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Effects of some GABAergic agents on quinine-induced seizures in mice

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Abstract. The effects of some GABAergic agents on seizures induced by quinine were studied in mice. Muscimol, AOAA, DABA and baclofen significantly protected mice against quinine-induced convulsions. Bicuculline effectively enhanced quinine-induced convulsions, and significantly attenuated the protective effects of muscimol, AOAA and DABA against convulsions induced by quinine. Diazepam and phenobarbitone significantly protected mice against convulsions induced by quinine. However, phenytoin did not affect quinine-induced seizures to any significant degree. These results indicate that the convulsant effect of quinine may be due to a disturbance in the status of the GABAergic system.
Key words. Quinine; seizures; GABAergic agents; GABAergic mechanism; diazepam; phenobarbitone; phenytoin.

Quinine, a cinchona alkaloid, is very consistent and effective in the treatment of severe malaria caused by chloroquine-resistant *Plasmodium falciparum* [1–4]. However, quinine in toxic doses can cause convulsions in patients [5]. The mechanism of the induction of seizures by antimalarial drugs is uncertain [6]. Since GABA is the major inhibitory neurotransmitter in the mammalian nervous system, impairment of GABA-mediated neurotransmission is a major factor underlying epileptic phenomena [7, 8]. The purpose of this study is, therefore, to investigate the influence of muscimol, aminooxyacetic acid (AOAA), diaminobutyric acid (DABA), baclofen, bicuculline, diazepam, phenobarbitone and phenytoin on quinine-induced seizures in mice, with a view to elucidating the mechanism underlying quinine seizures.

Materials and methods

Animals. Male albino mice (inbred in our Animal House, University of Zimbabwe, Harare) weighing 20–30 g were used in the study. The mice were normally housed in groups of eight per cage and maintained on tap water and food ad libitum. Each mouse was used for one experiment only.

Drugs. The following drugs were used: quinine hydrochloride (Sigma Chemical Co.), muscimol (Sigma Chemical Co.), aminooxyacetic acid hemihydrochloride (AOAA, Sigma Chemical Co.), DL-2,4-diamino-n-butyric acid dihydrochloride (DABA, Sigma Chemical Co.), baclofen (Sigma Chemical Co.), 5,5-diphenylhydantoin sodium salt (Phenytoin, Sigma Chemical Co.) and phenobarbitone (Paris Chemical), all dissolved in physiological saline; (+)bicuculline (Sigma Chemical Co.) suspended in Tween 80 and adjusted to the appropriate volume with physiological saline; diazepam (Valium, Roche Products) dissolved in a minimum amount of polyethylene glycol 400 (Fluka AG, Buchs) and adjusted to the appropriate volume with physiological saline. All drugs were administered intraperitoneally (i.p.) in a volume of 1 ml per 100 g body weight. Control animals received equal-volume injections of the vehicle.

The activity of the drugs used was not affected by the vehicle. Fresh drug solutions were prepared daily throughout the study. The pretreatment times prior to the injection of quinine were as follows: muscimol 1 h, AOAA 20 min, DABA 30 min, baclofen 30 min, bicuculline 10 min, diazepam 20 min, phenobarbitone 10 min and phenytoin 30 min. The pretreatment times as well as the doses used were established by preliminary studies in our laboratory.

Convulsant activity assessment. The method of Vellucci and Webster[9] was modified and used for the assessment of the convulsant activity of quinine. Eight mice per dose of drug were used. Mice were placed singly in a transparent perspex cage (25 cm × 15 cm × 15 cm) for 30 min to acclimatize to the new environment prior to administration of drug. The animals were observed for 30 min following the administration of quinine. The time taken for the onset of tonic convulsions and the numbers of animals convulsing were noted. Animals that did not convulse within 30 min were recorded as not convulsing. Both the control and test experiments were performed in a quiet room with an ambient temperature of 25 + 2 °C, between 08.00 and 20.00 h each day to avoid behavioral changes resulting from circadian rhythm.

Statistical analysis. The data for the time taken for the onset of tonic convulsions was compared by the paired Student's t-test. However, the proportion of mice that exhibited tonic convulsions was compared by Chi-squared test.

Results

Convulsant effect of quinine in mice. Quinine (200–350 mg/kg, i.p.) elicited dose-dependent tonic seizures in mice. The onset of seizures was shortened and the number of animals convulsing increased markedly with increases in quinine dose (table 1). Following administration of convulsant doses of quinine, there was intermittent increased exploratory activity by the mice, followed by random running; the mice then appeared to be stunned, and this was followed by tonic convulsions with the hindlimbs extended. All animals which convulsed in this study died within 10 s of tonic convulsion.

Effects of muscimol, AOAA, DABA and baclofen on quinine-induced seizures in mice. Pretreatment with muscimol (1.25–2 mg/kg, i.p.), AOAA (20 mg/kg, i.p.), DABA (16 mg/kg, i.p.) and baclofen (8 mg/kg, i.p.) significantly delayed the onset of quinine (250 mg/kg, i.p.)-induced seizures and also significantly reduced the number of animals convulsing. DABA (8 mg/kg, i.p.) and baclofen (4 mg/kg, i.p.) effectively reduced the number of animals convulsing but only weakly delayed the onset of quinine seizures. Baclofen (16 mg/kg, i.p.) completely protected mice against seizures elicited by quinine (250 mg/kg, i.p.; table 2).

Effects of bicuculline and its interactions with muscimol, AOAA and DABA on seizures induced by quinine in mice. Bicuculline (5 mg/kg, i.p.) effectively potentiated the convulsant effect of quinine (235 mg/kg, i.p.). The number of animals convulsing was significantly increased, and the time before the onset of seizures was slightly shortened by bicuculline (5 mg/kg, i.p.). Bicuculline at this dosage also profoundly shortened the time to the onset of seizures induced by quinine (250 mg/kg, i.p.). The protective effects of muscimol (2 mg/kg, i.p.), AOAA (20 mg/kg, i.p.) and DABA (8 mg/kg, i.p.) against seizures induced by quinine (250 mg/kg, i.p.) were significantly antagonized by bicuculline (5 mg/kg, i.p.), which effectively increased the number of animals convulsing and also shortened the time to the onset of the seizures (table 3).

Effects of diazepam, phenobarbitone and phenytoin on seizures induced by quinine in mice. Diazepam (0.5–1.0 mg/kg, i.p.) markedly reduced the number of animals convulsing in response to quinine (250 mg/kg, i.p.) and also effectively prolonged the time before the onset of the tonic seizures. Similarly, phenobarbitone (12.5 mg/kg, i.p.) profoundly delayed the onset of tonic seizures and reduced the numbers of animals convulsing due to quinine (250 mg/kg, i.p.). Phenobarbitone (15 mg/kg,

Table 1. Convulsant effect of quinine in mice

Quinine (mg/kg, i.p.)	No. convulsed/ No. used	Onset of tonic convulsion (min) Mean	±	SEM
200	0/8	-		
235	2/8	15.5		1.77
250	8/8	9.63		0.85
300	8/8	8.75		1.01
350	8/8	8.01		0.73

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

Drug and dose (mg/kg, i.p.) Quinine	Muscimol	AOAA	DABA	Baclofen	No. convulsed/ No. used	Onset of tonic convulsion (min) Mean	±	SEM
250	-	-	-	-	8/8	9.63		0.85
250	0.60	-	-	-	8/8	10.54		0.79
250	1.25	-	-	-	$3/8^{+}$	15.0**		1.41
250	2.0	-	-	-	$1/8^{+++}$	25.0****		0
250	-	5.0	-	-	8/8	7.25		0.86
250	-	10.0	-	-	7/8	7.43		1.10
250	-	20.0	-	-	$2/8^{++}$	19.0***		2.12
250	-	-	4.0	-	6/8	9.67		0.73
250	-	-	8.0	-	$3/8^{+}$	11.67		0.72
250	-	-	16.0	-	$3/8^{+}$	14.67*		1.19
250	-	-	-	4.0	$3/8^{+}$	13.0		0.94
250	-	-	-	8.0	$1/8^{+++}$	24.0****		0
250	-	-	-	16.0	$0/8^{++++}$	-		

*$p < 0.02$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$ respectively compared with quinine control, Student's t-test. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.005$, $^{++++}p < 0.001$ respectively compared with quinine control, Chi-squared test.

Table 3. Effects of bicuculline on seizures induced by quinine in mice and its interactions with muscimol, AOAA and DABA

Drug and dose (mg/kg, i.p.)					No. convulsed/	Onset of tonic convulsion (min)		
Quinine	Bicuculline	Muscimol	AOAA	DABA	No. used	Mean	±	SEM
235	-	-	-	-	2/8	16.02		1.81
250	-	-	-	-	8/8	9.34		0.78
235	2.5	-	-	-	3/8	14.95		1.03
235	5.0	-	-	-	$6/8^{++}$	11.87		1.29
250	5.0	-	-	-	8/8	4.63**		0.55
250	-	2.0	-	-	1/8	27.14		1.04
250	5.0	2.0	-	-	$5/8^{+++}$	11.39**		0.26
250	-	-	20	-	2/8	19.61		1.90
250	5.0	-	20	-	$5/8^{+}$	14.70*		1.12
250	-	-	-	8.0	3/8	12.25		0.53
250	5.0	-	-	8.0	$7/8^{+++}$	7.85*		0.71

*$p < 0.05$ compared with quinine plus DABA or AOAA, Student's t-test. **$p < 0.001$ compared with either quinine (250 mg/kg, i.p.) control alone or plus muscimol, Student's t-test. $^{+}p < 0.05$ compared with quinine (250 mg/kg, i.p.) plus AOAA, Chi-squared test. $^{++}p < 0.025$ compared with quinine (235 mg/kg, i.p.) control, Chi-squared test. $^{+++}p < 0.01$ compared with quinine (250 mg/kg, i.p.) plus muscimol or DABA, Chi-squared test.

Table 4. Effects of diazepam, phenobarbitone and phenytoin on seizures induced by quinine in mice

Drug and dose (mg/kg, i.p.)				No. convulsed/No. used	Onset of tonic convulsion (min)		
Quinine	Diazepam	Phenobarbitone	Phenytoin		Mean	±	SEM
250	-	-	-	8/8	8.47		0.60
250	0.25	-	-	8/8	14.11*		1.04
250	0.50	-	-	$2/8^{++}$	15.95*		1.10
250	1.0	-	-	$1/8^{+++}$	20.04**		1.07
250	-	10.0	-	6/8	13.83*		1.05
250	-	12.5	-	$3/8^{+}$	19.19**		1.0
250	-	15.0	-	0.8^{++++}	-		
250	-	-	8.0	8/8	10.23		0.96
250	-	-	16.0	8/8	10.61		1.01
250	-	-	32.0	8/8	8.98		1.12

*$p < 0.02$, **$p < 0.001$ respectively compared with quinine control, Student's t-test. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.005$, $^{++++}p < 0.001$ respectively compared with quinine control, Chi-squared test.

i.p.) completely abolished quinine (250 mg/kg, i.p.)-induced seizures. However, phenytoin (8–32 mg/kg, i.p.) did not alter the incidence or onset of seizures induced by quinine (250 mg/kg, i.p.; table 4).

Discussion

It is generally believed that GABA is the major inhibitory neurotransmitter in the brain, and the concept that the impairment of GABA-mediated neurotransmission is associated with convulsions is now widely accepted [7, 8, 10, 11]. Inhibitory effects of GABA are mediated in part by the $GABA_A$ receptors which are associated with epilepsy, among other things [8, 12]. Muscimol, a selective $GABA_A$ receptor agonist, mimics the inhibitory effects of GABA by interacting with $GABA_A$ receptors in the brain [12, 13]. In this study, muscimol (1.25–2 mg/kg, i.p.) antagonized the convulsant effect of quinine (250 mg/kg, i.p.), which suggests the probable involvement of $GABA_A$ receptors in quinine seizure. It is significant that the protective effect of muscimol against quinine seizure was antagonized by bicuculline, a potent $GABA_A$ receptor antagonist [12, 14] which also enhanced the convulsant effect of quinine. This further implicates the involvement of GABA receptors in the action of quinine. The present data are in agreement with the observation of Meldrum [15], who reported the anticonvulsant effect of muscimol against sound-induced seizures in mice.

In this study, AOAA (20 mg/kg, i.p.) protected mice against quinine (250 mg/kg, i.p.)-induced seizure. AOAA is a powerful inhibitor of GABA transaminase (GABA-T), an enzyme which metabolizes GABA [16, 17] thereby leading to an increase in brain GABA levels. The increased brain GABA levels might be responsible for the antagonism of quinine seizures. DABA is a potent and selective inhibitor of GABA uptake [18, 19], which results in the accumulation of GABA in the brain. In the present study, DABA (8–16 mg/kg, i.p.) significantly protected mice against quinine (250 mg/kg, i.p.)-induced seizures. The antagonism of quinine seizures by DABA might be due to an increase in the brain levels of free GABA available at the postsynaptic receptor sites. Bicuculline, a potent $GABA_A$ receptor antagonist, significantly antagonized the protective effects of both AOAA and DABA against quinine seizures in this study, which further supports the involvement of GABA mechanisms in quinine seizures.

Interestingly, in the present study, baclofen (4–16 mg/kg, i.p.) effectively protected mice against quinine (250 mg/kg, i.p.)-induced seizures. Baclofen is a potent $GABA_B$ receptor agonist used mainly to treat spasticity [20, 21]. However, Kerwin and Pycock [17] reported that baclofen enhanced the release of GABA from slices of rat globus pallidus. It is, therefore, possible that the observed GABAmimetic effects of baclofen in quinine

seizures may be due to its enhancement of the release of GABA, which accumulates at postsynaptic receptor sites to overcome the seizures. These data agree with the observation of Meldrum [15] and of Bein [22] who reported the anticonvulsant effect of baclofen in mice against sound-induced seizures, and against metrazol and thiosemicarbazide seizures, respectively.

Diazepam (0.5–1.0 mg/kg, i.p.) and phenobarbitone (12.5–15 mg/kg, i.p.) significantly protected animals against quinine (250 mg/kg, i.p.)-induced seizures. Postsynaptic $GABA_A$ receptors are functionally linked to benzodiazepine receptors, barbiturate receptors and chloride channels to form a GABA-chloride ionophore complex which is involved in the modulation of GABAergic inhibitory transmission [23–25]. Since benzodiazepines and barbiturates may increase chloride flux through chloride channels at $GABA_A$ receptor sites to enhance GABAergic inhibition [23–25], the antagonism of quinine seizures by diazepam and phenobarbitone is not surprising.

Interestingly, in the present study phenytoin, a standard antiepileptic drug [26], did not affect seizures induced by quinine. Phenytoin is thought to exert its antiepileptic action by blocking sodium channels and inhibiting the generation of repetitive action potentials [26, 27].

The data accumulated in this study suggest that enhancement of GABAergic mechanisms protected mice against quinine seizures, while blockade enhanced seizures. Thus, quinine may be producing seizures by interfering with GABA neurotransmission in the central nervous system. Furthermore, since bicuculline enhanced quinine seizures and also attenuated the protective effects of muscimol, aminooxyacetic acid and diaminobutyric acid against the seizures, the quinine effect may be at the postsynaptic level.

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