Attenuation of pentylenetetrazol kindling of epileptogenesis in rats by dopaminergic agents

Vijay Lakshmi MEHTA, Sarita BHALLA, Zafar Ahmed WAFAI (*Neuropharmacology Laboratory, Department of Pharmacology, Maulana Azad Medical College, New Delhi 110002, India*)

ABSTRACT Kindling model of seizure development was induced in the rats by chronic administration of pentylenetetrazol. On chronic administration of dopamine receptor agonists such as *l*-dopa, amphetamine and amantadine, the average convulsive score with pentylenetetrazol was attenuated in biphasic manner. Initial protection from convulsions changed to higher convulsive scores.

Chronic administration of dopamine receptor blocking agent such as haloperidol, delayed the development of kindled seizures in contrast to control rats. Metoclopramide, another dopaminergic receptor blocking agent did not attenuate the kindled seizures.

These observations lend support to the hypothesis that dopamine plays a role in seizure suppression.

KEY WORDS epilepsy; convulsions; pentylenetetrazol; levodopa; amphetamine; amantadine; haloperidol; metoclopramide

Kindling, an animal model of epilepsy, refers to the phenomenon whereby repeated administration of an initially subconvulsive stimulus eventually results in generalized motor seizures. Goddard coined the term 'kindling', because of the analogy to lighting a fire and recognized its potential as a model of human epileptogenesis[1]. A general hypothesis that kindling gradually induces a state of inadequate neural inhibition that participates in the development of generalized seizures[2], may serve as a model for pharmacological anticonvulsant testing.

Kindling can be induced by electrical stimulation or by repeated administration of sub-convulsant doses of various stimulant drugs including pentylenetetrazol[3].

Received 1986 Oct 22 Accepted 1987 Oct 26

Inhibitory amino acids, GABA and taurine, play a minor role in the pathogenesis of pentylenetetrazol-induced kindling. Catecholamines are believed to be inhibitory to the development of seizures, including those due to amygdaloid kindling. This is suggested by the observation that depletion of catecholamines by reserpine and 6-hydroxydopamine facilitated the development of kindled seizure[2,4] and also long-lasting depletion of catecholamine[5], dopamine and its increased turnover has been reported in the brain of kindled rats[6].

Recent evidence has focussed attention on the role of alterations in receptor sensitivity in kindled seizure development[7] and evidence for the possible involvement of altered dopamine receptors in amygdaloid kindling has been provided[8]. Since relatively little is known about the influence of pentylenetetrazol-induced kindling of epileptogenesis on dopaminergic mechanisms of the brain, we studied the effect of various dopaminergic agonists and antagonists on pentylenetetrazol-induced kindling model of epilepsy.

METHODS

56 adult ♂ albino rats kept at a constant weight (156 ± 22 g) throughout the study by partial reduction of food intake, water was made available *ad lib*, were selected for the study. All rats were allowed to acclimate to the laboratory environment for at least 24-28 h prior to the experiments.

Effect of dopaminergic agents on pentylenetetrazol kindling 32 rats were taken and the effect of dopaminergic agents was seen on pentylenetetrazol kindling. The

influence of dopaminergic agonists, *l*-dopa (50 mg/kg), *d*-amphetamine (1 mg/kg) and amantadine (10 mg/kg) on kindling induced by chronic pentylenetetrazol (25 mg/kg sc) administration every 4 d for a total of 21 treatments was seen and then the rats (8 in each group)were maintained drug free for 3 wk, followed by retest with pentylenetetrazol (22.5 mg/kg) and the convulsive score each time was recorded in a 10-min period following sc pentylenetetrazol. The criterion for scoring[3] was: 0) no response; 1) ear and facial twitching; 2) 0-50 myoclonic jerks; 3) 50-100 myoclonic jerks; 4) full clonic convulsion.

The test drugs and normal saline were injected (ip) 1 h before sc pentylenetetrazol. All drugs were injected ip in 10 ml/kg. *l*-Dopa was dissolved in dilute HCl, *d*-amphetamine and amantadine were prepared in 0.9% saline and haloperidol was prepared daily in tartaric acid 0.1 mol/L and adjusted to pH 6.5 with NaOH. Pentylenetetrazol and metoclopramide were used as official solution of Bengal Immunity Co Ltd and IPCA Laboratories Pvt Ltd, respectively.

Influence of pretreatment with haloperidol and metoclopramide on pentylenetetrazol kindling Twenty-four rats were selected and the effect of haloperidol and metoclopramide pretreatment on pentylenetetrazol-induced kindling was seen. The rats were divided into 3 groups of 8 each receiving daily treatment for 18 d. Group A received haloperidol 5 mg/kg, group B was given metoclopramide 1 mg/kg, while group C (control) received saline 1 ml/kg. Pentylenetetrazol (25 mg/kg) was injected every d 4, 50 min after haloperidol/metoclopramide/saline injection, and response parameters were recorded.

RESULTS

The figures in this study provide the data observed in rats after chronic treatment with the dopaminergic agonists *l*-dopa, *d*-amphetamine and amantadine (Tab 1) and dopaminergic receptor blocking agents haloperidol and metoclopramide (Tab 2).

Table 1 shows the average convulsive score obtained by administering dopaminergic agonists on pentylenetetrazol kindled rats and were compared with control groups treated with pentylenetetrazol alone. On chronic administration of *l*-dopa, the average convulsive score was attenuated in a biphasic manner. After the first 8 injections of pentylenetetrazol, rats were found to be protected when *l*-dopa was injected but as the therapy with *l*-dopa was continued and number of days of treatment increased the protective response went on declining and then the average convulsive score began to mount up. In the last few treatments with *l*-dopa the average convulsive score was much more than in control rats and on the 21 st treatment the average convulsive score was much higher than control ($p<0.01$).

With amphetamine on chronic administration, the response was like with *l*-dopa. Initially, amphetamine decreased the average convulsive score but as the treatment with amphetamine continued, there was an increase in convulsive score as compared to the control rats.

Amantadine showed a uniform pattern of average convulsive activity. From the very first day of therapy it enhanced the average convulsive score when compared with the rats which were treated with pentylenetetrazol and saline.

Retest with pentylenetetrazol after 3 wk of drug-free interval did not reduce the average convulsive score, rather there was a marked increase, showing that the kindling phenomenon was established with pentylenetetrazol in all the groups (Tab 1). Comparison between the 1st and 22nd treatment (retest) is shown in Tab 1.

In haloperidol-treated rats (Tab 2) the response with pentylenetetrazol was again biphasic. With control rats (treated only

Tab 1. Effects of *l*-dopa, *d*-amphetamine and amantadine on PTZ (pentylenetetrazol) kindled rats. A subconvulsive dose (25 mg/kg) of PTZ was administered during 1-21st treatments. n = 8 rats, $^{*}p<0.05$, $^{**}p<0.05$, $^{***}p<0.01$ *vs* control.

Expt	Convulsive score ($\bar{x}\pm$SD) Control (PTZ)	*l*-Dopa 50 mg/kg	Amphetamine 1 mg/kg	Amantadine 10 mg/kg
1	0.0±0.0	0.0±0.0*	0.0±0.0*	1.625±1.06***
2	0.625±0.51	0.0±0.0*	0.125±0.35*	2.0±1.06***
3	0.375±0.51	0.0±0.0*	0.125±0.35*	2.25±1.03***
4	0.625±0.51	0.0±0.0***	0.0±0.0***	1.0±0.0**
5	1.125±0.64	0.0±0.0***	0.0±0.0***	2.0±1.19**
6	0.5±0.07	0.0±0.0**	0.0±0.0**	2.875±0.99***
7	0.25±0.46	0.0±0.0*	0.875±0.64**	1.75±0.46***
8	0.125±0.35	0.0±0.0*	1.25±0.46***	2.875±1.24***
9	1.0±0.0	1.0±0.92*	0.375±0.51*	1.75±1.66*
10	0.875±0.35	0.5±0.07*	0.25±0.46**	1.875±0.77***
11	0.875±0.35	0.5±0.07*	1.25±0.46*	1.75±1.58*
12	0.75±0.46	1.0±0.31*	0.375±0.51*	2.25±1.48***
13	0.625±0.51	1.125±0.83*	1.25±0.7*	1.875±0.77***
14	0.875±0.35	1.375±1.3*	1.375±0.74*	2.0±1.41**
15	1.25±0.7	1.0±0.75*	1.25±0.7*	2.875±0.99***
16	0.875±0.83	0.875±0.83*	1.0±0.0*	2.25±1.035***
17	0.875±0.35	1.25±0.96*	1.0±0.0*	2.0±1.06***
18	0.75±0.7	1.25±1.03*	1.0±0.0*	2.375±2.25**
19	1.0±0.0	1.25±0.7*	1.0±0.0*	2.375±2.12**
20	1.25±0.46	1.5±1.19*	1.25±0.7*	2.75±1.16***
21	1.125±0.64	2.125±0.99**	1.375±0.51*	2.625±1.18***
PTZ 22.5 mg/kg was given during retest after a gap of 3 wk.				
22	2.25±0.46	2.625±1.3*	2.375±1.18*	3.5±0.75**

with saline and pentylenetetrazol), there was an increase in the average convulsive score as the number of days of challenge with pentylenetetrazol increased, while with haloperidol in the initial few treatments, the average convulsive score was much higher and it kept on declining as the number of challenges increased. With haloperidol in both the initial treatment and the last treatment with pentylenetetrazol, there was a statistical difference from the control rats.

Tab 2. Convulsive scores in chronic treatment with haloperidol and metoclopramide given every day for 18 d on PTZ kindling and rats were challenged with PTZ every d 4. Number of rats in parentheses. $\bar{x}\pm$SD, $^{*}p>0.05$, $^{**}p<0.05$, $^{***}p<0.01$ *vs* control.

Expt	Control PTZ 25 mg/kg (8)	Metoclopramide 1 mg/kg (8)	Haloperidol 5 mg/kg (7-8)
1	0.625±0.51	0.625±0.51*	1.428±0.92**
2	0.625±0.51	0.75±0.46*	1.285±0.46**
3	0.375±0.51	0.125±0.35*	1.285±0.48***
4	0.625±0.51	0.25±0.46*	0.714±0.48*
5	1.125±0.64	0.625±0.51*	0.571±0.53*
6	1.25±0.7	0.5±0.07**	0.571±0.53**
7	1.625±1.18	1.0±0.53*	0.428±0.53**

With metoclopramide, there was initially not much difference from the control rats but later on a tendency to decrease in the average convulsive score ($p<0.05$).

DISCUSSION

Dopaminergic and other neurotransmitter receptors in general show supersensitivity ("up-regulation") when transmitter occupation of the receptor are decreased by various experimental manipulations such as inhibition of synthesis, chronic receptor blockade by specific receptor antagonist, denervation by lesioning with chemical neurotoxins or intense electrical stimulation[9]. Subsensitivity ("down-regulation") occurs after prolonged enhancement of neurotransmitter levels by prolonged exposure to agonists, inhibition of re-uptake or catabolism and sustained release[8].

Dopaminergic receptor blocking agents,

haloperidol[8] and metoclopramide[10], were selected to induce supersensitivity in the rats. In a parallel series of experiments, *l*-dopa, amphetamine and amantadine were used to induce receptor subsensitivity.

Dopamine receptor supersensitivity decreases the rate of amygdaloid kindling[8,11] which supports our findings with haloperidol in case of pentylenetetrazol-induced kindling. With metoclopramide there was a tendency for the convulsive score to decline but the effect on the development of kindling was not significant as compared to the controls, probably because haloperidol inhibits both D_1 and D_2 receptors while metoclopramide does not inhibit adenylate cyclase linked to D_1 site[10].

In as much as dopamine receptor supersensitivity resulted in retarded rates of seizure development, opposite effect would have been expected with dopamine receptor subsensitivity. *l*-Dopa, amphetamine and amantadine were administered chronically to determine if the chronic drug treatment with these dopaminergic agents, which results in receptor subsensitivity, would alter the rate of kindling development.

The dopaminergic agonists, *l*-dopa, amphetamine and amantadine have not suppressed the development of kindling phenomenon which occurred as in the case of control. Initially, *l*-dopa (for the first 8 injections) and amphetamine (for the first 6 injections) suppressed the average convulsive response, which was found to be much less with *l*-dopa than in control rats (Tab 1) and on continuation of further treatment, convulsive score was increased and was more than the control group, rather it prolonged the kindled seizure.

l-Dopa and haloperidol act on both D_1 and D_2 subtypes of dopaminergic receptors, *l*-dopa is an agonist and stimulates adenylate cyclase at D_1 site while haloperidol is an antagonist and inhibits adenylate cyclase[12]. In our study on kindling phenomenon due to pentylenetetrazol the effect of these 2 agents was absolutely in opposite direction, thus confirming their action on the same receptor subtype.

In case of amphetamine, our results are comparable with those of others[13] who have shown that *d*-amphetamine tends to prolong established amygdaloid convulsions in rats.

Amantadine increases the release of dopamine from central sites and delays the re-uptake of dopamine by neural cells. It induced a distinct increase in convulsive response on chronic administrations, thus showing this agent is proconvulsive rather than anticonvulsive.

Supersensitivity and subsensitivity of dopaminergic receptors in the present study has attenuated the rate of kindling, as one expects if dopamine plays a role as an inhibitory neurotransmitter to seizure development and the data suggest that dopamine has an important role in the suppression of kindled seizure activity due to pentylenetetrazol.

REFERENCES

1 Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* 1969; 25 : 295

2 Corcoran ME, Fibiger HC, McCaughran JA Jr, Wada JA. Potentiation of amygdaloid kindling and metrazol-induced seizures by 6-hydroxydopamine in rats. *Ibid* 1974; 45 : 118

3 Fabisiak JP, Schwark WS. Aspects of the pentylenetetrazol kindling model of epileptogenesis in the rat. *Ibid* 1982; 78 : 7

4 Arnold PS, Racine RJ, Wise RA. Effects of atropine, reserpine, 6-hydroxydopamine, and handling on seizure development in the rat. *Ibid* 1973; 40 : 457

5 Engel J Jr, Sharpless NS. Long-lasting depletion of dopamine in the rat amygdala induced by kindling stimulation. *Brain Res* 1977; 136 : 381

6 Wilkison DM, Halpern LM. Turnover kinetics of dopamine and norepinephrine in the forebrain after kindling in rats. *Neuropharmacology* 1979; 18 : 219

7 McNamara JO. Selective alterations of

regional β-adrenergic receptor binding in the kindling model of epilepsy. *Exp Neurol* 1978; 61 : 582

8 Gee KW, Killam EK, Hollinger MA. Effects of haloperidol-induced dopamine receptor supersensitivity on kindled seizure development. *J Pharmacol Exp Ther* 1983; 225 : 70

9 Schwartz JC, Costentin J, Martres MP, Protais P, Baudry M. Modulation of receptor mechanisms in the CNS : Hyper- and hyposensitivity to catecholamines. *Neuropharmacology* 1978; 17 : 665

10 Roufogalis BD, Thornton M, Wade DN. Specificity of the dopamine sensitive adenylate cyclase for antipsychotic antagonists. *Life Sci* 1976; 19 : 927

11 Sato M, Tomoda T, Hikasa N, Otsuki S. Inhibition of amygdaloid kindling by chronic pretreatment with cocaine on methamphetamine. *Epilepsia* 1980; 21 : 497

12 Snyder SH. Drug and neurotransmitter receptors in the brain. *Science* 1984; 224 : 22

13 Babington RG, Wedeking PW. The pharmacology of seizures induced by sensitization with low intensity brain stimulation. *Pharmacol Biochem Behav* 1973; 1 : 461

中国药理学报 1988年1月; **9** (1) : 7-11

多巴胺能药物减少戊四唑诱发大鼠癫痫的发作

Vijay Lakshmi MEHTA, Sarita BHALLA, Zafar Ahmed WAFAI (*Neuropharmacology Laboratory, Department of Pharmacology, Maulana Azad Medical College, New Delhi 110002, India*)

提要 大鼠慢性 sc 戊四唑，使癫痫发作。慢性给予左旋多巴、苯丙胺和金刚胺等多巴胺受体激动剂，可减少戊四唑引起的癫痫发作。这种作用呈双相，即起初阻止惊厥，继而使惊厥次数增多。慢性给予多巴胺受体阻断剂，如氟哌啶醇可使癫痫发作较对照组推迟出现。但另一多巴胺能受体阻断剂甲氧普胺，不能减少发作。这些观察支持多巴胺参与抑制癫痫发作的假说。

关键词 癫痫；惊厥；戊四唑；左旋多巴；苯丙胺；金刚胺；氟哌啶醇；甲氧普胺

* * * * * *