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EFFECT OF SYSTEMICALLY ADMINISTERED NITRIC OXIDE DONOR, SODIUM NITROPRUSSIDE ON PICROTOXIN-INDUCED CONVULSIONS IN RATS

KARTHIK RAJASEKARAN, P. LEEMA REDDY AND VANAJA PAUL*

Department of Pharmacology and Environmental Toxicology,
Dr. ALM Post Graduate Institute of Basic Medical Sciences,
University of Madras, Taramani,
Chennai – 600 113

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Abstract : Nitric oxide (NO), the gaseous neurotransmitter has been reported to have an endogenous anticonvulsant property. This has prompted proposals to develop NO donors as anticonvulsant drugs. In the present study, the effect of NO donor, sodium nitroprusside (SNP) on picrotoxin (PCT)-induced convulsions was investigated. A convulsant dose of PCT (5 mg/kg) was administered 5, 10, 15 and 30 min after intraperitoneal injection of graded doses (0.7, 1.25 and 2.5 mg/kg) of SNP. SNP at doses 0.7 and 1.25 mg/kg increased dose dependently the severity of PCT-induced convulsions. But, pretreatment with the higher dose (2.5 mg/kg) of SNP was protective against PCT-induced convulsions. However, post treatment (5 and 10 min) with the same dose exacerbated convulsions and caused death of the animals. These results indicate that the vasodilator effect of SNP and an increased perfusion of PCT into brain may be responsible for the proconvulsant action of SNP. A decreased entry of PCT because of marked vasodilation and hypotension has been speculated for an inhibition of convulsions in animals pretreated with a higher dose of SNP. In conclusion, the results reveal the non-suitability of SNP to be developed as an anticonvulsant.

Key words : nitric oxide sodium nitroprusside convulsions

INTRODUCTION

The role of nitric oxide (NO) as neurotransmitter/neuromodulator was established in 1989 when Garthwaite et al. (1) reported the ability of neurons to synthesise NO in response to activation by N-methyl-D-aspartate (NMDA) receptors. NO is produced by the enzymatic conversion of L-arginine to L-citrulline. NO acts as a neurotransmitter in the brain by stimulating soluble guanylate cyclase to form the second messenger molecule cyclic guanosine monophosphate (cGMP) (2).

NO has been proposed as an endogenous anticonvulsant (3). This prompted proposals

*Corresponding Author

to develop NO donors as anticonvlsant agents (4). Supportingly, the NO donor, sodium nitroprusside (SNP), which is used in hypertensive emergencies due to its vasodilator action, has been reported by Marangoz et al. (5) to have an anticonvulsant action against penicillin-induced epileptiform activity when administered intracerbroventricularly. However, the effect of systemically administered SNP against convulsions has not been reported. In the present study, picrotoxin (PCT)-induced convulsive responses were determined in animals pretreated intraperitoneally (i.p) with graded doses of SNP.

METHODS

Colony bred adult male Wistar rats (150–170 g) were used for the study. Test (n = 10) and control (n = 10) animals were chosen randomly. The animals were housed in groups of 3–4 per cage under controlled conditions of light/dark cycle (12:12 hrs), and room temperature (29–31°C). Standard pelleted food (Gold Mohur, Mumbai) and water were provided ad libitum and was withdrawn one and a half hour prior to commencement of the experiment. All experiments were conducted between 0900 and 1400 hrs. The experiments were performed in accordance to the Guidelines for Breeding and Conducting Animal Experiments, 1998 defined by the Ministry of Social Justice Empowerment, Government of India.

A convulsion-inducing dose (5 mg/kg, i.p) (6) of PCT (Sigma Chem. Co., St. Louis, M. O., USA) was administered 5, 10, 15 and 30 min after injecting graded doses (0.7, 1.25 and 2.5 mg/kg, i.p) of SNP (SRL Fine Chemicals, Mumbai, India). The doses of SNP were selected on the basis of an earlier report (7) that 1 mg/kg SNP administered intraperitoneally could reverse the NOS inhibiting effects of N^{ω}-L-arginine. SNP was prepared in light-protected vials and used immediately due to their photosensitivity and short half life. The drugs were dissolved in physiological saline in such a way so as to inject 0.2 ml/100 g body weight. Control animals received an equivalent volume of saline at appropriate times. The latencies of onset of myoclonus and convulsions and the frequency of convulsions were the specific parameters determined. Myoclonus latency is the time between the injection of PCT and the appearance of the first myoclonic movement characterised by a sudden jerky movement of the whole body. Convulsion latency is the time between the injection of PCT and the appearance of the first clonic convulsion of the whole body. The frequency of convulsions was recorded using a convulsion monitoring (8) which recorded vibrations caused by the clonic movement of the animals. After PCT injection, the animal was placed in the convulsion monitoring chamber and the apparatus was switched on at the onset of clonic convulsions.

Statistical analysis were performed by One way ANOVA and Tukey's multiple comparison test. P values less than 0.05 was considered statistically significant. The results were expressed as mean ± SEM of 10 animals.

RESULTS

All saline pretreated animals responded to PCT and exhibited myoclonus and clonic convulsions. Five, 10 and 15 min pretreatment of SNP at 0.7 and 1.25 mg/kg shortened myoclonus latency ($P<0.01$; Fig 1A, B) and convulsion latency ($P<0.001$; Fig 2A, B). Convulsion frequency was significantly increased following 5 ($P<0.01$). 10 ($P<0.01$) and 15 min ($P<0.001$) pretreatment with 1.25 mg/kg SNP in these animals (Fig 3B). A dose-dependent increase in the frequency of convulsions was observed (Fig 3A, B).

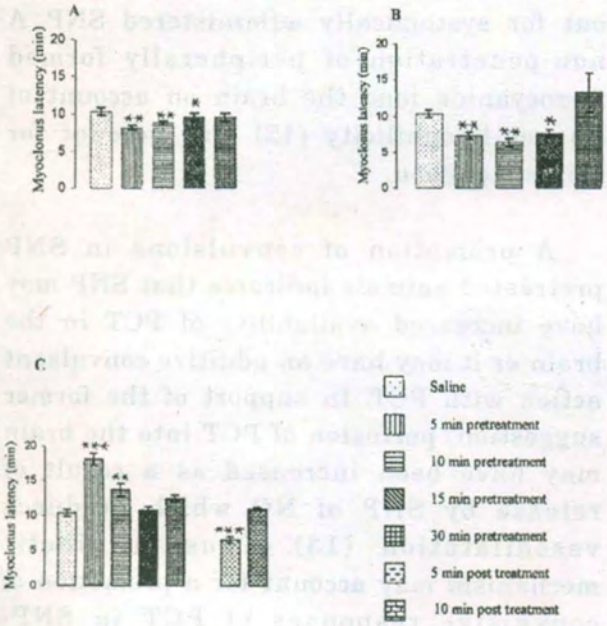

Fig. 1: Myoclonus latency following PCT administration in animals treated with 0.5 (A), 1.25 (B) and 2.5 (C) mg/kg of SNP. Results indicate mean ± SEM of 10 animals. *P<0.05, **P<0.01 and ***P<0.001 compared to control (One way ANOVA and Tukey's test).

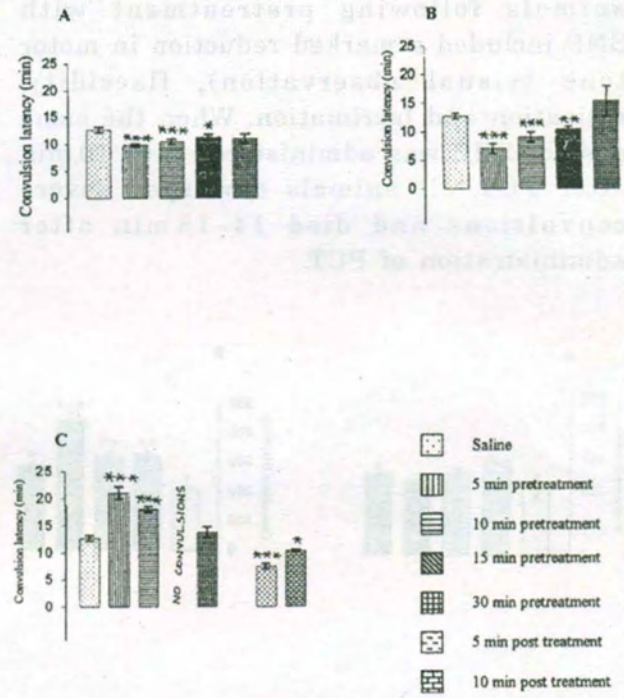

Fig. 2: Convulsion latency following PCT administration in animals pretreated with 0.5 (A), 1.25 (B) and 2.5 (C) mg/kg of SNP. Results indicate mean ± SEM of 10 animals. *P<0.05, **P<0.01 and ***P<0.001 compared to control (One way ANOVA and Tukey's test).

An inhibition of convulsive response was found in animals pretreated with 2.5 mg/kg SNP. Five and 10 min pretreatment prolonged the latency of both myoclonus (Fig 1C) and convulsions (Fig 3C). Fifteen min pretreatment completely abolished convulsions (Fig 3C) although these animals exhibited myoclonus (Fig 1C). No protection was observed in 30 min pretreated animals. In these animals, myoclonus latency (Fig 1C) and convulsion latency (Fig 2C) and the frequency of convulsions (Fig 3C) did not differ significantly from control animals. Behavioural changes observed in the

animals following pretreatment with SNP included a marked reduction in motor tone (visual observation), flaccidity, salivation and lacrimation. When the same dose of SNP was administered 5 or 10 min after PCT, all animals developed severe convulsions and died 14–18 min after administration of PCT.

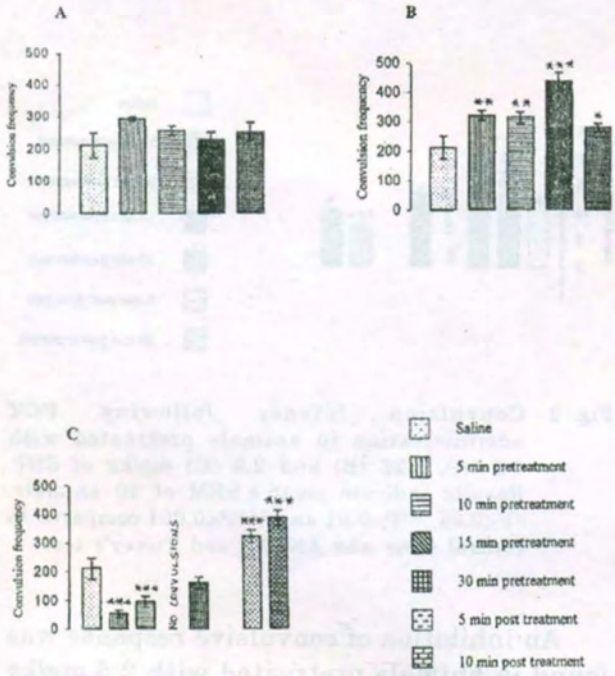

Fig. 3: Convulsion frequency following PCT administration in animals pretreated with 0.5 (A), 1.25 (B) and 2.5 (C) mg/kg of SNP. Results indicate mean ± SEM of 10 animals. *P<0.05, **P<0.01 and ***P<0.001 compared to control (One way ANOVA and Tukey's test).

DISCUSSION

In the present study, the lower doses of systemically administered SNP promoted the convulsive response of PCT and an inhibition of convulsions was achieved with a higher dose of it. However, previous studies have demonstrated an anticonvulsant property of intracerbroventricularly administered SNP against pencillin-induced epileptiform activity (5). SNP has been demonstrated to have a neuroprotective action in neuronal cultures (9). Further, SNP is known to produce ferrocyanide ion, which has NMDA blocking action (10), prior to the release of NO (11). Thus a neuroprotective action of SNP by release of ferrocyanide accounted for the protective effects of intracerebroventricularly administered SNP. Since, in the present study, promotion and inhibition of convulsions were observed with smaller and higher doses respectively, an involvement of ferrocyanide has been ruled out for systemically administered SNP. A non-penetration of peripherally formed ferrocyanide ions the brain on account of its non-lipophilicity (12) may account for this suggestion.

A promotion of convulsions in SNP pretreated animals indicates that SNP may have increased availability of PCT in the brain or it may have an additive convulsant action with PCT. In support of the former suggestion, perfusion of PCT into the brain may have been increased as a result of release by SNP of NO which produces vasodilatation (13). Thus, a kinetic mechanism may account for a promotion of convulsive responses of PCT in SNP-pretreated animals.

SNP is likely to have a proconvulsant action because it increases formation of cGMP (14), which has a convulsion inducing property (15). An increased release of NO

by SNP may account for this action because NO has been long known to activate soluble guanylate cyclase to promote production of cGMP (16). Further evidence for the involvement of cGMP in SNP-induced convulsive activity comes from the report of DeSarro et al (17) who have demonstrated that inhibition of cGMP resulted in prevention of SNP-induced seizures.

An elevation of cGMP by SNP (18) was found to suppress GABA-ergic activity (19). Further, SNP has been reported to have a direct GABA inhibiting action (20) and a short-term stimulatory effect on excitatory amino acids in the brain (17). These properties of SNP also contribute to its additive action with PCT, which is known to induce convulsions by inhibiting GABA (21).

Surprisingly, a higher dose of SNP (2.5 mg/kg) pretreatment was protective against PCT-induced convulsion. The effect was more marked in 15 min pretreated group as compared to 5 and 10 min pretreated groups. If the vasodilator effect of SNP by release of NO becomes profound leading to hypotension (22), then, the delivery of PCT is likely to be impaired resulting in an inhibition of convulsive responses in these animals. At the same time, it is also possible that the flaccidity produced with this dose could have masked convulsions. Since, in the present study, the protective effect was observed in 15 min and not in 30 min pretreated animals, it appears that the protective effect caused by hypotensive action remains only for a short time.

Post-treatment of the higher dose of SNP failed to inhibit convulsions. On the contrary, there was an exacerbation of convulsive responses resulting in death of animals. Since PCT was administered 5 and 10 min prior to administration of SNP, convulsions are likely to be produced as in control animals. During convulsions, there is an increased metabolic demand and a corresponding increase in cerebral blood flow (23). The potent hypotension induced by SNP post-treatment may have decreased blood supply, which in turn may have lead to a mismatch between cerebral blood flow and brain metabolism.

This condition may have aggravated the convulsant action of PCT resulting in death of the animals. Further, an increase of cGMP (14) levels and suppression of GABA-ergic activity by SNP (19) may have also contributed to the aggravation of PCT-induced convulsions.

In conclusion, this is the first report on the effect of systemically administered SNP on PCT-induced convulsions. SNP has both pro-and anti-convulsive actions on PCT-induced convulsions depending upon the dose and time of administration. An increased release by SNP of NO and a direct action of SNP accounted for these actions. The results reveal the non-suitability of SNP to be developed as an anticonvulsant, even if NO has a endogenous anticonvulsant property.

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