NORADRENERGIC MECHANISMS IN THE PROCONVULSANT EFFECTS OF BUSPIRONE

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Abstract : The effect of buspirone on the protection of chemically and electrically-induced seizures by anticonvulsant drugs was studied in mice. We found antagonism to the protective action of sodium valproate (300 mg/kg, po), carbamazepine (420 mg/kg, po) and phenytoin (23.2 mg/kg, po) against picrotoxin (3.5 mg/kg, ip) and maximal electroshock (54 mA, 0.2s) induced seizures by BUS (20 mg/kg, po) and a reversal of such antagonism by pretreatment with clonidine (1 mg/kg, po). The effects appear to be elicited through central α_2 adrenoceptor mechanisms.

Key words : buspirone   proconvulsant   central α_2-adrenoceptor

INTRODUCTION

Buspirone (azaspirodecanedione) has many advantages over benzodiazepines, the conventional anxiolytics viz lack of sedation, euphoria, muscle relaxation, psychomotor and cognitive effects and interaction with alcohol (1). While several reports are available on lack of anticonvulsant effects in buspirone against chemically and electrically induced seizures (1–3), one *in vitro* study against magnesium-depleted hippocampal slices suggested the presence of such activity (4). It has also been stated to exhibit a tendency to lower the seizure threshold (5). Information on interactions of buspirone with conventional anti-epileptic drugs are lacking. Pilot experiments in our laboratory indicated pro-convulsant effects of buspirone against MES and picrotoxin induced seizures in mice. Therefore, experiments were designed to find out the possible interaction of buspirone with conventional antiepileptic drugs and the mechanisms involved in such interactions.

METHODS

Animals : Swiss strain male albino mice (25–32 g), raised at the Central Animal House Facility of Jamia Hamdard, Delhi, were housed in polypropylene cages kept under controlled environmental conditions (temperature $23\pm2°C$ and humidity 50–55%) with a natural light-dark cycle. The mice were fed on a standard pellet diet (Goldmohar, Lipton India Ltd., Calcutta) and water *ad libitum*. Each experimental group consisted of 10 mice.

Induction of seizures : Mild clonic convulsions were induced by sub-threshold dose of picrotoxin (PTX, Sigma, USA 3.5mg/kg, ip) (8) and both tonic and clonic convulsions by

maximal electroshock (MES, 54 mA for 0.2 sec) in mice using electroconvulsiometer (INCO, Ambala) and crocodile ear clips (9).

Drugs and treatment schedules : All drugs were dissolved in distilled water except phenytoin which was suspended using 1% gum acacia. The solutions were freshly prepared and administered in a volume not exceeding 1 ml/100 g body weight. The anticonvulsant doses of sodium valproate, carbamazepine, and clonidine were selected in pilot experiments to give not less than 80% protection against PTX-induced seizures. The pre-treatment times were determined on the basis of their reported time of peak action following oral administration (10, 11).

Picrotoxin induced seizures

Experiments with sodium valproate, carbamazepine, buspirone and clonidine :

Seven groups of 10 mice each were treated as follows: Group I: no pre-treatment; Group II: sodium valporate (SVP) (Torrent, Ahmedabad, 300 mg/kg, po) administered 90 min before PTX; Group III: SVP (as in Gr II) followed by buspirone (BUS) (Merind, Mumbai, 20 mg/kg, po) 40 min before PTX; Group IV: clonidine (CLN) (Sigma, USA, 1 mg/kg, po) given 180 min before PTX followed by SVP and BUS (as in Gr III). Group V: Carbamazepine CBZ (Ciba-Geigy, Mumbai, 420 mg/kg po) administered 90 min before PTX; Group VI: CBZ (as in Gr V) followed by BUS (20 mg/kg po) 40 min before PTX; and Group VII: CLN (1 mg/kg, po) given 180 min before PTX followed by CBZ and BUS (as in Gr VI).

MES-induced seizures

Experiments with phenytoin, buspirone and clonidine :

Four groups of 10 mice each were treated as follows: Group I: no pre-treatment; Group II: Phenytoin (PHEN) (Sigma, USA 23.2 mg/kg, po) administered 120 min before MES; Group III: PHEN (as in Gr II) followed by BUS (20 mg/kg, po) 40 min before MES; and Group IV: CLN (1 mg/kg, po) given 180 min before MES followed by PHEN and BUS (as in Gr III).

Observational parameters : The animals were observed for latency and duration of convulsions. The severity was asessed by a combination of two reported methods (12, 13) as follows: 1: hyperlocomotion, piloerection, 2: stunning, catatonic posture, 3: clonic body tremors, 4: prolonged or repetitive clonic tremors, 5: tonic forelimb convulsions (flexion) followed by clonus, 6: repetitive tonic forelimb convulsions (flexion) followed by clonus, and 7: tonic extension of both fore and hind limbs folowed by clonus. In Experiment on MES-induced seizures, animals were observed for number of animals showing extension phase and mortality. Animals showing no evidence for limb jerks, clonus or stupor were considered to be recovered.

Statistics : The results were expressed as mean±SEM and student's unpaired t-test was used for analysis of data; P values<0.05 were considered significant.

RESULTS

PTX-induced convulsions

Sub-threshold dose of PTX (3.5 mg/kg, ip) elicited hyperlocomotion, tremors,

jerky movements, piloerection, mild clonic convulsion in all mice and tonic seizures in 10% of animals without mortality.

Effect of sodium valporate (SVP) and carbamazepine (CBZ) on PTX-induced seizures, its modulation by buspirone (BUS) and the effect of pre-treatment with clonidine (CLN) on such modulation :

The results are presented in Table I. SVP protected against PTX-induced convulsions; the treatment caused a significant increase in latency (P<0.05) and decrease in duration (P<0.01) of seizures. BUS antagonized the protective action of SVP. The mice under combined treatment

of SVP and BUS showed a highly significant decrease in onset time (P<0.001) and significant increase in duration (P<0.05) and severity (P<0.02) of convulsions vs mice treated with SVP alone. Pre-treatment with CLN caused a complete reversal of the antagonistic effect of BUS. Changes observed in onset time and duration indicating such effects were statistically significant (P<0.05 to <0.001).

CBZ showed protective effect against PTX-induced seizures. While a significant increase in latency (P<0.01) was observed, differences in duration of convulsions and severity scores were not found to be statistically significant. BUS markedly

TABLE I : Picrotoxin-induced convulsions in mice: Antagonism to the protective effect of Sodium valproate and Carbamazepine by Buspirone and its reversal by Clonidine.

Group (n=10)	Treatment[a]	Dose (mg/kg)	Convulsions (Mean±SEM)		
			Latency (min)	Duration (min)	Severity (score)
I	Picrotoxin	3.5	9.2±0.51	34.8±3.74	3.2±0.13
II	Sodium valproate + Picrotoxin	300 3.5	13.0±1.41	23.0±1.67	3.3±0.21
III	Sodium valproate+ Buspirone + Picrotoxin	300 20 3.5	5.9±0.48	33.5±3.81	4.2±0.25
IV	Clonidine + Sodium valproate + Buspirone + Picrotoxin	1 300 20 3.5	12.0±0.84	25.0±0.80	3.9±0.24
V	Carbamazepine + Picrotoxin	420 3.5	21.5±3.72	31.7±3.64	3.5±0.27
VI	Carbamazepine + Buspirone + Picrotoxin	420 20 3.5	10.7±1.23	73.0±2.25	6.0±0.42
VII	Clonidine + Carbamazepine + Buspirone + Picrotoxin	1 420 20 3.5	44.9±5.47	32.2±5.21	3.4±0.22

[a]Picrotoxin administered ip, other drugs given po. Latency before picrotoxin: Sodium valproate–90 min, Buspirone–40 min, Clonidine–180 min,
*P<0.05;   **P<0.01;   ***P<0.001

antagonized the protective effect of CBZ. This is evidenced by significant decrease in latency (P<0.02) and a highly significant increase in duration and severity of convulsions (P<0.001) vs CBZ alone group. The mice under combined treatment exhibited squeaking and violent clonic and tonic convulsions. Pre-treatment with CLN caused a reversal of such antagonistic action. The values revealed a highly significant increase in onset time and decrease in severity scores (P<0.001). A significant decrease in duration of convulsions was also noted (P<0.01).

MES-induced convulsions

Immediate seizures were observed with 54 mA for 0.2s in 100% mice without mortality. All phases (tonic flexion, extension and clonus) were observed and recovery noted within 4–5 min. Some animals showed hypersensitivity to touch and aggressive behaviour even after recovery.

Effect of phenytoin (PHEN) on MES-induced seizures, its modulation by BUS and the

effect of pre-treatment with CLN on such modulation :

The results are presented in Fig. 1 and Table II. PHEN afforded marked protection against MES seizures. The duration of extensor phase in the treated group (3.30±1.91 seconds) was significantly (P<0.001) lower vs that in the control animals (15.80±1.24 seconds). A significant decrease in recovery time was also noted (control: 232.40±14.78 second, treated: 129.80±40.65 seconds, P<0.05). The percentage of animals showing extensor phase was also reduced. None of the mice in control of PHEN treated groups died. Pre-treatment with BUS completely reversed the protective effect of PHEN; the values for extensor phase (15.20±2.32 seconds) and recovery times (239.00±14.29 seconds) were not found to be significantly different from those in the MES control group. Further the % of animals showing extensor phase and mortality in the group under combined treatment were much higher vs those in the PHEN-treated group. CLN markedly reversed the antagonistic action of BUS and restored the protective effect of PHEN. This

TABLE II : Maximal electroshock-induced seizures in mice: Antagonism to the protective effect of Phenytoin by Buspirone and its reversal by Clonidine.

Group (n = 10)	Treatment[a]	Dose (mg/kg)	% Animals	
			Extensor phase	Mortality
I	MES	–	100	0
II	Phenytoin + MES	23.2	30	0
III	Phenytoin + Buspirone + MES	23.2 20	90	30
IV	Clonidine + Phenytoin + Buspirone + MES	1 23.2 20	30	0

[a]Latency before MES (54 mA, 0.2 sec), Phenytoin–120 min, Buspirone–40 min, Clonidine–180 min.
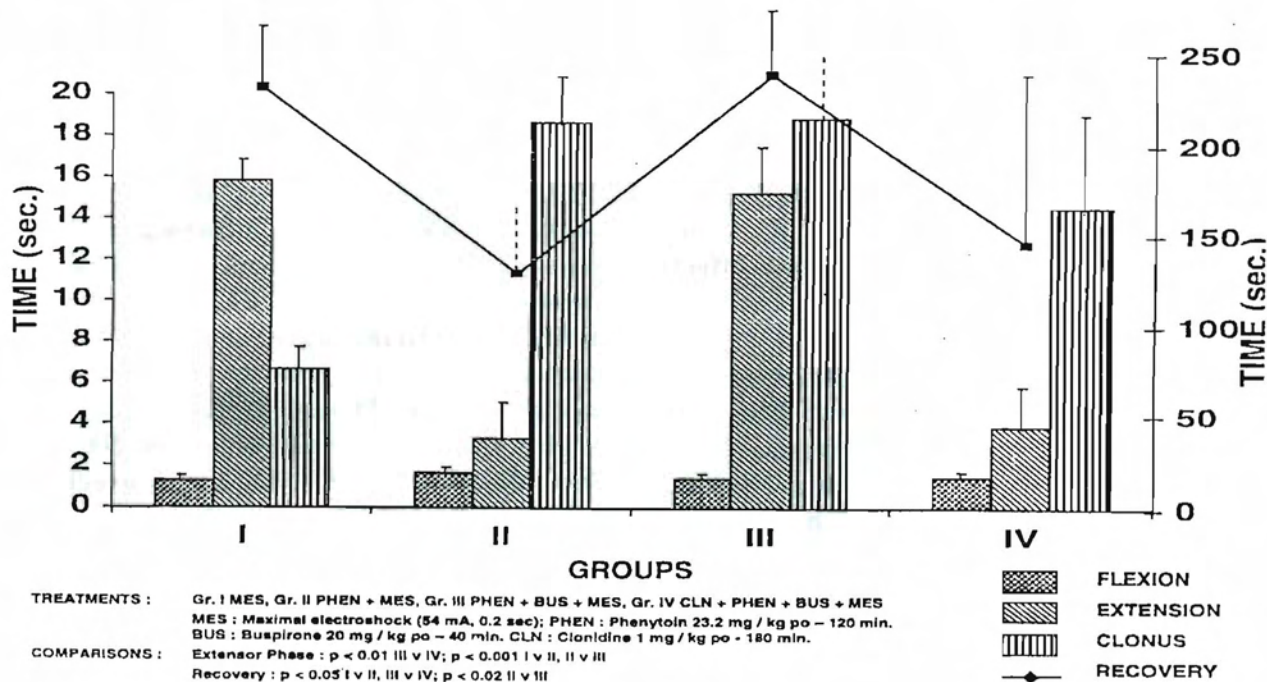
Fig. 1 : Maximal electroshock-induced seizures in mice: Antagonism to the protective effect of phenytoin by buspirone and its reversal by clonidine (α–2 receptor agonist).

is evidenced by the values for extensor phase (3.90±2.15 seconds) and recovery times (145.30±37.03 seconds) in this group which were not found to be statistically different from those in the PHEN alone group.

DISCUSSION

BUS is known to be devoid of anticonvulsant properties. This fact has been well documented by lack of such effects against varied convulsants (pentylenetetrazol, picrotoxin, bicuculline, strychnine and maximal electroshock) in experimental animals (1–3). This anxiolytic agent is rather stated to lower the seizure threshold suggesting a tendency for proconvulsant effects (5). Pilot studies in our laboratory showed that BUS potentiated PTX and MES-induced seizures (data not shown). The antagonism to the protective action of SVP, CBZ and PHEN by BUS, observed in our study, get support from its lack of anticonvulsant effect (1–3) as also our observations in pilot experiments. SVP is known to elicit its anticonvulsant effects through multiple mechanisms: sodium and calcium channels and GABAergic system while the anticonvulsant actions of CBZ and PHEN are mediated through sodium channels (14). Significant reversal of the anticonvulsant effects of SVP, CBZ and PHEN, shown by us, suggests that the pro-

convulsive effects are elicited through other mechanisms. We demonstrated marked reversal of the antagonistic action of BUS, against the protective effects of three clinically used anticonvulsants, by CLN: an α_2-adrenergic receptor agonist. This suggests the involvement of α_2-adrenoceptors in the proconvulsive effects of BUS.

While BUS is inactive at nor-adrenergic α_2-adrenoceptors (15), its metabolite 1–PP is known to possess potent presynaptic α_2-blocking properties (16, 17). Role of biogenic amines in seizure mechanisms is well documented (18). The susceptibility to convulsions increase when brain monoamine contents are lowered (19). NE release occurs upon massive neuronal discharges during seizures (18) and prejunctionally located α_2-adrenoceptors are known to modulate this release (20) These reports give a clue to mode of protective action elicited by CLN in the present model. Further, Electrophysiologic studies indicate that BUS increase the firing of noradrenergic neurons in the *locus ceruleus* (LC) (21). The noradrenergic receptors of the LC possess catecholamine receptors on or near the cell bodies that have pharmacological characteristic of 'presynaptic' α or α_2-adrenoceptors (22, 23). CLN is known to inhibit the firing rate of noradrenergic neurons in the LC (19), an effect opposite to that of BUS. Though CBZ is reported to inhibit the uptake and release of NE (24), this effect is mild with no appreciable influence on the firing rate of noradrenergic neurons in the LC (25).

The study suggests that buspirone has pro-convulsive property and this effect is modulated by clonidine. Therefore, it is concluded that central α_2-adrenergic mechanism may be involved in the pro-convulsive effects of buspirone.

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