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EFFECT OF MEDROXYPROGESTERONE ON DEVELOPMENT OF PENTYLENETETRAZOLE-INDUCED KINDLING IN MICE

S. A. NASIR,[a] A. SHARMA,[b] R. KHANAM[a] AND D. VOHORA[a]*

[a]*Neurobehavioral Pharmacology Laboratory, Department of Pharmacology, Faculty of Pharmacy, Hamdard University, New Delhi 110062, India*

[b]*Institute of Genomics & Integrative Biology(IGIB), New Delhi, India*

Abstract—In the present study, the effect of medroxyprogesterone (MPA) is evaluated for its effect on pentylenetetrazole (PTZ) kindling model of epileptogenesis in mice followed by evaluation on kindling-induced changes in cognitive and motor functions. To explore whether the effects are mediated via progesterone receptors, a selective antagonist of progesterone (mifepristone, MIF) was also taken. Kindling was induced by once every 2 days treatment with PTZ (25 mg/kg, i.p.) for 5 weeks. The seizure severity during induction of kindling and % incidence of animals kindled at the end of 5 weeks were recorded. The motor function was assessed using a grip strength meter, whereas spatial memory was assessed in a cross maze. MPA (5 and 10 mg/kg, i.p.) significantly reduced the seizure severity scores and produced a significant decrease in the incidence of animals kindled at the end of 5 weeks ($P<0.01$). A higher efficacy was observed against male mice as compared with females following MPA. MIF neither reduced nor delayed the development of PTZ-induced kindling in mice. Also, it couldn't reverse the antiepileptogenic effects of MPA. On grip strength test (GST) and spontaneous alternation behavior (SAB), a significant decline in GST and % alternation was observed in kindled mice which was reversed by pre-treatment with MPA. MIF, however, could reverse only the reduced % alternation and not grip strength (GS) in PTZ-kindled animals. The study shows that MPA has antiepileptogenic effects against development of PTZ-induced kindling in mice that may not be mediated via progesterone receptors.

Key words: medroxyprogesterone, mifepristone, diazepam, PTZ kindling, grip strength, spontaneous alternation behavior.

Literature suggests that progestins can have profound effect on seizure processes (Murialdo et al., 2009; Frye, 2010). Both progesterone and its metabolite have been shown to have antiseizure effect in various animal models (Lonsdale and Burnham, 2007; Singh et al., 2010). Progesterone has shown to be inhibitory to CNS, and it decreases epileptiform activity in various animal models like pentylenetetrazole- (PTZ) induced seizure in mice (Frye et al., 2002), maximal electroshock- (MES) induced seizure in rats (White et al., 1995), kainic acid-induced seizure in rats (Nicoletti et al., 1985) and amygdala-kindled seizure in rats (Lonsdale and Burnham, 2003) and so forth. In addition to experimental studies, there are some clinical reports like decreasing catamenial epilepsy in women (Reddy, 2005) suggesting the antiseizure effects of progesterone. The widely accepted mechanism of anticonvulsant action of progesterone is via conversion to a neurosteroid allopregnalonone (3α, 5α, tetra hydroprogesterone), which is a potent allosteric modulator of $GABA_A$ receptors (Frye, 1995).

It is interesting to note that unlike progesterone, medroxyprogesterone (MPA, synthetic progesterone) doesn't get metabolized to allopregnanolone, which is thought to be responsible for the antiepileptic effects of progesterone via $GABA_A$ receptors (Ciriza et al., 2006). However, MPA has interestingly reported efficacy in some experimental and clinical studies including improvement of seizures in catamenial epilepsy in women (Mattson et al., 1984), reduction of kainic acid-induced seizures in male and female rats (Nicoletti et al., 1985) suggesting it has antiepileptic effects.

In the present study, the effect of medroxyprogesterone is evaluated for its effect on PTZ-kindling model of epileptogenesis in mice followed by evaluation on kindling-induced changes in cognitive and motor functions. Because gender can influence the effect of progesterone on seizures, the study was conducted in both male and female mice. To explore whether the effects are mediated via progesterone receptors, an antagonist of progesterone (mifepristone) was also administered.

EXPERIMENTAL PROCEDURES

Animals

Swiss albino mice weighing between 25 and 35 g and raised at the Central animal house facility of Jamia Hamdard were used. They were housed in polypropylene cages and maintained at 25–30 °C and 50–55% humidity in a natural light/dark cycle. They were fed on a standard pellet diet (Amrut rat and mice feed; Chakan Oil Mills, Pune, India) and water *ad libitum*. All procedures involving animals were conducted in accordance with the guidelines of the Animal Ethics Committee, Hamdard University (project # 681). Utmost care was taken to ensure that minimum required animals are used in the study and they are treated in most humane manner.

Drugs

The studies used the following drugs and chemicals: PTZ and mifepristone (Sigma, USA); MPA acetate (Pfizer, USA); diazepam (Ranbaxy laboratories, India). MPA was administered half an hour before PTZ at doses of 5 mg/kg and 10 mg/kg converted by the

*Corresponding author. Tel: +91-11-26059688 (ext. 5657).
E-mail address: dvohra@jamiahamdard.ac.in or divyavohora@hotmail.com (D. Vohora).

Abbreviations: AED, antiepileptic drug; ANOVA, analysis of variance; GS, grip strength; GST, grip strength test; MIF, mifepriston; MPA, medroxyprogesterone; PTZ, pentylenetetrazole; SAB, Spontaneous alternation behavior.

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Table 1. Effect of medroxyprogesterone and mifepristone on the incidence of animals kindled following repeated treatment with a subconvulsant dose of pentylenetetrazole in mice

S. No.	Groups	% incidence of seizures in male mice (mean±SEM)	% incidence of seizures in female mice (mean±SEM)
1	Control	0.00$^{\$\$}$	0.00$^{\$\$}$
2	VEH+PTZ	89.29±5.05**	96.42±3.57**
3	MPA1+PTZ	71.43±6.52**$^{\$\$}$	75.00±9.44**
4	MPA2+PTZ	50.00±0.00$^{**,\$\$}$	82.14±7.14**
5	MIF+MPA1+PTZ	64.29±5.05$^{**,\$\$}$	60.71±5.05$^{**,\$\$}$
6	MIF+PTZ	92.86±4.61**	92.85±4.61**
7	DZP+PTZ	14.29±5.05$^{\$\$}$	10.71±5.05$^{\$\$}$

PTZ (25 mg/kg) was administered once every 2 d for 5 wk, whereas MPA1 (5 mg/kg) and MPA2 (10 mg/kg) and MIF (2.5 mg/kg) were administered daily. All treatments were given by i.p. route.

The total number of animals in a group was seven; ** $P<0.01$, when compared with normal control (Group 1), $^{\$\$}P<0.01$, when compared with toxic control (Group 2), significant by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

Abbreviations: VEH, Vehicle (Sterile water for injection); MPA, medroxyprogesterone; MIF, mifepristone; DZP, diazepam; PTZ, pentylenetetrazole.

method of Freireich et al. (1966) from corresponding rat dose exhibiting protection against kainic acid-induced seizures (Nicoletti et al., 1985). Mifepriston (MIF) was given at a dose of 2.5 mg/kg, 2 h before the administration of PTZ (Borowicz et al., 2002). The solution of MIF was made in dimethylsulfoxide. Diazepam was taken as standard and was given at 3 mg/kg 1.5 h before PTZ (Ali et al., 2006). All drugs were administered intraperitoneally between 9 AM to 12 noon every day.

Induction of kindling

Kindling was induced according to the method of Ali et al. (2006). Pentylenetetrazole (PTZ, 25 mg/kg, i.p.) was injected once every 2 days for 5 weeks to induce kindling. The intensity of seizure response was scored as 0=no response, 1=mouth and facial jerks, 2=nodding or myoclonic body jerks, 3=forelimb clonus, 4=rearing, falling down (loss of postural control), hind limb clonus and forelimb tonus, 5=tonic extension of hind limb, status epilecticus, and/or death. Animals were observed for 30 min after PTZ injection. When the animals had a seizure score of 4 on three consecutive administrations, they were defined as being kindled and PTZ treatment was then discontinued. The effect of various treatments on the seizure severity during induction of kindling and % incidence of animals kindled at the end of 5 weeks was recorded.

Grip strength test

The neuromuscular function was determined with the aid of a grip strength meter. The mouse was allowed to hold the grip with its forepaws. The mouse was then pulled back horizontally until it releases its grip. The grip strength reading was directly read from the digital meter (Ali et al., 2004).

Spontaneous alternation behavior (SAB)

Spontaneous alternation behavior (SAB) in a cross maze was assessed using the method of Ragozzino and coworkers (1998) suitably modified by us for mice (Vohora et al., 2005). A four-arm wooden cross maze (height: 50 cm, arms: length 23.5 cm, breadth 8 cm, wall height 10 cm) with a central platform (8.8 cm) was used. The dimensions of the maze were similar to the elevated plus maze for mice as per the method of Itoh et al. (1991). However, unlike the elevated plus maze, the cross maze consisted of four arms with 10-cm walls all of which were open as per the method described for rats (Ragozzino et al., 1998). After being placed in the central platform, mice were allowed to traverse the maze freely for 6 min. The number and sequence of entries were recorded; an alternation is defined as entry into four different arms on an overlapping quintuple set. Five consecutive arm choices within the total set of arm choices constitute a quintuple set. A quintuple set consisting of arm choices B, A, C, B, D comprised an alternation, whereas the set with B, A, D, B, A did not. Percentage alternation was calculated as actual alternations/possible alternations×100, where possible alternation was number of arm entries minus four.

Statistical analysis

Incidence of seizures, grip strength, and % alteration is expressed as mean±SEM and analyzed by ANOVA followed by Dunnett's *t* test. Seizure severity is presented as median seizure score with upper and lower quartiles and analyzed by Kruskal–Wallis one-way analysis of ranks test.

RESULTS

Effect of MPA on development of PTZ kindling

Repeated treatment with PTZ at a subconvulsant dose (25 mg/kg, i.p.), three times a week, induced chemical kindling (Table 1, Figs. 1 and 2). Animals treated with diazepam (3 mg/kg, i.p.) exhibited a significant reduction in incidence ($P<0.01$, Table 1) and severity of seizures ($P<0.01$, Figs. 1–2). MPA (5 and 10 mg/kg, i.p.) effectively delayed the development of PTZ-induced kindling in mice. This was demonstrated by a significant reduction in the seizure severity scores in mice (Figs. 1–2) and a significant decrease in the incidence (Table 1) of seizures at the end of 5 weeks. A dose of 10 mg/kg was more efficacious. A higher efficacy was observed against male mice as compared with females following MPA. Mifepristone (MIF) neither reduced nor delayed the development of PTZ-induced kindling in mice. Pre-treatment with MIF couldn't reverse the antiepileptogenic effects of MPA.

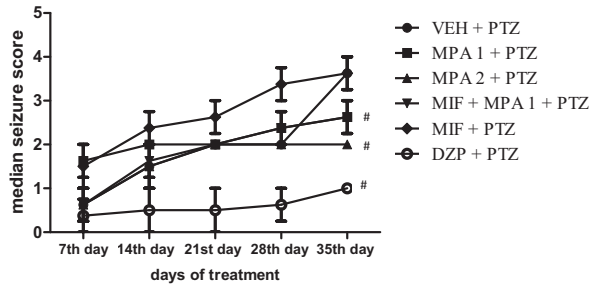

Fig. 1. Effect of medroxyprogesterone and mifepristone on seizure severity during induction of kindling by PTZ in male mice. PTZ (25 mg/kg) was administered once every 2 d for 5 wk, whereas MPA1 (5 mg/kg) and MPA2 (10 mg/kg) and MIF (2.5 mg/kg) were administered daily. All treatments were given by i.p. route. Data are presented as median seizure score with upper and lower quartiles. # $P<0.05$ vs. VEH+PTZ by Kruskal–Wallis one-way analysis by ranks (at the end of 35th day of treatment). The total numbers of animals in a group were seven. VEH: Vehicle (Sterile water for injection); MPA: medroxyprogesterone; MIF: mifepristone; DZP: diazepam; PTZ: pentylenetetrazole.