Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 82 (2005) 241 – 245
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Possible role of free radicals in theophylline-induced seizures in mice

Kavita Gulati, Arunabha Ray *, Giridhari Pal, V.K. Vijayan

Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, P.O. Box: 2101, Delhi-110 007, India
Clinical Research Centre, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110 007, India

Received 12 February 2005; received in revised form 20 April 2005; accepted 20 June 2005

Abstract

Theophylline is a methylxanthine bronchodilator with a narrow therapeutic index and is prone to induce seizures, the mechanisms for which are not clearly defined. Free radicals have considerable neurotoxic potential and the present study evaluated the possible involvement of these bioactive moieties in aminophylline-induced seizures in mice. Aminophylline (50–250 mg/kg) induced convulsions and mortality in mice in a dose-dependent manner. The anti-oxidants, melatonin (25–100 mg/kg) and N-actylcysteine (100 and 200 mg/kg) attenuated aminophylline seizures and mortality. Similar antagonism of aminophylline seizures was also observed after pretreatments with nitric oxide (NO) synthase inhibitors, L-NAME (3 and 10 mg/kg) and 7-nitroindazole (10 and 30 mg/kg). Further, combined treatment with otherwise sub-effective doses of melatonin and L-NAME or 7-nitroindazole produced marked protective effects against these seizures. Aminophylline-induced seizures enhanced malondialdehyde (MDA) concentrations and NO metabolite (NO_x) levels in the brain homogenates of mice, and these were attenuated by melatonin and L-NAME pretreatments. The results are suggestive of the possible involvement of free radicals (reactive oxygen and reactive nitrogen species) in the convulsiogenic effects of aminophylline.

Keywords: Aminophylline; Convulsions; Melatonin; Nitric oxide; Free radicals

1. Introduction

Theophylline, a methylxanthine used in the treatment of bronchial asthma and chronic obstructive pulmonary disease, has a narrow therapeutic index, and a high propensity to induce toxicity. The toxic manifestations include cardiotoxicity and neurotoxicity, which has resulted in its restricted use. However, the recent demonstration of anti-inflammatory and immunomodulatory effects of the drug has generated renewed interest for the use of this potent and pharmacoeconomically viable bronchodilator (Barnes and Pauwels, 1994; Barnes, 1998), and strategies to overcome its toxicity potential warrant consideration.

Theophylline is a potent CNS stimulant and seizures are a potentially fatal complication of theophylline toxicity (Nakada et al., 1983). Theophylline induced convulsions are usually not preceded by other milder, warning symptoms and are relatively refractory to conventional anticonvulsant agents (Czuczwar et al., 1987). In an attempt to evaluate the mechanisms involved in this phenomenon, several mechanisms have been proposed, but no clearcut consensus regarding the toxicodynamics of theophylline-induced seizures has emerged (Hornfeldt and Larson, 1994; Chakrabarti et al., 1993, 1998), and thus, the treatment of its overdose/toxicity still remains far from satisfactory.

Free radicals play a crucial role in health and disease (Hollan, 1995; Lohr and Browning, 1995). Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) constitute the most complex interactive systems, aimed at maintaining the homeostasis, in different situations (Tomasian et al., 2000; Darley-Usmar et al., 1995). Any disturbance/change in this balance results in biochemical and cellular alterations leading to development of various pathophysiological states such as rheumatoid arthritis, septic shock, ischaemia reperfusion injury, atherogenesis, carcinogenesis and diabetes mellitus (Wardle, 1990). These reactive species are also known to be involved in several CNS disorders viz. neuropsychiatry, traumatic brain injury, and Alzheimer's disease (Lohr and Browning, 1995; Zhang and Snyder,

* Corresponding author. Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, P.O. Box: 2101, Delhi-110 007, India. Tel.: +91 11 27662155; fax: +91 11 27667420.
E-mail address: arunabha14@yahoo.co.in (A. Ray).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.06.019

1995). Further, excitotoxicity and oxidative stress are shown to be closely related (Frantseva et al., 2000). Nitric oxide (NO), a free radical and an important neuromodulator in the CNS, has been implicated as an endogenous anticonvulsant on one hand, and is shown to sensitize/promote the convulsive effects of cocaine on the other (Buisson et al., 1993; Itzhak, 1994). However, the involvement of ROS and RNS during theophylline neurotoxicity, with specific reference to seizures, have not been studied.

In view of the above, the present experimental study was designed to explore the possible involvement of ROS and RNS, and their interactions, during the convulsiogenic effects of theophylline. Convulsions were induced in mice by aminophylline (ethylenediamine salt of theophylline), and their modulation by drugs affecting ROS and RNS activity, were assessed. Malondialdehyde (MDA), which is a marker of lipid peroxidation, and nitrates and nitrites (NO_x), which are stable metabolites of NO, were assayed in brain homogenates of mice for corroborative evidence.

2. Materials and methods

2.1. Subjects

Swiss albino mice (15–25 g) of either sex were used. Our pilot studies have shown that no significant differences existed across male and female mice with reference to seizure susceptibility and intensity. The animals were housed in standard laboratory conditions of light dark schedule (12 h light–12 h dark, lights on at 7 AM), and room temperature of 22±2 °C. They had free access to food and water (except during the observation period). Each experimental group comprised of 10 animals ($n=10$). Animal care was as per Guidelines for Care and Use of Animals in Scientific Research formulated by the Indian National Science Academy, and the study protocol was approved by the Institutional Animal Ethical Committee.

2.2. Aminophylline-induced convulsions

Experiments were carried out with graded single doses of aminophylline from 50–250 mg/kg administered intraperitoneally (ip) to mice and subsequently put into individual perspex cages (25 × 25 × 10 cm). The mice were then observed for 60 min, and the following parameters were studied:

a. Percent animals having clonic/tonic–clonic convulsions
b. Latency of onset of convulsions (min)
c. Percent mortality at 1 and 24 h.

Appropriate vehicle treated groups served as controls. All experiments were carried out in a quiet room between 10 AM and 3 PM. On the basis of the results obtained with various doses of aminophylline, a dose producing tonic–clonic seizures in 100% of animals was selected for subsequent experiments to investigate the modulatory effects of different drugs. In mice, which did not show any seizures during the observation period of 1 h, the seizure latency was taken as 60 min for the purpose of data analysis.

2.3. Biochemical studies

After observing the percent incidence of convulsions and mortality for 1 h, the animals were decapitated under ether anesthesia. The brains were quickly removed, cleaned with ice cold saline and stored at −80 °C. Brain samples were thawed and homogenized with 10 ml of ice cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates were used to determine MDA and NO_x.

MDA was measured by the method of Liu et al. (1990). The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue samples, and heated at 100 °C for 60 min. The mixture was cooled with tap water and 5 ml of n-butanol/pyridine (15:1), 1 ml of distilled water was added. The mixture was vortexed and after centrifugation at 4000 rpm for 10 min, the organic layer was separated, and absorbance was measured at 532 nm using a UV–VIS Spectrophotometer (UV 5740 SS, ECIL). Protein estimation was done by Lowry's method (Lowry et al., 1951) and the data were expressed as nmol/mg protein.

For NO_x assay (Tracey et al., 1995) brain homogenates were centrifuged at 10,000 $\times g$ for 15 min at 4 °C. Fifty microlitres of supernatant was mixed with 20 μl of 0.86 mM of NADPH, 0.11 mM FAD and 20 mU of nitrate reductase. Samples were allowed to incubate for 1 h at room temperature in the dark. Then 5 μl of 1 M $ZnSO_4$ was added and the samples were centrifuged at 6000 $\times g$ for 5 min at 4 °C, and the supernatants were removed. One hundred microlitres of Greiss reagent (1:1 mixture of 1% sulfanilamide in 5% H_3PO_4 and 0.1% N-(1-naphthyl)ethylenediamine) was added to 50 μl of supernatant, and the mixture was incubated for 10 min at room temperature. Absorbance was measured at 540 nm in a microplate reader (MS 5605A, ECIL), and converted to NO_x content using a nitrate standard curve. The data were expressed as nmol/mg protein.

2.4. Drugs

The drugs used were: Aminophylline, L-NAME (Nω-nitro-L-arginine methyl ester), 7-nitroindazole (7-NI), L-arginine HCl, N-acetylcysteine (all from Sigma Chemical Co., USA), and glyceryl trinitrate (GTN, Panacea Biotec). Melatonin was a gift from Dabur Research Foundation (India). Aminophylline, N-acetyl cysteine and L-NAME were dissolved in distilled water. Melatonin was dissolved in 5% ethanol and 7-nitroindazole in a drop of Tween 20, and then diluted/suspended in normal saline. All drugs were injected intraperitoneally (ip) in a volume of 10 ml/kg body weight of mice. The pretreatment times for melatonin, N-acetylcysteine, L-arginine and GTN was 60 min, and that for NO synthase inhibitors (L-NAME or 7-NI) was 30 min. The doses and pretreatment times of the various drugs were selected on the basis of earlier studies.

2.5. Statistical analysis

The quantal data (incidence of seizures and mortality) was analysed using the Fisher's Exact Test. The latency data was analysed by the Kruskal–Wallis one way ANOVA for nonparametric data followed by Mann–Whitney *U* test (two-tailed) for inter group comparisons. The results of the biochemical assay (MDA and NO_x) were also analysed using the Mann–Whitney *U* test (two-tailed). A *p* value of at least 0.05 was considered as the level of significance in all statistical tests.

3. Results

Aminophylline (50–250 mg/kg, ip) administration induced convulsions and mortality in mice in a dose dependent manner. At 50 mg/kg of the drug there was increased exploratory activity, and none of the mice showed convulsions or mortality. At the dose level of 100 mg/kg, convulsions were seen in 20% of animals with no mortality. At a higher dose of aminophylline (200 mg/kg), 40% of mice showed clonic/tonic–clonic convulsions and mortality, and at still higher doses (250 mg/kg) of the drug, all animals in the group exhibited tonic–clonic convulsions with 90% mortality (Table 1). The results of the aminophylline (250 mg/kg) group were significantly different from the vehicle control group ($p<0.05$, Fisher's test). Accordingly, for subsequent experiments, the dose of 250 mg/kg was selected for studying possible protective effects of drugs against aminophylline seizures, and a dose of 100 mg/kg of the drug was used as a subconvulsive dose for studying potentiating effects of drugs. Vehicle (5% ethanol and/or tween 20 in saline) pretreatment, per se, had no significant effect on aminophylline seizures (data not shown). Further, none of the mice showed any significant neurobehavioral effects when observed up to 1 h. Earlier studies have also used these solvents for dissolving melatonin or 7-NI in neurobehavioral studies (Borowicz et al., 1999; Masood et al., 2005).

Table 1
Effects of antioxidants and NO modulators on aminophylline (A) seizures in mice

Treatment (mg/kg, ip)	Incidence %	Latency (min) (Mean+SE)	Mortality (24 h) %
Controls (V)	0	60.0±0.0	0
A (100)	20	54.0±3.9	0
A (250)	100*	20.0±2.3*	90*
Mel (25)+A	90	25.8±4.5	80
Mel (50)+A	70	32.5±6.0	40[a]
Mel (100)+A	50	50.0±4.6[a]	30[a]
NAC (100)+A	80	28.6±5.3	60
NAC (200)+A	60	40.9±5.3[b]	30
L-NAME (3)+A	90	23.8±4.0	80
L-NAME (10)+A	20[a]	53.0±4.6[a]	0[a]
7-NI (10)+A	100	18.2±2.3	60
7-NI (30)+A	80	28.0±5.4	50
L-Arg (1000)+A (100)	60	37.2±6.1	20
GTN (10)+A (100)	50	42.5±5.7	60[c]
L-NAME (3) + Mel (25)+A	60[a]	39.7±5.5[a]	0[a]
7-NI (10) + Mel (25)+A	90	34.0±4.7[a]	30[a]

n=10, per group; V — vehicle; A — A (250 mg/kg); Mel — Melatonin; NAC — N-acetylcysteine; 7-NI — 7-nitroindazole; L-Arg — L-Arginine; GTN — Glyceryl trinitrate.
* $p<0.02$ (compared to controls); [a]$p<0.05$; [b]$p<0.02$ (compared to respective A-250 mg/kg values); [c]$p<0.05$ (compared to respective Amino-100 mg/kg values).

Overall analysis of latency data showed that the mean latencies for onset of seizures were significantly different across all groups [$H(15)=51.8$, $p<0.001$, Kruskall–Wallis test]. As shown in Table 1, the latency for onset of seizures in the aminophylline (250 mg/kg) group was also significantly lower when compared to the vehicle treated group ($p<0.02$, Mann–Whitney *U* test).

Melatonin (25–100 mg/kg, ip) pretreatment exhibited a dose-related protective effect against aminophylline (250 mg/kg) seizures, with maximum protection against convulsions and mortality being seen with the dose of 100 mg/kg. Further, latency of onset of convulsions was also significantly greater with this dose, when compared to the vehicle+aminophylline group ($p<0.05$, Mann–Whitney *U* test). Dose related attenuations of aminophylline (250 mg/kg) seizures were also seen after N-acetylcysteine (100 and 200 mg/kg). Whereas the lower dose was ineffective in reducing the seizure incidence, mortality or onset latency, the higher dose of 200 mg/kg of the drug significantly delayed the onset of seizures and reduced the 24 h post-ictal mortality ($p<0.05$).

Pretreatment with the NO synthase inhibitor, L-NAME (3 and 10 mg/kg) attenuated aminophylline (250 mg/kg) convulsions and mortality, the protection with L-NAME (10 mg/kg) being statistically significant, when compared to the vehicle treated aminophylline group ($p<0.05$) (Table 1). Further, when sub-effective doses of L-NAME (3 mg/kg) was combined with an ineffective dose of melatonin (25 mg/kg), the combination produced a significant protective effect against aminophylline seizures ($p<0.05$). The neuronal NO synthase inhibitor, 7-nitroindazole (10 and 30 mg/kg), reduced the mortality to 60% and 50%, respectively but these changes were not statistically significant ($p>0.05$). When sub-effective doses of 7-nitroindazole (10 mg/kg) and melatonin (25 mg/kg) were combined, an enhancement of anti-convulsant effect was observed, i.e. the percent mortality was further reduced to 30% as compared to 90% seen in the vehicle+aminophylline treated group ($p<0.05$). Administration of the NO precursor, L-arginine (1000 mg/kg) prior to subconvulsive doses of aminophylline (100 mg/kg) induced seizures in 60% animals with 20% mortality and a 32% reduction in seizure latency. However, these data were not significantly different from the vehicle treated aminophylline (100 mg/kg) treated group ($p>0.05$). Similar convulsiogenic effects were also seen when the NO donor, glyceryl trinitrate (10 mg/kg) was combined with the subthreshold dose of aminophylline, though a significant enhancement (of 60%) in the 24 h post-seizure mortality was observed ($p<0.05$).

Biochemical data showed that both MDA levels (an index of lipid peroxidation), and total nitrates and nitrites (NO_x, an index of NO activity), in brain homogenates were higher in the

Table 2
Effects of melatonin and L-NAME on brain MDA and NO_x levels during aminophylline seizures in mice

Dose (mg/kg, ip)	NO_x (nmol/mg protein)	MDA (nmol/mg protein)
Vehicle	15.0±2.8	3.0±0.8
A (100)	26.8±6.2*	4.8±0.5*
A (250)	48.2±5.2*	7.9±1.6*
L-NAME (10)+A	14.5±2.0[a]	4.8±1.1[a]
Mel (100)+A	34.3±6.1	5.1±2.0[a]
Mel (25)+L-NAME(3)+A	13.5±1.2[a]	3.5±1.2[a]

All data are expressed as Mean±S.E.
$n=10$, per group, A — Aminophylline, 250 mg/kg, Mel — Melatonin.
* $p<0.05$ (compared to Vehicle), [a]$p<0.05$ compared to aminophylline 250 mg/kg) — Mann–Whitney U test.

aminophylline (100 and 200 mg/kg) treated groups, as compared to vehicle controls (Table 2). The baseline values in the vehicle group were 3.0±0.8 and 15.0±2.8 nmol/mg protein for MDA and NO_x, respectively. Aminophylline (250 mg/kg) raised these levels to 7.9±1.6 and 48.2±5.2 nmol/mg protein, respectively ($p<0.05$). Pretreatment with melatonin (100 mg/kg) and L-NAME (10 mg/kg) attenuated the aminophylline-induced changes in brain MDA and NO_x levels. Further, combination treatment with sub-effective doses of melatonin and L-NAME, induced significant reductions in the above biochemical parameters assayed. At the doses used, melatonin and L-NAME, per se, did not induce any significant modulations in MDA and NO_x activity in the brain homogenates, when compared to the vehicle group ($p>0.05$) (data not shown).

4. Discussion

The therapeutic use of theophylline is associated with a significant incidence of intractable seizures and mortality (Barnes and Pauwels, 1994; Barnes, 1998). However, the seizurogenic effect of the drug could not be satisfactorily explained by mechanisms like adenosine antagonism and phosphodiesterase inhibition (Hornfeldt and Larson, 1994; Gulati et al., 2004). Recently, free radicals have been implicated in many drug and chemical induced toxicities (Wardle, 1990; LeBel and Bondy, 1991), and thus the involvement of free radicals during theophylline seizures was explored. The xanthine–xanthine oxidase system is an important pathway of generation of ROS in biological systems (Cotgreave et al., 1988) and one of the major metabolites of theophylline is a substrate for xanthine oxidase (Lohmann and Miech, 1976). It is thus possible that the increased production of ROS during metabolism of high doses of theophylline could result in oxidant/anti-oxidant imbalance and thus precipitate neurotoxicity.

Aminophylline administered in high doses of 250 mg/kg consistently induced seizures in mice, an effect that was not seen at lower doses (50 and 100 mg/kg) of the drug. Interestingly, theophylline and aminophylline, in low doses, have been reported to act as anti-oxidants (Lapenna et al., 1995; Mahomed et al., 1998). Melatonin acts as an antioxidant by scavenging hydroxyl radicals, peroxyl radicals, neutralizes superoxide anion, peroxyl radical and NO, and also stimulates several antioxidative enzymes viz. superoxide dismutase, glutathione peroxidase and glutathione reductase (Reiter et al., 2002). In order to investigate the involvement of free radicals, melatonin was administered in graded doses (25–100 mg/kg) prior to aminophylline, in separate groups. The protection offered by melatonin is suggestive of the involvement of ROS in aminophylline seizures.

Reactive nitrogen species (RNS) like NO have been shown to be an important neuromodulator in the CNS (Zhang and Snyder, 1995), and studies have suggested both neuroprotective and neuroexcitatory roles for NO (Rundfeldt et al., 1995). For example, in various models of experimental convulsions, NO has been shown to play both inhibitory and excitatory roles (Przegalinski et al., 1994, 1996). In the present experiments, NO synthase inhibitors, L-NAME and 7-nitroindazole, administered prior to aminophylline, significantly blocked the incidence of seizures and mortality, suggesting that NO may also play a role in aminophylline seizures. However, Urbanska et al. (1996) reported that another NO synthase inhibitor, NG-nitro-L-arginine (NNA), at high doses, enhanced aminophylline seizures, and pharmacokinetic interactions were proposed. This possibility appears unlikely in our present study, as both NO synthase inhibitors, L-NAME and 7-NI, even at low doses, were able to protect against aminophylline seizures. Further, NNA was also reported to either antagonize of have no effect on other seizure models (Tutka et al., 1996). Nevertheless, other studies have also reported protective effects for NO synthase inhibitors in various seizure models, and our results are in agreement with the same. It thus appears that the NO synthase inhibitors have a complex profile in relation to various seizure models. Involvement of NO in other aminophylline/theophylline effects like thermoregulation and uterine relaxation in experimental animals has also been reported (Apaydin et al., 1998; Zarrindast et al., 2002).

NO, which by itself is a relatively non-toxic molecule, could lead to generation of highly reactive nitrogen species such as peroxynitrite (OONO) ion in the presence of/combination with superoxide anion (Squadrito and Pryor, 1998). Interactions between ROS and RNS are reported in different situations and a balance between the two is a critical determinant of several disease processes (Darley-Usmar et al., 1995; Tomasian et al., 2000). In the present study, combined treatment of sub-effective doses of melatonin and NOS-inhibitors, when given prior to aminophylline, showed a synergistic effect against aminophylline seizures and mortality, which is suggestive of involvement of both ROS and RNS in this phenomenon. However, pharmacokinetic interactions between these agents cannot be totally discounted, though a study has shown that melatonin does not influence brain and plasma levels of anticonvulsants (Borowicz et al., 1999). The results of the biochemical assay further support this hypothesis originating from pharmacological studies. The MDA and NO_x levels were elevated in the brain homogenates of aminophylline treated mice, a dose which produced maximal convulsiogenesis and mortality. Further, pretreatment with the antioxidant, melatonin and the NO synthase inhibitor, L-NAME, reverted the changes

in these biochemical parameters. It is well known that NO in the presence of superoxides forms a highly toxic peroxynitrite (OONO) which induces lipid peroxidation and is responsible for several pathophysiological states (Ischiropoulos and al-Mehdi, 1995; Szabo, 1996). In view of the pharmacological and biochemical data obtained, the same may be possible and substantiates the involvement of both ROS and RNS during aminophylline seizures. The fact that sub-effective doses of either melatonin or L-NAME, when given together, were effective (a) in antagonizing aminophylline seizures, and (b) lowering levels of MDA and NO_x in brain homogenates, further points towards the synergistic involvement of both ROS and RNS, in this phenomenon.

Acknowledgements

The financial support from the Council of Scientific and Industrial Research (CSIR), New Delhi, is gratefully acknowledged. The authors thank Dabur India Limited for the generous gift of melatonin and Dr. Priyanka Narayan and Mr. Rishi Pal for their assistance during the experiments.

References

Apaydin S, Gonen C, Guven H. The probable role of nitric oxide on the relaxations obtained by caffeine and aminophylline in rat uterus. Pharmacol Res 1998;38(5):387–92.

Barnes PJ. Theophylline. In: Barnes PJ, Rodger IW, Thomson NC, editors. Asthma: basic mechanisms and clinical management. 3rd edition. San Diego: Academic Press; 1998. p. 689–706.

Barnes PJ, Pauwels RA. Theophylline in the management of asthma: time for reappraisal. Eur Respir J 1994;7(3):579–91.

Borowicz KK, Kaminski R, Gasior M, Kleinrok Z, Czuczwar SJ. Influence of melatonin upon the protective action of conventional antiepileptic drugs against maximal electroshock in mice. Eur Neuropsychopharmacol 1999;9(3):185–90.

Buisson A, Lakhmeche N, Verrecchia C, Plotkine M, Boulu RG. Nitric oxide: an endogenous anticonvulsant substance. NeuroReport 1993;4(4):444–6.

Chakrabarti A, Chugh Y, Sharma PL. Search for antidote to electroconvulsion and lethality in aminophylline-sensitized mice. Eur J Pharmacol 1993;235(1):133–6.

Chakrabarti A, Saini HK, Garg SK. Dose finding study with nimodipine: a selective central nervous system calcium channel blocker, on aminophylline induced seizure models in rats. Brain Res Bull 1998;45(5):495–9.

Cotgreave IA, Moldeus P, Orrenius S. Host biochemical defense mechanisms against prooxidant. Annu Rev Pharmacol Toxicol 1988;28:189–212.

Czuczwar SJ, Janusz W, Wamil A, Kleinrok Z. Inhibition of aminophylline-induced convulsions in mice by anti-epileptic drugs and other agents. Eur J Pharmacol 1987;144(3):309–15.

Darley-Usmar V, Wiseman H, Haliwell B. Nitric oxide and oxygen radicals: a question of balance. FEBS Lett 1995;369:131–5.

Frantseva MV, Perez Velazquez JL, Tsoraklidis G, Mendonca AJ, Adamchik Y, Mills LR, et al. Oxidative stress is involved in seizure induced neurodegeneration in the kindling model of epilepsy. Neuroscience 2000;97(3):431–5.

Gulati K, Anand S, Wardhan N, Vijayan VK, Ray A. Studies on the toxicodynamics of theophylline seizures: in search for an antidote. Clin Exp Pharmacol Physiol 2004;31(Suppl. 1):A187–8.

Hollan S. Free radicals in health and disease. Haematologia 1995;26(4): 177–89.

Hornfeldt CS, Larson AA. Adenosine receptors are not involved in theophylline-induced seizures. J Toxicol Clin Toxicol 1994;32(3):257–65.

Ischiropoulos H, al-Mehdi AB. Peroxynitrite-mediated oxidative protein modifications. FEBS Lett 1995;364(3):279–82.

Itzhak Y. Blockade of sensitization to the toxic effects of cocaine in mice by nitric oxide synthase inhibitors. Pharmacol Toxicol 1994;74(3):162–6.

Lapenna D, De Gioia S, Mezzetti A, Ciofani G, Festi D, Cuccurullo F. Aminophylline: could it act as an antioxidant in vivo? Eur J Clin Invest 1995;25(7):464–70.

LeBel CP, Bondy SC. Oxygen radicals: common mediators of neurotoxicity. Neurotoxicol Teratol 1991;13(3):341–6.

Liu J, Edamatsu R, Kabuto H, Mori A. Antioxidant action of guilingji in the brain of rats with $FeCl_3$ induced epilepsy. Free Radic Biol Med 1990;9:451–4.

Lohmann SM, Miech RP. Theophylline metabolism by the rat liver microsomal system. J Pharmacol Exp Ther 1976;196(1):213–25.

Lohr JB, Browning JA. Free radical involvement in neuropsychiatric illnesses. Psychopharmacol Bull 1995;31:159–65.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.

Mahomed AG, Theron AJ, Anderson R, Feldman C. Anti-oxidative effects of theophylline on human neutrophils involve cyclic nucleotide and protein kinase A. Inflammation 1998;22(6):545–57.

Masood A, Banerji BD, Vijayan VK, Ray A. Pharmacological and biochemical studies on the possible role of nitric oxide in stress adaptation in rats. Eur J Pharmacol 2005;493:111–5.

Nakada T, Kwee IL, Lerner AM, Remler MP. Theophylline induced seizures: clinical and pathophysiologic aspects. West J Med 1983;138(3):371–4.

Przegalinski E, Baran L, Siwanowicz J. The role of nitric oxide in the kainate-induced seizures in mice. Neurosci Lett 1994;170(1):74–6.

Przegalinski E, Baran L, Siwanowicz J. The role of nitric oxide in chemically- and electrically-induced seizures in mice. Neurosci Lett 1996;217:145–8.

Reiter RJ, Tan DX, Allegra M. Melatonin: reducing molecular pathology and dysfunction due to free radicals and associated reactions. Neuroendocrinol Lett 2002;23(Suppl. 1):3–8.

Rundfeldt C, Koch R, Richter A, Mevissen M, Gerecke U, Loscher W. Dose dependent anticonvulsant and proconvulsant effects of nitric oxide synthase inhibitors on seizure threshold in a cortical stimulation model in rats. Eur J Pharmacol 1995;274:73–81.

Squadrito GL, Pryor WA. Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite and carbon dioxide. Free Radic Biol Med 1998;25:392–403.

Szabo C. Physiological and pathophysiological roles of nitric oxide in the central nervous system. Brain Res Bull 1996;41(3):131–41.

Tomasian D, Keaney JF, Vita JA. Antioxidants and the bioactivity of endothelium-derived nitric oxide. Cardiovasc Res 2000;47:426–35.

Tracey WR, Tse J, Carter G. Lipopolysaccharide induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. J Pharmacol Exp Ther 1995;272:1011–5.

Tutka P, Klonowski P, Dzieciuch J, Kleinrok Z, Czuczwar SJ. NG-nitro-L-arginine differentially affects glutamate or kainate induced seizures. NeuroReport 1996;7(10):1605–8.

Urbanska EM, Drelewska E, Borowicz KK, Blaszczak P, Kleinrok Z, Czuczwar SJ. NG-nitro-L-arginine, a nitric oxide synthase inhibitor, and seizure susceptibility in four seizure models in mice. J Neural Transm 1996;103(10):1145–52.

Wardle EN. Free radicals and lipid peroxidation in relation to pathophysiological mechanisms. Saudi Med J 1990;11:181–6.

Zarrindast MR, Adl M, Sharifzadeh M, Bahreini T. Role of nitric oxide in systemic effects of theophylline on mouse body temperature. Eur J Pharmacol 2002;451(1):63–8.

Zhang J, Snyder SH. Nitric oxide in the nervous system. Annu Rev Pharmacol Toxicol 1995;35:213–33.