responsible for inhibition of uptake of dexfenfluramine into serotonergic neurons. Less amount of dexfenfluramine enters into serotonergic neurons after pretreatment with 5-HT reuptake inhibiting doses of duloxetine. This results in less release of 5-HT from serotonergic neurons with resultant decrease in the intensity of dexfenfluramine induced behavioural syndrome. Based on the above results, all doses of duloxetine used in the studyeffectively exert neuronal reuptake blocking activity in a dose dependent manner.

CONCLUSIONS

Duloxetine at all doses (5, 10, 20 mg/kg) appear to inhibit uptake of dexfenfluramine into neurons. Thesedoses of duloxetine significantly decreased the intensity of dexfenfluramine induced behavioral syndrome by exerting SERT inhibiting effect.Only 20 mg/kg dose of duloxetine inhibited the neuronal reuptake of spilt 5-HT significantly by inhibiting SERT. This causes an increase in 5-HT concentration to greater level in the synaptic gap with resultant potentiation of behavioral syndrome due to 5-HTP.

ACKNOWLEDGEMENT

Authors acknowledge to Macleods Pharmaceuticals Ltd, Mumbai for providing drug samples of Duloxetine and Dr. S. V. Kakade, Associate Professor, Department of Preventive and Social Medicine, Krishna Institute of Medical Sciences, Karad for his valuable support in the statistical analysis.

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