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ORIGINAL ARTICLE

Effect of phosphamidon on convulsive behavior and biochemical parameters: modulation by progesterone and 4′-chlorodiazepam in rats

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Abstract Phosphamidon (PHOS) has been shown to affect nervous system adversely. The present study was designed to explore the modulation of the effects of PHOS on convulsions by neurosteroids, progesterone (PROG), and 4′-chlorodiazepam (4′-CD), in both acute and chronic seizure models. In acute study, seizures were induced by either pentylenetetrazole (PTZ) injection or maximal electroshock seizures, while in the chronic study, kindling was induced by injecting PTZ (30 mg/kg, s.c.) on alternate days three times in a week. Oxidative stress was assessed in the brain by measuring the levels of malondialdehyde (MDA), acetylcholinesterase (AChE), and non-protein thiol (NP-SH). PROG and 4′-CD were able to modulate the PHOS-induced convulsions in acute PTZ convulsions as well as in chronic kindling model. However, they failed to reverse the derangements in oxidative stress parameters of MDA and NP-SH produced by PHOS in kindled animals. PROG significantly increased the AChE activity in untreated rats, while PROG and 4′-CD reversed the AChE activity inhibition induced by PHOS. The study indicates a possible anticonvulsive mechanism of neurosteroids, since both PROG and 4′-CD reversed PHOS-induced inhibition of AChE activity. The neurosteroids seem to play a protective role in PHOS-induced convulsions besides their antioxidant property.

Keywords Pentylenetetrazole · Maximal electroshock seizures · Phosphamidon · Progesterone · 4′-chlorodiazepam

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Introduction

Phosphamidon (PHOS) is a non-cumulative systemic organophosphorus pesticide with a broad spectrum of activity. It is an acetylcholinesterase (AChE) inhibitor and acts via interaction of nucleophilic active site serine of the enzyme to form a phosphorylated enzyme derivative (Naqvi and Hasan 1990). Exposure of the general population to this pesticide may occur through consumption of foods treated with pesticides or harvested before pesticide residues have declined to acceptable levels, from contact with treated areas during structural applications or by drift from aerial spraying. Several cases of suicidal or occupational poisoning have been reported (Tarbah et al. 2004). Hence, PHOS has generated considerable concern about its potential to cause adverse health effects in human and animals. The metabolism of certain classes of pesticide also results in the generation of oxygen-free radicals (OFRs), such as superoxide anion and hydrogen peroxide, and many pesticides induce cytochrome P450 and also elicit an increase in the rate of OFR production by liver microsomes (Lu and West 1979). The reaction of OFRs with polyunsaturated lipids is a particularly toxic event because it initiates the membrane-damaging chain reaction process of

lipid peroxidation. Oral administration of PHOS increased serum malondialdehyde and decreased erythrocyte superoxide dismutase, catalase activity, and whole-blood glutathione levels showing PHOS-induced oxidative stress (Suke et al. 2008). Since PHOS acts by inhibiting AChE (Chattopadhyay et al. 1986) and acetylcholine has been shown to play important role in epileptogenesis (De Condole et al. 1953; Rickett et al. 1986), PHOS-induced convulsive behavior may be linked with its anti-AChE property. Studies with laboratory animals, case reports, accidental or acute poisonings, and epidemiological studies have provided significant information about the toxicological properties and pharmacodynamics of this pesticide (Chuang et al. 2002). It has been reported in earlier studies that the exposure to various organophosphate pesticides causes inadvertent alteration of immune responses (Banerjee et al. 1997; Seth et al. 2002). Several previous studies have also reported that significant exposure to various carbamate or organophosphate pesticides induces convulsions (Kadriu et al. 2009; Kalkan et al. 2005; Gupta et al. 2001). However, the effect of PHOS on convulsions has been less investigated.

Neurosteroids (NS) occur in the brain in various forms like non-conjugated steroids, sulfated esters, and fatty acid esters. The important NS are progesterone (PROG), allopregnanolone (AP), allotetrahydrodeoxycorticosterone, pregnenolone (PREG), pregnenolone sulfate, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), etc. They are highly lipophilic and hence rapidly cross the blood brain barrier (Balieu and Robel 1990; Paul and Purdy 1992). They have been proposed to act through various modes of action, like modulation of $GABA_A$ receptors, *N*-methyl-D-aspartate (NMDA) receptor, and glycine, sigma, kainate, serotonergic, and neuropeptide receptors (Krueger and Papadopoulos 1992; Korneyev et al. 1993; Fontaine-Lenoir et al. 2006). NS have a role in neuroprotection and induction of neurite outgrowth, dendritic spines, and synaptogenesis (Sasahara et al. 2007). They are also known to be involved in various physiological and pathological states like behavior, stress, depression, anxiety, memory, convulsions, and neurodegenerative disorders (Rhodes and Frye 2005; Kulkarni and Reddy 1995).

PROG has been known to have anticonvulsant activity in animal seizure models (Pence et al. 2009; Frye 2008). In clinical studies, PROG has been found to reduce the frequency of interictal spikes and lessen the risk of seizures (Landgren et al. 1978). It has been reported that AP, a metabolite of PROG, is responsible for its acute anticonvulsant profile (Lonsdale and Burnham 2007). In addition, NS are highly efficacious against cocaine, ethanol, diazepam, and neurosteroid withdrawal seizures, indicating a unique broad-spectrum antiseizure activity (Reddy et al. 2001). α-Estradiol, DHEA, and DHEA-S have been shown to protect neurons against NMDA-induced neurotoxicity and reported to be neuroprotective (Akwa et al. 1991; Majewska 1992). NS may exert anticonvulsant and neuroprotective effects by various mechanisms such as decreasing the oxygen utilization by neurons or by direct action on $GABA_A$ receptor in the brain (Pence et al. 2009; Lonsdale and Burnham 2007). Recently, PROG has been shown to have antiseizure effect due to decreased levels of adenosine deaminase, which in turn results in increased adenosine levels that exerts anticonvulsant effect via $GABA_A$ receptors (Pence et al. 2009).

4′-Chlorodiazepam (4′-CD) is not an NS in true sense, but it acts by increasing the production of NS through the mitochondrial diazepam binding inhibitor receptor complex (Korneyev et al. 1993). It acts by increasing the delivery of cholesterol to the NS biosynthetic pathway. 4′-CD has been shown to facilitate intramitochondrial flux of cholesterol to increase the PREG synthesis, in adrenalectomized and castrated male rats (Stoffel-Wagner 2001).

With the possibility that oxidative stress may play a role in development of epilepsy by PHOS and NS, being antioxidant, may modulate the effect of PHOS, the present study was designed to evaluate the effect of PROG and 4′-CD on PHOS-induced convulsive behavior and oxidative stress in rats.

Material and methods

Animals

Male Wistar rats weighing between 150 and 220 g, obtained from the Central Animal House of the University College of Medical Sciences, Delhi, were used in the study. The animals were housed in polypropylene cages (30×15×15 cm) in groups of five rats per cage with free access to pellet diet and water and kept under controlled environmental condition (temperature 22±2°C, humidity 50–55%, natural light/day cycle). All the experiments were performed during the light phase between 0930 and 1530 hours. The study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication no. 86-23, revised 1985) and with the recommendations and approval of the Institutional Animal Ethics Committee, University College of Medical Sciences, Delhi.

Drugs and dosing schedule

PHOS and PROG were obtained from Sigma chemicals (Kansas, MO, U.S.A). 4′-CD was procured from Fluka (USA). All other chemicals used were of laboratory grade. PHOS was given in a dose of 1.74 mg/kg per day p.o., with distilled water as a vehicle. PROG and 4′-CD were

administered intraperitoneally in a dose of 15 and 0.5 mg/kg per day, respectively, after dissolving in distilled water with two drops of Tween 80 added per 10 ml of suspension. Each animal received 0.5 ml/100 g of suspension per day. All animals received PHOS or vehicle for PHOS p.o. for 6 weeks. PROG or its vehicle was administered intraperitoneally for 2 weeks after treatment with PHOS. The different experimental groups were evaluated for the latency and duration of convulsive behavior (Tables 1 and 2).

Assessment of convulsive behavior

Acute studies

Electroshock seizure

Seizures were induced in the rats by delivering electroshock of 120 mA for 0.2 s by means of a convulsiometer through a pair of ear clip electrodes. Duration of tonic hind limb extension (THLE) was measured for comparison in between the groups (Rao et al. 2005).

PTZ-induced convulsions

Subcutaneous (s.c.) administration of PTZ in a dose of 60 mg/kg produces convulsions, and the latency to preclonic convulsions and the duration of clonic convulsions were recorded (Gupta et al. 2008).

Chronic studies

PTZ-induced kindling

PTZ (30 mg/kg, s.c.) was administered on alternate days at the same time (thrice a week), and the animals were observed for the appearance of seizure activity. When the animal reached stages 4 or 5 of epileptogenesis at three consecutive injections at 72-h interval, it was defined as kindled and the treatment was discontinued. Most of the rats fulfilled the kindling criteria after 5–6 weeks. After this, the effect on the expression of convulsions following the administration of the test compounds was observed. PROG and 4′-CD were administered 15 and 30 min prior to the PTZ injection in the kindled animals, respectively (Sarro et al. 1999).

Assessment of oxidative stress

At the end of the study period, animals were sacrificed by deep ether anesthesia. Whole brains were quickly dissected out in toto, washed with ice-cold sodium phosphate buffer, weighed, and stored over ice. The brains were further processed within half an hour of dissection and the estimation of oxidative stress done on the same working day. Brain tissue was homogenized with 10 times (*w*/*v*) sodium phosphate buffer (pH 7.4 ice-cold mixture of KH_2PO_4 and Na_2HPO_4). The homogenate was centrifuged at 3,000 rpm for 15 min, and the supernatant was used for the estimation of malondialdehyde (MDA) and non-protein thiol (NP-SH) levels.

Estimation of malondialdehyde

MDA, an indicator of lipid peroxidation, was estimated as described by Ohkawa et al. (1979). A sample containing 0.5 ml of supernatant was mixed with 1 ml trichloroacetic acid (20%, pH 3.5), 1.5 ml thiobarbituric acid (0.8%), and 0.2 ml sodium lauryl sulfate (8.1%) and heated at 100°C for 1 h. After cooling with tap water, 5 ml of butanol/pyridine (15:1, *v*/*v*) and 1 ml of distilled water were added. The mixture was vortexed vigorously and was centrifuged at 4,000 rpm for 10 min. Thereafter, the organic layer was withdrawn and absorbance measured at 532 nm using a spectrophotometer.

Estimation of non-protein thiol

NP-SH was estimated by the method as described by Ellman (1959). To 0.5 ml of the supernatant obtained above, 1 ml trichloroacetic acid (5%) was added, and the mixture was centrifuged to remove the proteins. To 0.1 ml of this homogenate, 4 ml of phosphate buffer (pH 8.4), 0.5 ml of 5,5-dithiobis, 2-nitrobenzoic acid (DTNB), and 0.4 ml double-distilled water were added. The mixture was vortexed and absorbance was read at 412 nm within 15 min.

Table 1 Treatment schedule for acute studies (maximal electroshock seizures and PTZ-induced convulsions)

Group	PHOS/vehicle	PROG/4′-CD	Convulsive stimulus
Control	Vehicle (p.o.)	Vehicle (i.p.)	MES or PTZ
PHOS	PHOS (1.74 mg/kg, p.o.)	Vehicle (i.p.)	MES or PTZ
Only PROG	Vehicle for PHOS	PROG (15 mg/kg/d, i.p.)	MES or PTZ
Only 4′-CD	Vehicle for PHOS	4′-CD (0.5 mg/kg/d i.p.)	MES or PTZ
PHOS+PROG	PHOS (1.74 mg/kg, p.o.)	PROG (15 mg/kg/d, i.p.)	MES or PTZ
PHOS+4′-CD	PHOS (1.74 mg/kg, p.o.)	4′-CD (0.5 mg/kg/d i.p.)	MES or PTZ

PHOS phosphamidon, *PROG* progesterone, *4′-CD* 4′-chlorodiazepam, *PTZ* pentylenetetrazole, *MES* maximal electroshock seizures

Table 2 Treatment schedule for chronic studies (PTZ kindling)

Group	PTZ/PHOS/vehicle for 6weeks (on alternate days)	PROG/4′-CD/vehicle for 2weeks
Control	Vehicle (p.o.)	Vehicle (i.p.)
PTZ+PHOS	PTZ (30 mg/kg, s.c.)+PHOS (1.74 mg/kg, p.o.)	Vehicle (i.p.)
PTZ+PROG	PTZ (30 mg/kg, s.c.)	PROG (2.5 mg/kg, i.p.)
PTZ+4′-CD	PTZ (30 mg/kg, s.c.)	4′-CD (0.5 mg/kg, i.p.)
PTZ+PHOS+PROG	PTZ (30 mg/kg, s.c.)+PHOS (1.74 mg/kg, p.o.)	PROG (2.5 mg/kg, i.p.)
PTZ+PHOS+4′-CD	PTZ (30 mg/kg, s.c.)+PHOS (1.74 mg/kg, p.o.)	4′-CD (0.5 mg/kg/d, i.p.)
PTZ	PTZ (30 mg/kg, s.c.)+vehicle for PHOS (p.o.)	Vehicle (i.p.)
PHOS	PHOS (1.74 mg/kg, p.o.)	Vehicle (i.p.)
PROG	Vehicle for PHOS (p.o.)	PROG (2.5 mg/kg, i.p)
4′-CD	Vehicle for PHOS (p.o.)	4′-CD (0.5 mg/kg, i.p)

PHOS phosphamidon, *PROG* progesterone, *4′-CD* 4′-chlorodiazepam, *PTZ* pentylenetetrazole

Estimation of acetylcholinesterase (AChE) activity

Brain AChE activity was estimated by the method as described by Ellman et al. (1961). Following centrifugation, 1 ml of the supernatant was mixed with 9 ml of sucrose solution (0.32 M) to get a 15 postmitochondrial supernatant (PMS). AChE estimation was done in this 1% PMS. Then, 2.7 ml phosphate buffer (0.1 M, pH 8.0), 0.1 ml DTNB solution (10 mM), and 0.1 ml of 1% PMS were taken in a test tube. This constituted the test solution, while the blank was constituted of all the solutions except PMS. Both the solutions were preincubated for 5 min, and absorbance was taken at 412 nm. Following this, 0.1 ml acetylcholine iodide (30 mM) was added in both the solutions to initiate the reaction, and absorbance at 412 nm was recorded for 3 min after every 1-min interval. AChE activity was calculated using the following formula: R=volume of assay×A/CO, where R=rate in moles of substrate hydrolyzed per minute per gram tissue, A=change in absorbance per minute, and CO=original concentration of the tissue (mg/ml).

Statistical analysis

All the values are expressed as mean±standard error of the mean (SEM). Data were analyzed using one-way ANOVA with post hoc Tukey's test. P values less than 0.05 were considered significant.

Results

Acute studies

Effect of PHOS, PROG, and 4′-CD on maximal electroshock seizures

On maximal electroshock seizures (MES), PHOS did not cause any modification in the duration of THLE. PROG and 4′-CD also did not produce any significant difference on the duration of THLE as compared to the control group. Administration of PROG and 4′-CD in animals pretreated with PHOS showed no effect on THLE (Fig. 1).

Effect of PHOS, PROG, and 4′-CD on PTZ-induced latency to onset of preclonic convulsions

In PHOS-treated group, the latency to onset of preclonic convulsions was significantly decreased as compared to the control group. Administration of PROG and 4′-CD per se showed significant anticonvulsant effect on PTZ-induced convulsions. PROG and 4′-CD caused significant delay in the onset of preclonic convulsions as compared to the control and PHOS-treated groups. Administration of PROG and 4′-CD to the PHOS-treated group significantly antagonized the effect of PHOS on PTZ-induced latency to onset of preclonic convulsions (Fig. 2).

Effect of PHOS, PROG, and 4′-CD on duration of PTZ-induced clonic convulsions

The duration of clonic convulsions was significantly increased in the group of animals that received PHOS alone as compared to the control group. PROG and 4′-CD treatment per se produced significant decrease in the duration of clonic convulsions as compared to the control and PHOS-treated groups. Further, they also significantly attenuated the effect of PHOS on the duration of clonic convulsions in group pretreated with PHOS, when compared to the control group (Fig. 3).

Chronic studies

Effect of PHOS, PROG, and 4′-CD on the latency to onset of preclonic convulsions in PTZ-kindled rats

The PTZ-kindled group administered with PHOS showed significant decrease in the latency to onset of preclonic convulsions as compared to the control group. PROG and

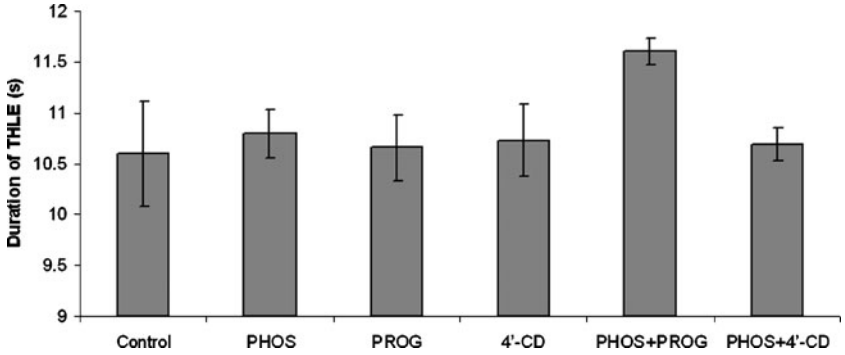

Fig. 1 Effect of phosphamidon (*PHOS*), progesterone (*PROG*), and 4′-chlorodiazepam (*4′-CD*) on maximal electroshock seizures. The duration of tonic hind limb extension (*THLE*) was measured. All animals received PHOS or vehicle for PHOS (1.74 mg/kg per day, p.o.). PROG, 4′-CD, or their vehicles were administered i.p. in doses of 15 and 0.5 mg/kg per day, respectively. Values are expressed as mean ± SEM, for ten animals in each group. $p>0.001$ in all the groups

4′-CD per se produced significant delay in the onset of preclonic convulsions as compared to the control and PHOS-treated groups. Furthermore, they also produced significant delay on the onset of convulsions in the groups of animal which received PHOS along with PTZ (Fig. 2).

Effect of PHOS, PROG, and 4′-CD on duration of PTZ-induced clonic convulsions in PTZ-kindled rats

The group of animals that received PHOS alone showed significant increase in the duration of clonic convulsions as compared to the PTZ-kindled (control group) rats. PROG and 4′-CD treatment significantly decreased the duration of convulsions in the PTZ-kindled rats. In addition, they significantly reversed the effect of PHOS in group of animals which received PHOS along with PTZ in the respective groups (Fig. 3).

Oxidative stress

Effect of PHOS, PROG, and 4′-CD on brain levels of MDA in PTZ-kindled rats

There was a marked and significant increase in the brain MDA levels in the PHOS-treated group as compared to the control group, which indicates that PHOS does cause oxidative stress and increases lipid peroxidation. Furthermore, there was a significant increase in the MDA levels in PTZ+PHOS-treated group as compared to the PHOS-alone group. The MDA levels were significantly lowered in the PROG- and 4′-CD-treated kindled group of animals as compared to the PHOS-alone group. However, PROG and 4′-CD per se showed no effect on the brain MDA levels when compared to the control group. PROG and 4′-CD failed to modulate the effect of PHOS on MDA levels in the kindled brains (Table 3).

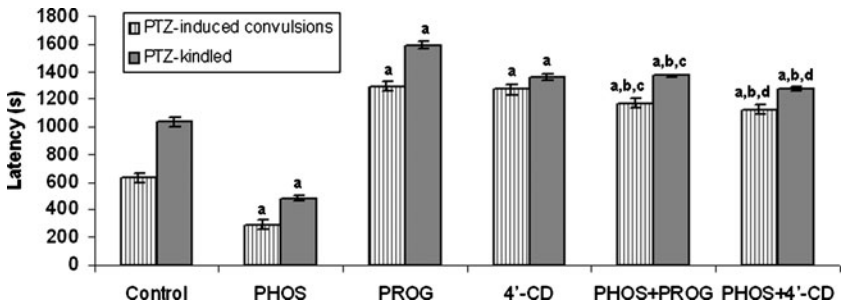

Fig. 2 Effect of phosphamidon (*PHOS*), progesterone (*PROG*), and 4′-chlorodiazepam (*4′-CD*) on the latency to onset of preclonic convulsions (in seconds) in PTZ-kindled and PTZ-induced convulsions in rats. All animals received PHOS or vehicle for PHOS (1.74 mg/kg per day, p.o.). PROG, 4′-CD, or their vehicles were administered i.p. in doses of 15 and 0.5 mg/kg per day, respectively. PTZ-kindled animals received PHOS or vehicle for PHOS for 6 weeks, followed by PROG, 4′-CD, or their vehicles for 2 weeks. Values are expressed as mean ± SEM, for ten animals in each group. *a*, $p<0.001$ as compared to control group; *b*, $p<0.001$ as compared to PHOS group; *c*, $p<0.001$ as compared to PROG group; *d*, $p<0.001$ as compared to 4′-CD group

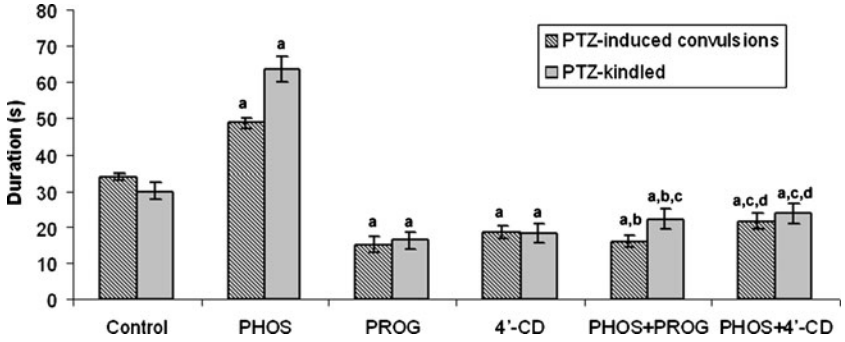

Fig. 3 Effect of phosphamidon (*PHOS*), progesterone (*PROG*), and 4′-chlorodiazepam (*4′-CD*) on the duration of clonic convulsions (in seconds) in PTZ-kindled and PTZ-induced clonic convulsions in rats. All animals received PHOS or vehicle for PHOS (1.74 mg/kg per day, p.o.). PROG, 4′-CD, or their vehicles were administered i.p. in doses of 15 and 0.5 mg/kg per day, respectively. PTZ-kindled animals received PHOS or vehicle for PHOS for 6 weeks, followed by PROG, 4′-CD, or their vehicles for 2 weeks. Values are expressed as mean ± SEM, for ten animals in each group. *a*, $p<0.001$ as compared to control group; *b*, $p<0.001$ as compared to PHOS group; *c*, $p<0.001$ as compared to PROG group; *d*, $p<0.001$ compared to 4′-CD group

Effect of PHOS, PROG, and 4′-CD on brain levels of NP-SH in PTZ-kindled rats

NP-SH levels were significantly reduced in the group that received PHOS alone as compared to the control group. There was no difference in NP-SH levels of groups that were administered with PROG and 4′-CD per se as compared to the control group. Further, both PROG and 4′-CD failed to increase the NP-SH levels in kindled brain tissue. Moreover, they failed to modulate the decrease in NP-SH levels produced by PTZ and PHOS treatment (Table 3).

Effect of PHOS, PROG, and 4′-CD on brain levels of AChE in PTZ-kindled rats

There was a marked and significant decrease in the brain AChE activity in the PHOS-treated group as compared to the control and PTZ-only group, which indicates that PHOS inhibits the AChE enzyme. The AChE activity was also significantly higher in the PROG-only group as compared to the control, PHOS-only, PTZ-only, and PTZ+PHOS groups. This indicates that PROG not only reversed the inhibitory effect of PHOS on AChE activity but also increased the normal AChE activity. Further, 4′-CD also

Table 3 Effect of PHOS, PROG, and 4′-CD on the brain levels of MDA, NP-SH, and AChE activity in PTZ-kindled rats

Group	Treatment (mg/kg, route)	MDA (nmol/g wet brain tissue)	NP-SH (μg/g wet brain tissue)	AChE activity (mol/min/g protein)
Control	Vehicle for PHOS (p.o.)+vehicle for PROG and 4′-CD (i.p.)	171.23±10.2	395.56±11.92	126.59±5.03
PHOS	1.74 mg/kg, p.o. on alternate day	505.48±10.4*	195.29±12.2*	45.05±2.23*·***
PTZ	30 mg/kg, s.c.	495.73±10.2*	199.1±11.12*	103.38±2.45**·****
PTZ+PHOS	30 mg/kg, s.c.+1.74 mg/kg, p.o	593.46±14.8*·**	152±8.3*·**	44.79±5.0*·***
PROG	15 mg/kg, i.p.	166.75±9.2**·***·****	405.16±11.7**·***·****	168.89±6.11*·***·****
4′-CD	0.5 mg/kg, i.p.	168.2±9.1**·***·****	396.63±7.9**·***·****	146.44±3.33**·***·****
PTZ+PROG	30 mg/kg, s.c.+15 mg/kg, i.p.	500.46±8.9*	216.2±7.81*·**	161.53±5.9*·***·****
PTZ+ 4′-CD	30 mg/kg, s.c.+0.5 mg/kg, i.p.	498.36±9.6*	221.4±8.41*·**	115.77±5.59**·****
PTZ+PHOS+ PROG	30 mg/kg, s.c. + 1.74 mg/kg, i.p. + 15 mg/kg, i.p.	573.3±11.1*·**	175.63±7.95*·**	101.6±2.71**·****
PTZ+PHOS+ 4′-CD	30 mg/kg, s.c. + 1.74 mg/kg, i.p. + 0.5 mg/kg, i.p.	597.2±9.7*·**	171.42±7.86*·**	83.34±2.41*·**·****

Values are expressed as mean ± SEM

*$p<0.001$ as compared to control group; **$p<0.001$ as compared to PHOS-only group; ***$p<0.001$ as compared to PTZ-only group; **** $p<0.001$ as compared to PTZ+PHOS group

demonstrated the same properties except that it was unable to increase the AChE activity significantly in the control group. PROG, when administered with PTZ and PHOS, significantly increased the AChE activity as compared to the PHOS- and PTZ+PHOS-treated groups but were lower than the control group, though not significant (Table 3).

Discussion

Pesticides have been shown to influence brain function adversely. It has been reported as proconvulsant in various studies (Kadriu et al. 2009; Kalkan et al. 2005; Gupta et al. 2001). This is because they rapidly cross the blood brain barrier and alters the activity of various neuronal enzymes like AChE, Na^+-K^+-ATPase, and Mg^{2+}-ATPase (Naqvi and Hasan 1990; Chattopadhyay et al. 1986). They have also been found to bind with picrotoxin/TBPS receptors of $GABA_A$-activated Cl^- channel (Sahoo et al. 2000). Pesticides have also been shown to influence steroidogenesis by inhibiting the enzyme P450scc, thus inhibiting the conversion of cholesterol to PREG (Akgul et al. 2008). Further, they have been demonstrated to inhibit the activity of steroidogenic acute regulatory protein, which mediates an important step in steroidogenesis, the intramitochondrial transfer of cholesterol to the P450scc enzyme (Sircar and Lahiri 1990; Walsh and Stocco 2000; Sujatha et al. 2001). They have been found to produce sudden seizures of the mixed type, i.e., grand mal, petit mal, and myoclonus, predominating. Other effects include intention tremors, memory impairment, irritability, and aggression (Khare et al. 1977). Several previous studies have reported that significant exposure to various carbamate or organophosphate pesticides induces convulsions (Kadriu et al. 2009; Kalkan et al. 2005; Gupta et al. 2001). However, the effect of PHOS on convulsions has been less investigated.

NS like PROG, AP, DHEA, PREG, etc. are derivatives of cholesterol and are synthesized in the brain (Balieu and Robel 1990; Paul and Purdy 1992). They have been proposed to act through various modes of action, like modulation of $GABA_A$ receptors, NMDA receptor, and glycine, sigma, kainate, serotonergic, and neuropeptide receptors (Krueger and Papadopoulos 1992; Korneyev et al. 1993; Fontaine-Lenoir et al. 2006). Recently, PROG has been shown to have antiseizure effect due to decreased levels of adenosine deaminase, which in turn results in increased adenosine levels that exerts anticonvulsant effect via $GABA_A$ receptors (Pence et al. 2009). Over the past few decades, the role of NS has been demonstrated in a wide range of brain functions including epilepsy, memory, anxiety, depression, and neuroprotection (Krueger and Papadopoulos 1992). NS like PROG and AP have been shown to have anticonvulsant activity in various animal seizure models (Landgren et al. 1978; Holmes et al. 1984; Frye 1995; Kokate et al. 1996; Edwards et al. 2001). They have also been shown to be neuroprotective by exerting antioxidant effect and promote neurogenesis (Yu 1989; Jung-Testas et al. 1992, 1996; Kurata et al. 2004). It is well known that the frequency and intensity of seizures are altered in physiological state typically associated with cyclic changes in the secretion of steroid hormones. Hence, it is tempting to speculate that the cyclic changes in the PROG and AP contribute to changes in seizure susceptibility (e.g., catamenial epilepsy; Reddy et al. 2001; Reddy and Rogawski 2009). It is surprising that, among the various classes of compounds known to inhibit seizure activity, NS have been studied the least, even though their anticonvulsant properties have been known to be distinct from their hormonal effects.

Agonist of mitochondrial diazepam binding inhibitor receptor complex, Ro-5-4864 (4′-CD), has been shown to increase the brain PREG synthesis without any effect on the blood PREG concentration (Korneyev et al. 1993). The ligands of this receptor facilitate the intramitochondrial flux of cholesterol, thereby increasing the availability of cholesterol to P450scc, leading to an increased NS biosynthesis (Stoffel-Wagner 2001). 4′-CD has been implicated in various functions, including steroidogenesis, mitochondrial respiration, and cell growth and differentiation (Gavish et al. 1999).

In the present study, efforts were made to investigate the convulsiogenic potential of PHOS. We have studied the role of PROG and 4′-CD in modulating PHOS-induced convulsions by using two acute models of convulsions, i.e., PTZ-induced convulsions and MES, and a chronic model of epilepsy, i.e., chemical kindling by PTZ. Since PTZ has been reported to cause enhanced oxidative stress during convulsions (Frantseva et al. 2000), parameters of oxidative stress were also assessed to further understand the effect of PHOS and NS in modulating the oxidative activity in the present experimental setup. Assessment of oxidative stress was done in kindled rats by using MDA, AChE, and NP-SH as the parameters of oxidative stress.

PTZ is a powerful CNS stimulant believed to be acting by interference with the GABAergic inhibition (Frantseva et al. 2000). Rats treated with PTZ alone developed generalized tonic clonic convulsions without any mortality. The group treated with PTZ and PHOS showed decrease in the time taken for the onset of convulsions and increased the duration of seizures. This can be explained by the fact that pesticides may induce seizure by interfering with GABAergic inhibition (Sahoo et al. 2000).

The present data provide compelling evidence in support of the anticonvulsant potential of PROG and 4′-CD. The group of rats that received PROG or 4′-CD along with PTZ demonstrated an increase in the latency to onset and

decrease in the duration of convulsions. These results are in accordance with the previous reports where NS were found to have anticonvulsant activity (Landgren et al. 1978; Belelli et al. 1989; Frye 1995; Beekman et al. 1998). However, a recent study has reported that PROG has no effect on caffeine-induced epileptiform activity (Borekci et al. 2010). Further, in the group of rats that received NS along with PTZ and PHOS, an increase in the latency to onset and decrease in the duration of convulsions were observed as compared to the group that received PTZ+ PHOS. Since NS have been reported to show a free-radical scavenging action (Roof et al. 1997), it is possible that these agents would reduce the damage caused by the free radicals which are generated due to PTZ and PHOS administration by its free-radical extinguishing action.

Multiple lines of evidence indicate that 4′-CD indirectly modulates GABAergic transmission via increasing NS production (Korneyev et al. 1993). 4′-CD at high doses inhibits the GABA gating of $GABA_A$ receptor channel and may induce seizures (Weissman et al. 1984; Gavish et al. 1999). However, in this experiment, the low dose of 4′-CD was employed to render such action unlikely. The anticonvulsant action of 4′-CD on the proconvulsant effect of PHOS observed in the present study could be related to its anticonvulsant activity.

In the model of chemical kindling, the effect of PROG and 4′-CD was studied on the expression of convulsions. The study showed that PROG and 4′-CD delayed the latency to onset of convulsions and decreased the duration of convulsions in PTZ-kindled rats. In this study, PHOS treatment along with PTZ did not show any change in the time duration for the kindling to develop as compared to PTZ-alone-treated group. However, PHOS produced marked decrease in the latency and increase in the duration of clonic convulsions in the kindled rats, confirming the proconvulsant action of PHOS. The PROG and 4′-CD showed delay in the onset of convulsions and reduction in the duration of convulsions in the PHOS+PTZ-kindled rats, thus demonstrating anticonvulsant potential of PROG and 4′-CD on the proconvulsant effect of PHOS. To date, no other study relating to this aspect has been reported.

The steroid hormones circulating in the blood rapidly attain unrestricted access to all parts of the nervous system, where hormone may be metabolized and the metabolite can interact with the receptors to produce its effect. In addition to their well-known genomic action, steroids may have non-genomic action like altering the excitability of neuronal membrane rapidly (Balieu and Robel 1990; Paul and Purdy 1992; Compagnone and Mellon 2000). In the present study, rapid effect of PROG on the kindled rats coincides with the hypothesis that anticonvulsant activity of NS is being mediated through the membrane-bound $GABA_A$/BZD receptor ionophore complex.

Further, PROG and 4′-CD had no protective activity against MES in the doses used in this study. Similarly, PHOS-treated rats did not show any alteration in the duration of THLE. This action of PROG is in contrast to that of barbiturates (Belelli et al. 1989). This observation supports the hypothesis that PROG and barbiturates do not share a common site of action on the $GABA_A$/BZD receptor ionophore complex (Gee et al. 1988). There was no effect of PROG and 4′-CD on the MES test, thus suggesting that their main utility would be in the treatment of absence seizures.

In the present study, PHOS showed marked increase in oxidative stress in the brain which was assessed by measuring MDA, AChE, and NP-SH levels. These results are in accordance with the previous reports (Junqueira et al. 1997; Sahoo et al. 2000). MDA, a product of lipid peroxidation, is increased during xenobiotic-induced oxidative stress. The assay of MDA is often considered as an index of OFR generation. High levels of MDA in the PHOS-exposed rats indicate that this compound enhances lipid peroxidation and causes oxidative stress. The group of rats that received PTZ alone during chemical kindling showed a significant increase in the MDA levels as compared to the control group. This finding is in accordance with an earlier report which has shown oxidative potential of PTZ (Frantseva et al. 2000). There was marked increase in the MDA levels in the group of animals that received PHOS along with PTZ as compared to the control group, indicating the possibility of the PHOS exhibiting lipid peroxidation in the rat brain.

PHOS reduced the brain AChE activity, which was reversed by PROG and 4′-CD. PROG increased the brain AChE activity significantly even in comparison to the control group which shows that NS may reduce the brain ACh and may play a role in the treatment of epilepsy as ACh has been shown to have a role in epileptogenesis (De Condole et al. 1953; Rickett et al. 1986). Furthermore, PHOS has properties of epileptogenesis which can be explained by its inhibitory effect on AChE activity that can induce excitotoxicity by elevating ACh levels.

NP-SH is the most prevalent and important intracellular antioxidant. This compound is able to scavenge both singlet oxygen and hydroxyl radicals. Levels of NP-SH were observed to decrease in the PHOS-treated group as compared to the control group. The decrease in NP-SH levels were marked in the group of rats that received PTZ along with PHOS in kindled rats as compared to the control group, indicating the potential of PHOS to cause oxidative stress. In our study, PROG and 4′-CD failed to produce any effect on the MDA and NP-SH levels per se. They did not modulate the oxidative stress produced by PTZ and PHOS in the kindled rats in the administered doses. Although the antioxidant property of various NS likes DHEA, PROG,

and AP has also been hypothesized (Zisterer et al. 1992; Bucolo et al. 2005), the lack of antioxidant property of PROG and 4′-CD in kindled rats in this setup could be due to the use of insufficient doses for insufficient period of time to revert the oxidative stress caused by PTZ and PHOS.

In the present study, PHOS was found to be proconvulsant by decreasing the latency and increasing the duration of convulsions in both PTZ-induced acute convulsions and PTZ-induced kindling. PROG and 4′-CD were found to be protective against the proconvulsant action of PHOS in acute PTZ-induced convulsions and chemical kindling model, suggesting their anticonvulsant potential. In the chronic study, PHOS was also shown to adversely affect the oxidative stress parameters. However, PROG and 4′-CD in the administered doses failed to modulate the oxidative stress parameters. Hence, it is possible that some prolonged exposure of PROG and 4′-CD may be required to modulate the oxidative damage produced by PHOS. Furthermore, there is a possibility of the involvement of other mechanisms besides oxidative stress which might be involved in mediating the effect of NS on PHOS-induced epileptogenesis.

Thus, our study introduces us to a new concept of modulating PHOS-induced convulsions with the aid of NS.

Conclusion

The study shows the protective action of NS on PHOS-induced convulsions besides their antioxidant property. PROG significantly increased the AChE activity in untreated rats, while PROG and 4′-CD reversed the AChE activity inhibition induced by PHOS, indicating a possible anticonvulsive mechanism of NS.

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