Effect of Alpha-Methyl-P-Tyrosine Pretreatment on Stereotyped Behaviour Induced by Lamotrigine, Apomorphine, Dexamphetamine in Rats

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Research Article

Abstract: Alpha-methyl-p-tyrosine by inhibing tyrosine hydroxylase, decreases the synthesis of dopamine (DA) in the nigrostriatal DAergic neurons so less DA is available for release from these neurons. Lamotrigine(LTG), Dexamphetamine(DAM) and Apomorphine (APO) produce SB directly by stimulating DA receptors or indirectly by releasing DA from nigrostriatal DAergic neurons .Our aim is to study the effect of pretreatment of alpha methyl P tyrosine(AMT) on SB induced by LTG,DAM and APO in rats. Albino rats of either sex (120-180 g) were used by random distribution in group of 10 animals each. Intensity of SB is assessed by Costall and Naylor scoring system. Our results indicate that pretreatment with 100 mg/kg AMT significantly antagonised the SB induced by 5, 10, 20 and 40 mg/kg lamotrigine. Pretreatment with 200 mg/kg AMT abolished the SB induced by 5 mg/kg lamotrigine and significantly antagonised the SB induced by 10, 20 and 40 mg/kg lamotrigine. Pretreatment with 100 and 200 mg/kg AMT significantly antagonised the SB induced by 5 and 10 mg/kg dexamphetamine. Pretreatment with 100 and 200 mg/kg AMT did not significantly influence the intensity of SB induced by 1.5 and 3 mg/kg apomorphine.

Keywords: Alpha-methyl-P-tyrosine, Apomorphine, Dexamphetamine, Dopamine, Lamotrigine, Stereotyped Behaviour.

Introduction

The corpus striatum and the substantia nigra pars compacta (SNc) receive glutamatergic innervation from the cerebral cortex via the corticostriatal and corticonigral projections respectively [1]. In vitro and in vivo biochemical studies have shown that glutamate, via activation of the N-methyl-D-aspartate (NMDA) type of glutamate receptors, regulates the synthesis and release of dopamine (DA) from the nigrostrital DAergic neurons [2]-[4]. Lesions of the frontal cortex, leading to a decrease in striatal glutamate levels [5], were reported to enhance behavioural responses to amphetamine [6]-[7].Studies have demonstrated that NMDA receptor antagonist MK-801 [8], blocks NMDA receptors in the striatum and SNc and causes activation of the nigrostriatal DAergic neurons which increase in the synthesis and release of DA in the striatum [1]-[3]. Elevated DA levels lead to hyperfunctioning of the nigrostriatal DAergic system in the rat producing stereotyped behaviour (SB). The SB manifests as sniffing behaviour and of the oral movement variety (OMV) characterised by gnawing, biting and licking behaviour. High doses of the directly acting DA agonist apomorphine induce SB by directly stimulating the postsynaptic striatal D2 and D1 DA receptors. Indirectly acting DA agonists like amphetamines, in high doses, induces SB by releasing DA from the nigrostriatal DAergic neurons with resultant activation of the postsynaptic striatal D2 and D1 DA receptors by the released DA [9]. According to our studies Lamotrigine produces SB indirectly by releasing dopamine from nigrostriatal DAergic neurons. Alpha-methyl-p-tyrosine inhibits tyrosine hydroxylase enzyme and inhibits the synthesis of DA [10].Thus it depletes the stores of DA in the nigrostriatal DAergic neurons and makes less DA available for release. Since lamotrigine high dose apomorphine, dexamphetamine produce SB in rats, we planned this study with following objective:

- To study the effect of pretreatment of Alpha-methyl-p-tyrosine on stereotyped behavior induced by those drugs.

Materials and Methods

Animals

Albino rats of either sex (weighing 100-180 g), bred in Central Animal House Facility of the Institute, were used. The animals were maintained on a 12 hr light/dark cycle and had free access to food and water up to the time of experimentation. Atleast 1 hr before the experiments the animals were brought to the laboratory for acclimatization.. Each group consisted of 10 animals. Each animal was used only once. All observations were made between 10 and 17 hrs at 27°-30°C. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the

Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs

Drugs used were lamotrigine (Kopalle Ltd.), dexamphetamine sulphate (Koch-Light), apomorphine hydrochloride (Sigma), and alpha-methyl-p-tyrosine methyl ester hydrochloride (Sigma). Lamotrigine was dissolved in 2% solution of Tween 80 in distilled water. Dexamphetamine and alpha-methyl-p-tyrosine methyl ester hydrochloride were dissolved in distilled water. While apomorphine was dissolved in distilled water containing 0.2 mg/ml ascorbic acid. All drug solutions were prepared immediately before use and were injected intraperitoneally. The volume of injection was 5 ml/kg body weight for 2.5 to 40 mg/kg dose range of lamotrigine and alpha methyl P tyrosine. The volume of injection is 10 ml/kg body weight for 80 and 160 mg/kg doses of lamotrigine, while for the remaining drugs it was 2 ml/kg body weight. Doses refer to the forms mentioned. Drug doses, routes of administration and the testing time intervals were selected based on previous studies conducted in our laboratory and those reported in literature.

Observation of SB

For observation of SB, the rats were placed in individual cages made of wire netting, measuring 30×20×20 cm, 30 min before drug treatment to allow adaptation to the new environment. The intensity of SB was assessed over a 30 sec observation period at 10 min intervals throughout its duration, using the scoring system of Costall and Naylor [11], where periodic sniffing=score1, continuous sniffing=2, periodic biting, gnawing or licking =3, continuous biting, gnawing or licking =4. The maximum intensity of SB scored by each rat in the group was taken to compute the mean value of the group.

Statistical Analysis The results were statistically analysed by the Student's unpaired t-test with differences considered significant at $P< 0.05$.

Observations and Results

Comparison of the intensity of SB induced by lamotrigine, apomorphine and dexamphetamine in rats

The results are given in Table 1.

The intensity of SB induced by 5 and 10 mg/kg lamotrigine was significantly lower than the intensity of SB induced by 1.5 and 3 mg/kg apomorphine and that induced by 5 and 10 mg/kg dexamphetamine ($P<0.001$). The intensity of SB induced by 20 mg/kg lamotrigine was significantly lower than the intensity of SB induced by 1.5 mg/kg apomorphine and 5 mg/kg dexamphetamine ($P<0.05$) and that induced by 3 mg/kg apomorphine and 10 mg/kg dexamphetamine ($P<0.001$). There was no significant difference between the intensity of SB induced by 40 mg/kg lamotrigine, 1.5 mg/kg apomorphine and 5 mg/kg dexamphetamine ($P>0.05$). However, the intensity of SB induced by 40 mg/kg lamotrigine was significantly lower than that induced by 3 mg/kg apomorphine and 10 mg/kg dexamphetamine ($P< 0.01$).

Table 1: Comparison of the intensity of SB induced by lamotrigine (LTG), apomorphine (APO) and dexamphetamine (DAM) in rats

Treatment (dose mg/kg ip)	Intensity Score (Mean ± S.E.M.)
LTG 5	1.2 ± 0.13[a]
LTG 10	1.7 ± 0.15[a]
LTG 20	2.3 ± 0.15[b,c]
LTG 40	2.8 ± 0.13[d,e]
APO 1.5	2.9 ± 0.10
APO 3	3.8 ± 0.13
DAM 5	2.9 ± 0.10
DAM 10	3.7 ± 0.15

[a]$P< 0.001$ as compared to groups receiving 1.5 and 3 mg/kg APO and 5 and 10 mg/kg DAM.

[b]$P< 0.05$ as compared to groups receiving 1.5 mg/kg APO and 5 mg/kg DAM.

[c]$P<0.001$ ascompared to groups receiving 3mg/kg APO and 10 mg/kg DAM.

[d]$P< 0.01$ as compared to groups receiving 3 mg/kg APO and 10 mg/kg DAM.

[e]There was no significant difference between groups receiving 40 mg/kg LTG, 1.5 mg/kg APO and 5 mg/kg DAM.

Effect of alpha-methyl-p-tyrosine pretreatment on lamotrigine induced SB in rats

The results are given in Table 2.

Alpha-methyl-p-tyrosine 100 and 200 mg/kg did not induce SB in rats in any of the studies it was used. Lamotrigine (5 to 40 mg/kg) induced dose-dependent SB in rats. Pretreatment with 100 mg/kg alpha-methyl-p-tyrosine significantly antagonised the SB induced by 5, 10, 20 and 40 mg/kg lamotrigine. Pretreatment with 200 mg/kg alpha-methyl-p-tyrosine abolished the SB induced by 5 mg/kg lamotrigine and significantly antagonised the SB induced by 10, 20 and 40 mg/kg lamotrigine.

Table 2: Effect of alpha-methyl-p-tyrosine (AMT) pretreatment on lamotrigine (LTG) induced SB in rats

	Treatment (dose mg/kg ip)	Intensity Score (Mean ± S.E.M.)
A	1. DW + LTG 5	1.2 ± 0.13
	2. AMT 100 + LTG 5	0.6 ± 0.16*
	3. AMT 200 + LTG 5	0.0
B	1. DW + LTG 10	1.8 ± 0.13
	2. AMT 100 + LTG 10	1.2 ± 0.13*
	3. AMT 200 + LTG 10	0.5 ± 0.16**
C	1. DW + LTG 20	2.4 ± 0.16
	2. AMT 100 + LTG 20	1.8 ± 0.13*
	3. AMT 200 + LTG 20	1.1 ± 0.10**
D	1. DW + LTG 40	2.9 ± 0.10
	2. AMT 100 + LTG 40	2.3 ± 0.15*
	3. AMT 200 + LTG 40	1.6 ± 0.16**

*P<0.05; **P<0.001 as compared to respective distilled water pretreated control lamotrigine group by Student's unpaired t-test.
DW= Distilled Water (5 ml/kg ip).

Effect of alpha-methyl-p-tyrosine pretreatment on SB induced by high doses of apomorphine in rats

The results are given in Table 3.

Apomorphine (1.5 and 3 mg/kg) induced dose-dependent SB in rats. Pretreatment with 100 and 200 mg/kg alpha-methyl-p-tyrosine did not significantly influence the intensity of SB induced by 1.5 and 3 mg/kg apomorphine.

Table 3: Effect of alpha-methyl-p-tyrosine (AMT) pretreatment on SB induced by high doses of apomorphine (APO) in rats

	Treatment (dose mg/kg ip)	Intensity Score (Mean ± S.E.M.)
A	1. DW + APO 1.5	3.0± 0.00
	2. AMT 100 + APO 1.5	2.9± 0.10
	3. AMT 200 + APO 1.5	2.8± 0.13
B	1. DW + APO 3	3.9± 0.10
	2. AMT 100 + APO 3	3.7± 0.15
	3. AMT 200 + APO 3	3.8 ± 0.13

DW = Distilled Water (5 ml/kg ip)

Effect of alpha-methyl-p-tyrosine pretreatment on dexamphetamine induced SB in rats

The results are given in Table 4.

Dexamphetamine (5 and 10 mg/kg) induced dose-dependent SB in rats. Pretreatment with 100 and 200 mg/kg alpha-methyl-p-tyrosine significantly antagonised the SB induced by 5 and 10 mg/kg dexamphetamine.

Table 4: Effect of alpha-methyl-p-tyrosine (AMT) pretreatment on dexamphetamine (DAM) induced SB in rats

	Treatment (dose mg/kg ip)	Intensity Score (Mean ± S.E.M.)
A	1. DW + DAM 5	3.0 ± 0.00
	2. AMT 100 + DAM 5	2.4 ± 0.16*
	3. AMT 200 + DAM 5	1.7 ± 0.15**
B	1. DW + DAM 10	3.7 ± 0.15
	2. AMT 100 + DAM 10	3.1 ± 0.10*
	3. AMT 200 + DAM 10	2.5 ± 0.16**

*P<0.05; **P<0.001 as compared to respective distilled water pretreated control dexamphetamine group by Student's unpaired t-test.
DW = Distilled Water (5 ml/kg ip)

Discussion

In the present study we studied the effect of pretreatment of alpha methyl P tyrosine on SB induced by Lamotrigine, Dexamphetamine and high dose apomorphine. Our results indicate that pretreatment with 100 mg/kg AMT significantly antagonised the SB induced by 5, 10, 20 and 40 mg/kg lamotrigine. Pretreatment with 200 mg/kg AMT abolished the SB induced by 5 mg/kg lamotrigine and significantly antagonised the SB induced by 10, 20 and 40 mg/kg lamotrigine. Pretreatment with 100 and 200 mg/kg AMT significantly antagonised the SB induced by 5 and 10 mg/kg dexamphetamine. Pretreatment with 100 and 200 mg/kg AMT did not significantly influence the intensity of SB induced by 1.5 and 3 mg/kg apomorphine.This indicates that pretreatment with alpha-methyl-p-tyrosine antagonised the SB induced by lamotrigine and dexamphetamine but did not antagonise the SB induced by high doses (1.5 and 3 mg/kg) of apomorphine. Alpha-methyl-p-tyrosine by inhibiting tyrosine hydroxylase, decreases the synthesis of DA in the nigrostriatal DAergic neurons. Consequently the stores of DA in the nigrostriatal DAergic neurons are depleted and therefore less DA is available for release from the nigrostriatal DAergic neurons.[10]. As pretreatment with alpha-methyl-p-tyrosine antagonised lamotrigine induced SB it indicates that lamotrigine induces the SB indirectly by releasing DA from the nigrostriatal DAergic neurons with resultant stimulation of the postsynaptic striatal D2 and D1 DA receptors by the released DA,it supports our hypothesis regarding its action.

References

1. Carlsson M, Carlsson A. "Interactions between glutamatergic and monoaminergic systems within the basal ganglia - implications for schizophrenia and Parkinson's disease." Trends Neurosci, Vol 13, 272-276, 1990.
2. Cheramy A, Romo R, Godeheu G, Baruch P, Glowinski J. "In vivo presynaptic control of dopamine release in the cat caudate nucleus-II. Facilitatory or inhibitory influence of l-glutamate". Neuroscience Vol 19,1081-1090, 1986.
3. Imperato A, Scrocco MG, Bacchi S, Angelucci L. "NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus." Eur J Pharmacol Vol 18, 555-556,1990.
4. Krebs MO, Desce JM, Kemel ML, Gauchy C, Godeheu G, Cheramy A et al. "Glutamatergic control of dopamine release in the rat striatum: evidence for presynaptic N-methyl-D-aspartate receptors on dopaminergic nerve terminals." J Neurochem Vol 56 , 81-85,1991.
5. Kim JS, Hassler R, Haug P, Paik KS. "Effect of frontal cortex ablation on striatal glutamic acid level in the rat." Brain Res Vol 132 , 370-374,1977.
6. Iversen SD, Wilkinson S, Simpson B. "Enhanced amphetamine responses after frontal cortex lesions in the rat." Eur J Pharmacol Vol 13, 387-390,1971.
7. Ketter TA, Manji HK, Post RM. "Potential mechanisms of action of lamotrigine in the treatment of bipolar disorders." J Clin Psychopharmacol Vol 23 , 484-95 , 2003.
8. Dingledine R, Borges K, Bowie D, Traynelis SF. "The glutamate receptor ion channels." Pharmacol Rev Vol 51, 7-61, 1999.
9. Seiden LS, Sabol KE, Ricaurte GA. "Amphetamine: effects on catecholamine systems and behaviour." Annu Rev Pharmacol Toxicol Vol 33, 639-77,1993.
10. Spector S, Sjoerdsma A, Udenfriend S. Blockade of endogenous norepinephrine synthesis by alpha-methyl-p-

tyrosine, an inhibitor of tyrosine hydroxylase. J. Pharmacol Exp Ther 1965; 147: 86-95.

11. Costall B, Naylor RJ. "Mesolimbic involvement with behavioural effects indicating antipsychotic activity". Eur J Pharmacol Vol 27, 46-58,1974.