ELSEVIER

European Journal of Pharmacology 345 (1998) 257–260

ejp

Short communication

Chlormethiazole anticonvulsive efficacy diminished by *N*-methyl-D-aspartate but not kainate in mice

Slawomir Pilip [a], Ewa M. Urbanska [a,b], Stanislaw J. Czuczwar [a,b], Zdzislaw Kleinrok [a], Waldemar A. Turski [a,b,*]

[a] *Department of Pharmacology and Toxicology, Medical University School, Jaczewskiego 8, 20-090 Lublin, Poland*
[b] *Department of Clinical Toxicology, Institute of Agricultural Medicine, Jaczewskiego 2, 20-950 Lublin, Poland*

Received 4 December 1997; revised 10 February 1998; accepted 13 February 1998

Abstract

The aim of this study was to evaluate the effect of *N*-methyl-D-aspartate (NMDA) and kainate used at nonconvulsive doses upon protective efficacy of chlormethiazole against maximal electroshock-induced seizures. NMDA (50 mg/kg, i.p.) reduced the anticonvulsant potency of chlormethiazole increasing its ED_{50} value from 126.9 to 155.0 mg/kg. The effect of NMDA was completely reversed by the competitive NMDA receptor antagonist D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116) (0.06 mg/kg i.p.). Kainic acid (9 mg/kg i.p.) did not affect the anticonvulsive properties of chlormethiazole. Our results suggest that NMDA but not kainate receptor-mediated events participate in the anticonvulsant action of chlormethiazole. © 1998 Elsevier Science B.V.

Keywords: Chlormethiazole; Anticonvulsant; NMDA (*N*-methyl-D-aspartate); Kainate; Seizure; (Mouse)

1. Introduction

It is now widely accepted that pathogenesis of epileptic disorders might be related to the impaired inhibitory, mediated mainly by γ-aminobutyric acid (GABA), and/or enhanced excitatory (predominantly glutamatergic) transmission. Increased plasma levels of endogenous excitatory amino acid receptor agonists were reported in patients with epilepsy (Huxtable et al., 1983). Glutamate content in extracellular fluid was shown to increase prior to the occurrence of seizures in humans (During and Spencer, 1993). Experimental stimulation of ionotropic excitatory amino acid receptors of *N*-methyl-D-aspartic acid (NMDA) or kainic acid type may evoke seizures in rodents (Ben-Ari et al., 1980; Zaczek et al., 1981). NMDA and non-NMDA receptor antagonists are potent anticonvulsants in different experimental models of chemically or electrically induced seizures (Croucher et al., 1982; Turski et al., 1990; Chapman et al., 1991a,b). Moreover, they enhance the protective activity of conventional antiepileptics against maximal electroshock-induced seizures in mice (Czuczwar et al., 1984; Urbanska et al., 1991; Pietrasiewicz et al., 1993).

Rational use of antiepileptics has to be correlated with the knowledge concerning mechanisms of antiepileptic activity as well as recognizing factors that may alter the individual susceptibility to anticonvulsants. Chlormethiazole displays potent hypnotic, sedative and anticonvulsive properties and is able to interrupt epileptic seizures in humans and experimental animals. The drug is used in patients with acute alcohol-withdrawal syndrome, status epilepticus, eclampsia and pre-eclampsia. Mechanism of chlormethiazole antiepileptic action has been attributed mainly to enhanced GABA-ergic neurotransmission (see, for review, Smith and Jewkes, 1995). In the present study, we have evaluated the influence of enhanced NMDA and kainate receptor stimulation upon the anticonvulsive efficacy of chlormethiazole in maximal electroshock-induced seizures in mice.

2. Materials and methods

2.1. Animals

The experiments were performed on male Swiss mice, 20–26 g body weight. Animals were kept in colony cages under standard laboratory conditions (ambient temperature

* Corresponding author. Tel./fax: +48-81-747-89-24; e-mail: andreas@galen.imw.lublin.pl

of 20 ± 1°C, free access to food and tap water, natural light/dark cycle). Experimental groups consisting of 8–10 animals were chosen by means of a randomized schedule.

2.2. Drugs

Chlormethiazole (Heminevrin, Astra, Sweden) was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) and administered subcutaneously (s.c.) at doses of 110–180 mg/kg, 45 min before induction of seizures. *N*-methyl-D-aspartic acid (NMDA, RBI, Natick, MA, USA) was brought into solution with a minimum quantity of 1 M NaOH, further diluted with distilled water and given intraperitoneally (i.p.) at the doses of 30 and 50 mg/kg. Kainic acid (RBI) and D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116; kindly supplied by Mrs. A. Suter, Novartis Pharma, Basel, Switzerland) were dissolved in distilled water and administered at the dose of 9 mg/kg s.c. and 0.06 mg/kg i.p., respectively. NMDA, kainate and CGP 40116 were administered 15, 60 and 120 min before electroconvulsions, respectively. Studied doses of NMDA (50 mg/kg) and kainate (9 mg/kg) were equivalent to 75% of the CD_{16}, i.e., the convulsive dose inducing seizures in 16% of animals studied for both drugs and were established in preliminary experiments. Administration was designed prior to chlormethiazole at the interval corresponding to the peak latency of seizures following the application of NMDA and kainate alone. Injection volume was 0.05 ml/10 g body weight for drugs given s.c. and 0.1 ml/10 g body weight for substances administered i.p.

2.3. Electroconvulsions

Electroconvulsions were generated by a Hugo Sachs (Type 221, Freiburg, Germany) stimulator (50 Hz, the stimulus duration being 0.2 s, varying current strength) and delivered via ear-clip electrodes. Tonic hind-limbs extension was considered as the endpoint. The convulsive threshold (CS_{50}), i.e., the current strength (in mA), necessary to induce tonic hindlimb extension in 50% of the animals tested was estimated. Maximal electroshock-induced seizures were evoked by the stimulus of 25 mA strength.

2.4. Statistics

CS_{50}, ED_{50} (the dose of chlormethiazole required to prevent seizures in 50% of studied animals) and statistical analysis of the results were calculated by fitting the data by computerized probit analysis according to the method of Litchfield and Wilcoxon (1949). At least three groups of mice, each consisting of 8–10 animals, were used to estimate CS_{50} or ED_{50} value.

Table 1
Effects of kainate, NMDA and CGP 40116 upon the electroconvulsive threshold in mice

Treatment (mg/kg)	*n*	Electroconvulsive threshold (CS_{50} in mA)
Saline	32	5.9 (5.1–6.8)
Kainate (9)	24	5.3 (4.8–5.8)
Saline	32	5.3 (5.1–5.5)
NMDA (50)	24	5.4 (5.2–5.5)
Saline	24	5.9 (5.6–6.2)
CGP 40116 (0.06)	24	6.1 (5.7–6.5)

n = number of mice. The CS_{50} values (with 95% confidence limits) and statistical comparisons were calculated by fitting the data by computerized probit analysis based on the method of Litchfield and Wilcoxon (1949).

3. Results

NMDA used at 50 mg/kg i.p., kainate given at 9 mg/kg s.c. and CGP 40116 at 0.06 mg/kg i.p. did not affect the CS_{50} (Table 1). NMDA administered at the dose of 50 mg/kg i.p. increased the ED_{50} of chlormethiazole against maximal electroshock-induced seizures from 126.9 to 155.0 mg/kg i.p., ($P < 0.01$; Table 2). CGP 40116 given at the dose of 0.06 mg/kg i.p. did not affect the anticonvulsant activity of chlormethiazole against maximal electroshock-evoked convulsions (Table 2). The pretreatment with CGP 40116 reversed the effect of NMDA (50 mg/kg i.p.) upon chlormethiazole reducing its ED_{50} from 155.0 to 133.0 mg/kg, ($P < 0.05$; Table 2). Kainic acid applied at the dose of 9 mg/kg i.p. did not affect anticonvulsive properties of chlormethiazole against maximal electroshock-induced seizures (Table 2).

Table 2
Effects of kainate, NMDA and CGP40116 on the anticonvulsive action of chlormethiazole against maximal electroshock-induced seizures in mice

Treatment (mg/kg)	*n*	ED_{50} of chlormethiazole (mg/kg)
Saline	24	137.6 (125.9–150.4)
Kainate (9)	24	134.1 (113.7–158.2)
Saline	40	126.9 (116.2–138.7)
CGP 40116 (0.06)	32	128.7 (112.6–147.1)
NMDA (30)	24	127.2 (113.9–142.2)
NMDA (50)	32	155.0 (139.3–172.4)[a]
NMDA (50) + CGP 40116 (0.06)	32	133.0 (119.1–148.6)[b]

[a] $P < 0.01$ vs. respective saline-treated group; [b] $P < 0.05$, compared NMDA (50) + CGP 40116 (0.06) vs. NMDA (50)-treated group; *n* = number of mice. The ED_{50} values and statistical comparisons were calculated by fitting the data by computerized probit analysis based on the method of Litchfield and Wilcoxon (1949).

4. Discussion

Data presented here suggest that activation of excitatory amino acid receptors of NMDA type may diminish the anticonvulsive effects of chlormethiazole against maximal electroshock-induced seizures in mice. The competitive NMDA receptor antagonist CGP 40116 reversed the effect exerted by NMDA upon chlormethiazole antiepileptic activity. In contrary to NMDA, kainate receptor stimulation did not influence anticonvulsive properties of chlormethiazole under studied conditions. Both, NMDA and kainate, were used in the doses that evoked neither seizure response on their own nor alterations in the electroconvulsive threshold.

Basing on the observation that CGP 40116 was able to antagonize the effect of NMDA upon chlormethiazole action, it is reasonable to conclude that receptor activation contributes to this phenomenon. The fact that NMDA receptor antagonist reversed NMDA-induced alteration of chlormethiazole potency practically excludes the possibility of a pharmacokinetic interaction between chlormethiazole and NMDA.

Antiepileptic activity of chlormethiazole is considered to be mediated mainly via enhanced GABA-ergic transmission. It was suggested that chlormethiazole binds to picrotoxin binding site within $GABA_A$ receptor complex and prolongs the duration of Cl^- channel opening (Ögren, 1986). Electrophysiological studies demonstrated that chlormethiazole potentiates $GABA_A$-activated currents and at higher doses directly activates the $GABA_A$ receptors (Hales and Lambert, 1992).

There are sparse data on the excitatory amino acids system activity and the mechanism of action of chlormethiazole. Results obtained during binding studies did not suggest that the drug may exert its effects via interaction with glutamatergic receptors. Chlormethiazole did not affect the binding of the noncompetitive NMDA receptor antagonist, dizocilpine, to rat cortical membranes or the stimulation of this binding by glutamate, glycine or spermidine (Cross et al., 1993). Chlormethiazole was found to have a weak action on kainate binding within striatum (Ögren, 1986).

Contradictory data have been obtained during in vivo studies. Chlormethiazole was shown to diminish electrophysiological response to microiontophoretically coadministered glutamate in the brainstem (Gent and Wacey, 1983). On the other hand, chlormethiazole applied topically to the rat cortical surface or given i.p. did not influence *N*-methyl-DL-aspartate (NMDLA), kainate or quisqualate effects in the cerebral cortex (Addae and Stone, 1988). Another group, however, has demonstrated that intravenous administration of chlormethiazole antagonized NMDA-induced alterations in stimulation-evoked potential (Thoren and Sjolander, 1993). All of these results have been obtained from studies performed on anesthetized rats. Experiments carried out on mice have revealed that i.p. application of chlormethiazole blocked tonic seizures and prolonged the latency to the onset of clonic convulsions precipitated by systemic injection of NMDLA in mice (Cross et al., 1993). The inability of chlormethiazole to affect dizocilpine binding described in the same study has prompted the authors to conclude that chlormethiazole does not antagonize NMDA-induced seizures by interacting with NMDA receptor complex (Cross et al., 1993).

Our study provides new evidence suggesting that enhanced NMDA receptor-mediated transmission may alter the anticonvulsive activity of chlormethiazole. It also indicates that activation of excitatory amino acid receptors of NMDA but not kainate type contributes to chlormethiazole anticonvulsive efficacy.

Acknowledgements

This research was supported by grants D.S.323/97 and P.W.727/97.

References

Addae, J.I., Stone, T.W., 1988. Effects of anticonvulsants on responses to excitatory amino acids applied topically to rat cerebral cortex. Gen. Pharmacol. 19, 455–462.

Ben-Ari, Y., Tremblay, E., Ottersen, O.P., 1980. Injections of kainic acid into the amygdaloid complex of the rat: an electrographic, clinical and histological study in relation to the pathology of epilepsy. Neuroscience 5, 515–528.

Chapman, A.G., Graham, J., Patel, S., Meldrum, B.S., 1991a. Anticonvulsant activity of two orally active competitive *N*-methyl-D-aspartate antagonists, CGP 37849 and CGP 39551 against sound-induced seizures in DBA/2 mice and photically induced myoclonus in *Papio papio*. Epilepsia 32, 578–587.

Chapman, A.G., Smith, S.E., Meldrum, B.S., 1991b. The anticonvulsant effect of the non-NMDA antagonists, NBQX and GYKI 52466, in mice. Epilepsy Res. 9, 92–96.

Cross, A.J., Snape, M.F., Green, A.R., 1993. Chlormethiazole antagonizes seizures induced by *N*-methyl-DL-aspartate without interacting with the NMDA receptor complex. Psychopharmacology 112, 403–406.

Croucher, M.J., Collins, J.F., Meldrum, B.S., 1982. Anticonvulsant action of excitatory amino acid antagonists. Science 216, 899–901.

Czuczwar, S.J., Turski, L., Schwarz, M., Turski, W.A., Kleinrok, Z., 1984. Effects of excitatory amino acid antagonists on the anticonvulsant action of phenobarbital or diphenylhydantoin in mice. Eur. J. Pharmacol. 100, 357–362.

During, M.J., Spencer, D.D., 1993. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. Lancet 341, 1607–1610.

Gent, J.P., Wacey, T.A., 1983. The effects of chlormethiazole on the single unit activity in rat brain: interactions with inhibitory and excitatory neurotransmitters. Br. J. Pharmacol. 80, 439–444.

Hales, T.G., Lambert, J.J., 1992. Modulation of GABA and glycine receptors by chlormethiazole. Eur. J. Pharmacol. 210, 239–246.

Huxtable, R.J., Laird, H., Lippincott, S.E., Wolson, P., 1983. Epilepsy and the concentration of plasma amino acids in humans. Neurochem. Int. 5, 125–135.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Ögren, S.O., 1986. Chlormethiazole—mode of action. Acta Psychiatr. Scand. 73 (Suppl. 329), 13–27.

Pietrasiewicz, T., Czechowska, G., Dziki, M., Turski, W.A., Kleinrok, Z., Czuczwar, S.J., 1993. Competitive NMDA receptor antagonists enhance the antielectroshock activity of various antiepileptics. Eur. J. Pharmacol. 250, 1–7.

Smith, M., Jewkes, D.A., 1995. Chlormethiazole. In: Lewy, R.H., Meldrum, B.S. (Eds.), Antiepileptic Drugs, 4th edn., Raven Press, New York, pp. 953–962.

Thoren, P., Sjolander, M., 1993. Chlormethiazole attenuates the derangement of sensory evoked potential (SEP) induced by ICV administration of NMDA. Psychopharmacology (Berlin) 111, 256–258.

Turski, W.A., Urbanska, E., Dziki, M., Parada-Turska, J., Ikonomidou, C., 1990. Excitatory amino acid antagonists protect mice against seizures induced by bicuculline. Brain Res. 514, 131–134.

Urbanska, E., Dziki, M., Kleinrok, Z., Czuczwar, S.J., Turski, W.A., 1991. Influence of MK-801 on the anticonvulsant activity of antiepileptics. Eur. J. Pharmacol. 200, 277–282.

Zaczek, R., Collins, J., Coyle, J.T., 1981. *N*-methyl-D-aspartic acid: a convulsant with weak neurotoxic properties. Neurosci. Lett. 24, 181–186.