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CIMETIDINE-INDUCED SEIZURES IN MICE

ANTAGONISM BY SOME GABAERGIC AGENTS

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Abstract—The effects of muscimol, aminooxyacetic acid (AOAA), diamino-*n*-butyric acid (DABA), baclofen, bicuculline, picrotoxin, strychnine, diazepam, phenobarbitone and phenytoin on cimetidine-induced seizures were studied in mice. Cimetidine (400–1000 mg/kg, i.p.) induced dose-dependent tonic convulsion. Muscimol, AOAA and DABA effectively protected mice against cimetidine-induced seizures. Bicuculline and picrotoxin significantly potentiated the seizures induced by cimetidine and effectively antagonized the protective effects of muscimol, AOAA and DABA against the seizures. Diazepam and phenobarbitone significantly protected the mice against cimetidine-induced seizures while phenytoin and strychnine did not significantly alter the seizures. These results indicate that the attenuation of central γ-aminobutyric acid neurotransmission may underlie cimetidine-induced seizures in mice.

Cimetidine, a specific histamine H_2 receptor antagonist, is effective in the management of peptic ulcers [1]. Although rare, cimetidine convulsions have been reported in patients even at standard doses [2, 3]. According to Gerald and Richter [4], proconvulsant or convulsant effects of histamine H_2 receptor antagonists observed at very high doses might not be associated with the histaminergic system but might be secondary due to (unspecific) effects known of these drugs to occur at toxic doses. However, the mechanism underlying seizures induced by cimetidine still remains uncertain. Sibilia *et al.* [5] reported that the γ-aminobutyric acid (GABA†) system may be involved in the prolactin releasing ability of cimetidine. Since GABA is a major inhibitory neurotransmitter in the mammalian brain and impairment of GABA neurotransmission is associated with epilepsy [6, 7], this study was designed to investigate the involvement of the GABA system in cimetidine seizures by studying the influence of muscimol, aminooxyacetic acid (AOAA), diamino-*n*-butyric acid (DABA), baclofen, bicuculline, picrotoxin, diazepam and phenobarbitone on seizures in mice. Furthermore, the effects of phenytoin, a standard antiepileptic drug, which is claimed to exert its antiepileptic action by blocking sodium potentials [8], and strychnine, a selective antagonist of glycine, an inhibitory neurotransmitter in the spinal cord and lower brainstem [9], were also studied on seizures induced by cimetidine in mice.

MATERIALS AND METHODS

Animals. Female albino mice (inbred in our Animal House) weighing between 20 and 25 g were used. The mice were normally housed in groups of eight per cage and were maintained on tap water and food *ad lib.*

Drugs. Muscimol (Sigma Chemical Co., St Louis, MO, U.S.A.), AOAA hemihydrochloride (Sigma), DL-2, 4-DABA dihydrochloride (Sigma), baclofen (Sigma), 5,5-diphenylhydantoin sodium salt (phenytoin, Sigma), strychnine hydrochloride (Geddes, Zimbabwe) and picrotoxin (Sigma) were all dissolved in physiological saline. (+) Bicuculline (Sigma) was suspended in 3% (v/v) Tween 80 and adjusted to the appropriate volume with physiological saline. Diazepam (Dizam, Caps, Zimbabwe) was dissolved in a minimum amount of polyethylene glycol 400 (Fluka AG, Buchs, Switzerland) and adjusted to the appropriate volume with physiological saline. Cimetidine ampoule (Tagamet, Smith Kline and French) and phenobarbitone ampoule (Paris Chemicals) were both diluted appropriately with physiological saline. All drugs were administered i.p. in a volume of 1 mL per 100 g body wt. Control animals received equal-volume injections of appropriate solvent vehicle and cimetidine. Fresh drug solutions were prepared on each day of the experiment. The drug pretreatment times prior to the administration of cimetidine were muscimol (1 hr), AOAA (20 min), DABA (30 min), baclofen (30 min), bicuculline (10 min), picrotoxin (20 min), diazepam (20 min), phenobarbitone (10 min), strychnine (10 min) and phenytoin (30 min). The pretreatment times as well as the doses used were obtained from preliminary studies in our laboratory.

Experimental procedure. The modified method of Vellucci and Webster [10] was employed in the assessment of the convulsant activity of cimetidine. Eight mice were used per experiment. The mice were placed singly in cages (25 × 15 × 15 cm) 30 min before the start of the experiment so as to acclimatize

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† Abbreviations: AOAA, aminooxyacetic acid; DABA, diamino-*n*-butyric acid; GABA, γ-aminobutyric acid.

Table 1. Convulsant effect of cimetidine in mice

Cimetidine (mg/kg, i.p.)	No. convulsed/ no. used	Onset of tonic convulsion (min)*
400	0/8	—
500	3/8	16.80 ± 3.21
600	8/8	12.68 ± 1.08
800	8/8	5.89 ± 0.63
1000	8/8	5.17 ± 0.49

* Values are means ± SEM.

them to their new environment. The experiments were performed between 12:00 and 18:00 each day to avoid a possible fluctuating influence of circadian rhythm on the susceptibility of mice to cimetidine seizures. The experiments were performed in a quiet room with an ambient temperature of 23 ± 2°. Each mouse was used only once for the seizure experiment. The mice were observed for seizures for 30 min following the administration of cimetidine. The time taken for the onset of tonic seizures and the proportion of animals convulsing were noted. Animals that did not convulse within 30 min were regarded as not convulsing.

Statistical analysis. The data on the onset of seizures were analysed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Analysis of the proportion of mice that convulsed was carried out using χ-squared test with Yate's correction for continuity.

RESULTS

Cimetidine produced tonic seizures that were dose dependent. The onset of tonic seizures and the proportion of mice that convulsed were shortened and increased, respectively, with increase in dose of cimetidine (Table 1). Before the onset of tonic seizures, there were intensive exploration of the cage, wide running and stunning by the mice. The tonic seizures were preceded by myoclonic jerks. All the animals that convulsed throughout the experiment died within 5 min.

Muscimol dose-dependently and significantly prolonged the onset and significantly reduced the incidence of cimetidine-induced tonic seizures. Similarly, AOAA and DABA significantly delayed the onset and significantly reduced the incidence of seizures induced by cimetidine in a dose-related manner. Baclofen, at all the doses used, did not significantly affect cimetidine-induced seizures (Table 2).

Bicuculline, at all the doses used, did not produce seizures but increased the movement of the mice in the cages. However, bicuculline potentiated the tonic seizures induced by cimetidine by significantly shortening the onset and significantly increasing the incidence of the seizures. The protective effects of muscimol, AOAA and DABA against cimetidine-induced seizures were markedly attenuated by bicuculline (Table 3). Similarly, picrotoxin, at all the doses used, increased the movement of the animals in the cages without producing any seizures. Picrotoxin potentiated the seizures elicited by cimetidine by significantly shortening the onset and significantly increasing the incidence of the seizures. Picrotoxin also significantly antagonized the anti-convulsant effects of muscimol, AOAA and DABA (Table 4).

Diazepam significantly and dose-dependently reduced the incidence and prolonged the onset of cimetidine-induced seizures. Similarly, phenobarbitone significantly delayed the onset and reduced the incidence of seizures induced by cimetidine in a dose-related manner. Phenytoin did not significantly affect the onset and the incidence of cimetidine-induced seizures (Table 5). Furthermore, strychnine, at all the doses used, did not produce any seizures although the animals were excited. Strychnine also

Table 2. Influence of muscimol, AOAA, DABA and baclofen on cimetidine-induced seizures in mice

Drugs and doses (mg/kg, i.p.)						
Cimetidine	Muscimol	AOAA	DABA	Baclofen	No. convulsed/ no. used	Onset of tonic convulsions (min)*
600	—	—	—	—	8/8	12.93 ± 1.11
600	0.5	—	—	—	7/8	14.43 ± 2.56
600	1.0	—	—	—	4/8	17.16 ± 1.63
600	2.0	—	—	—	2/8§	25.32 ± 3.31†
600	—	5.0	—	—	8/8	13.13 ± 1.10
600	—	10.0	—	—	5/8	16.40 ± 2.30
600	—	20.0	—	—	3/8‡	20.70 ± 2.41†
600	—	—	4.0	—	8/8	13.41 ± 1.27
600	—	—	8.0	—	7/8	13.72 ± 1.87
600	—	—	16.0	—	3/8‡	19.85 ± 2.17†
600	—	—	—	4.0	8/8	13.97 ± 1.57
600	—	—	—	8.0	8/8	13.59 ± 1.04
600	—	—	—	16.0	8/8	13.82 ± 1.93

* Values are means ± SEM.
† $P < 0.01$ vs cimetidine, Duncan's test. ‡ $P < 0.05$, § $P < 0.01$ vs cimetidine, χ-squared test.

Table 3. Influence of bicuculline and its interaction with muscimol, AOAA and DABA on cimetidine-induced seizures in mice

Drugs and doses (mg/kg, i.p.)					No. convulsed/ no. used	Onset of tonic convulsions (min)*
Cimetidine	Bicuculline	Muscimol	AOAA	DABA		
—	1.25	—	—	—	0/8	—
—	2.50	—	—	—	0/8	—
—	5.0	—	—	—	0/8	—
500	—	—	—	—	3/8	17.02 ± 3.26
500	1.25	—	—	—	3/8	14.51 ± 1.98
500	2.50	—	—	—	4/8	13.99 ± 1.83
500	5.0	—	—	—	7/8‖	13.84 ± 1.66
600	—	—	—	—	8/8	12.47 ± 0.98
600	5.0	—	—	—	8/8	6.50 ± 0.86†
600	—	2.0	—	—	2/8	24.78 ± 2.61
600	5.0	2.0	—	—	6/8‡	13.23 ± 1.03†
600	—	—	20	—	3/8	21.14 ± 2.50
600	5.0	—	20	—	8/8§	10.46 ± 0.95†
600	—	—	—	16	3/8	21.28 ± 1.79
600	5.0	—	—	16	8/8§	11.36 ± 1.0†

* Values are means ± SEM.

† $P < 0.01$ vs muscimol, AOAA or DABA plus cimetidine (600 mg/kg) or vs cimetidine (600 mg/kg) alone, Duncan's test. ‡ $P < 0.025$, § $P < 0.005$ vs muscimol, AOAA or DABA plus cimetidine (600 mg/kg), χ-squared test. ‖ $P < 0.01$ vs cimetidine (500 mg/kg), χ-squared test.

Table 4. Influence of picrotoxin and its interactions with muscimol, AOAA and DABA on cimetidine-induced seizures in mice

Drugs and doses (mg/kg, i.p.)					No convulsed/ no. used	Onset of tonic convulsions (min)*
Cimetidine	Picrotoxin	Muscimol	AOAA	DABA		
—	0.25	—	—	—	0/8	—
—	0.5	—	—	—	0/8	—
—	1.0	—	—	—	0/8	—
500	—	—	—	—	3/8	16.39 ± 2.87
500	0.25	—	—	—	3/8	16.10 ± 1.75
500	0.5	—	—	—	7/8‖	15.04 ± 1.94
500	1.0	—	—	—	7/8‖	12.63 ± 1.48
600	—	—	—	—	8/8	13.40 ± 0.65
600	0.5	—	—	—	8/8	8.12 ± 0.44†
600	—	2.0	—	—	2/8	26.21 ± 3.55
600	0.5	2.0	—	—	5/8‡	14.37 ± 1.82†
600	—	—	20.0	—	3/8	20.49 ± 2.08
600	0.5	—	20.0	—	8/8§	10.76 ± 0.59†
600	—	—	—	16.0	3/8	20.15 ± 1.82
600	0.5	—	—	16.0	8/8§	11.36 ± 0.61†

* Values are means ± SEM.

† $P < 0.01$ vs muscimol, AOAA or DABA plus cimetidine (600 mg/kg) or vs cimetidine (600 mg/kg) alone, Duncan's test. ‡ $P < 0.05$, § $P < 0.005$ vs muscimol, AOAA or DABA plus cimetidine (600 mg/kg), χ-squared test. ‖ $P < 0.01$ vs cimetidine (500 mg/kg), χ-squared test.

did not affect the incidence and the onset of cimetidine-induced seizures significantly (Table 6).

DISCUSSION

The data from the present study show that cimetidine produced pronounced dose-related tonic seizures in mice. Muscimol was shown to protect mice against cimetidine seizures in this study. According to Lloyd [9] and Vellucci [11], muscimol, a selective GABA agonist, produces its effects by interacting with $GABA_A$ receptors in the brain. It is therefore possible that the anticonvulsant effect of muscimol against cimetidine seizures might be due to GABA receptor activation. This suggestion was supported by our findings which showed that bicuculline, a potent $GABA_A$ receptor antagonist [9, 12], effectively enhanced cimetidine-induced

Table 5. Influence of diazepam, phenobarbitone and phenytoin on cimetidine-induced seizures in mice

Drugs and doses (mg/kg, i.p.)				No. convulsed/ no. used	Onset of tonic convulsions (min)*
Cimetidine	Diazepam	Phenobarbitone	Phenytoin		
600	—	—	—	8/8	12.68 ± 1.08
600	0.25	—	—	8/8	15.15 ± 2.24
600	0.5	—	—	3/8‡	22.40 ± 1.11†
600	1.0	—	—	1/8§	25.33 ± 0 †
600	—	10	—	8/8	13.01 ± 0.93
600	—	15	—	3/8‡	25.73 ± 0.31†
600	—	20	—	0/8§	—
600	—	—	8.0	8/8	12.81 ± 0.92
600	—	—	16.0	8/8	11.09 ± 0.77
600	—	—	32.0	8/8	11.12 ± 0.38

* Values are means ± SEM.
† $P < 0.01$ vs cimetidine, Duncan's test. ‡ $P < 0.05$, § $P < 0.001$ vs cimetidine, χ-squared test.

Table 6. Influence of strychnine on cimetidine-induced seizures in mice

Drugs and doses (mg/kg, i.p.)		No. convulsed/ no. used	Onset of tonic convulsion (min)*	
Cimetidine	Strychnine			
—	0.125	0/8	—	—
—	0.25	0/8	—	—
—	0.50	0/8	—	—
500	—	3/8	14.67	4.01
500	0.125	3/8	13.67	2.84
500	0.25	3/8	15.0	3.56
500	0.50	3/8	14.33	2.13
600	—	8/8	11.13	1.23
600	0.50	8/8	12.00	1.59

* Values are means ± SEM.

seizures as well as antagonized the protective effect of muscimol against the seizures. The present data essentially agree with the observation of Meldrum [13] who reported the anticonvulsant effect of muscimol against sound-induced seizures in mice.

In this study, AOAA exhibited an anticonvulsant effect against seizures produced by cimetidine. AOAA, a potent inhibitor of GABA transaminase, is believed to increase brain GABA levels by inhibiting GABA metabolism [14, 15]. The increased brain GABA levels as a result of the activity of AOAA might account for its antagonism of cimetidine-induced seizures. According to Iversen and Kelly [16] and Horton [17], DABA is a potent and selective inhibitor of GABA uptake which accumulates GABA in the brain. In the present study, DABA protected mice against cimetidine seizures. The anticonvulsant effect of DABA might be due to an increase in the brain levels of free GABA, resulting from DABA activity, present at the post-synaptic receptor sites. It is not surprising, therefore, that bicuculline, a potent $GABA_A$ receptor antagonist [9, 12], effectively antagonized the protective effects of both AOAA and DABA against seizures elicited by cimetidine in this study which further implicates GABAergic mechanisms in cimetidine seizures.

Picrotoxin, a specific GABA antagonist [9, 12] acts by blocking the $GABA_A$-linked chloride ion channel which normally opens to allow increased chloride ion conductance when the $GABA_A$ receptor is activated by GABA [9, 12]. It is of particular significance in this study that picrotoxin markedly augmented seizures induced by cimetidine and also antagonized the protective effects of muscimol, AOAA and DABA against the seizures. These data further support the involvement of GABAergic system in cimetidine seizures.

Baclofen, a potent $GABA_B$ agonist used mainly in spasticity [18, 19], has been shown to have anticonvulsant effects in some animal models of epilepsy [13, 20, 21]. According to Kerwin and Pycock [15] baclofen produces a GABA-mimetic effect by releasing GABA from the globus pallidus in the brain which may activate post-synaptic $GABA_A$ receptors. However, in the present study, baclofen did not affect cimetidine-induced seizures at all the doses used.

Post-synaptic $GABA_A$ receptors are functionally linked to benzodiazepine receptors, barbiturate receptors and chloride (Cl^-) channels to form the GABA–chloride ionophore complex which is involved in the modulation of GABAergic inhibitory transmission [6, 22, 23]. Since benzodiazepines and

barbiturates may increase chloride flux through chloride channels at $GABA_A$ receptor sites to enhance GABAergic functions [6, 22, 23], the antagonism of cimetidine seizures by diazepam and phenobarbitone is not surprising. However, in the present study, phenytoin, a standard antiepileptic drug, which is thought to exert its antiepileptic action by blocking sodium channels and inhibiting the generation of repetitive action potentials [8], did not affect cimetidine-induced seizures in mice at all the doses used. Similarly, strychnine, a specific inhibitor of the glycine receptor [9], did not alter cimetidine seizures.

In conclusion, this series of experiments demonstrated the convulsant effects of cimetidine which were antagonized by muscimol, AOAA and DABA but potentiated by bicuculline and picrotoxin, both of which also antagonized the anticonvulsant effects of muscimol, AOAA and DABA. These results suggest that GABA might be involved in cimetidine seizures.

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