Neuroscience Letters, 157 (1993) 123–126

NSL 09628

N-Methyl-D-aspartate receptors contribute to guanidinosuccinate-induced convulsions in mice

Rudi D'Hooge, Yin-Quan Pei* and Peter P. De Deyn

Laboratory of Neurochemistry and Behavior, Born-Bunge Foundation, University of Antwerp, Antwerp (Belgium) and Department of Neurology, Middelheim General Hospital, Antwerp (Belgium)

(Received 8 February 1993; Revised version received 5 April 1993; Accepted 5 April 1993)

Key words: Guanidino compound; Uremia; Convulsant; Anti-epileptic drug; Epilepsy; NMDA receptor

Increased levels of the endogenous convulsant guanidinosuccinate (GSA) might contribute to the epileptic symptomatology presenting in patients with renal failure. Little is known, however, about the underlying epileptogenic mechanism of guanidinosuccinate-induced convulsions. In this paper, we present pharmacological evidence for a direct excitatory action of this compound. In particular, the close involvement of *N*-methyl-D-aspartate (NMDA) receptors in the pathogenesis of GSA-induced generalized convulsions is suggested. GSA potentiated NMDA-induced convulsions significantly, but not L-glutamate- or kainate-induced convulsions. Conversely, and in addition, NMDA receptor antagonists, like D(-)-2-amino-5-phosphonovalerate, CGP 37849 [DL)-(E)-2-amino-4-methyl-5-phosphono-3-pentenoate] or ketamine (but not kynurenate), blocked the convulsions induced by i.c.v. injection of GSA dose dependently whereas anti-epileptic drugs, like carbamazepine, diazepam, phenobarbital or valproate, only abolished the tonic extension phase of these convulsions. Thus, NMDA receptors appear to be involved, at least partly, in GSA-induced convulsions.

Although its exact biosynthetic pathway is still conjectural, guanidinosuccinate (GSA) is present in physiological fluids and tissues (including brain) of man and other ureotelic animals [16]. Increased GSA levels in patients with renal insufficiency [6] might contribute to the epileptic symptomatology presenting in renal failure [21]. Since the first report on the convulsive properties of guanidinobutyrate [11], members of our group and others have demonstrated that many guanidino compounds share the ability to induce epileptiform phenomena in different animal species [19]. We have recently shown that generalized clonic and tonic convulsions, and concomitant epileptiform electrocorticographic discharges, follow the i.p. injection of GSA in mice [8]. The excitant properties of GSA have been proposed to be due to the blockade of chloride channels associated with inhibitory glycine and γ-aminobutyric acid (GABA) receptors [5]. However, inhibitory properties could be explained by this guanidino group-dependent blocking of ionic channels as well. For example, the blockade of Na channels by tetrodotoxin is thought to be due to the presence of a guanidino group in the structural formula of this neurotoxin [1]. Vogel et al. [25] reported current-dependent blocking of endplate channels by monoguanidines and Reynolds and Rothermund [26] suggested that monoguanidines may block *N*-methyl-D-aspartate (NMDA) receptor-associated Ca channels in a similar manner to Mg^{2+}.

On the other hand, GSA bears a close structural resemblance to excitatory amino acid receptor ligands. A number of analogues of aspartate and glutamate, including GSA, have been found to depolarize cat spinal motoneurones in vivo [3]. Kizer et al. [12] have suggested that the structural requirements for both this kind of neuroexcitatory action as for neurotoxicity are the presence of a primary or a secondary amino group alpha to a carboxylic acid that is three to four bond lengths removed from another acidic group. Many succinate derivatives have been shown to induce convulsions after i.c.v. administration in mice [14]. Quinolinate is such a succinate derivative and an endogenous tryptophan metabolite, which has also been suggested to play a role in neurological complications of metabolic disorders. The convulsive effects of excitant kynurenines, like quinolinate, comprise frequent clonic seizures, often preceded by a phase of motor hyperactivity (e.g., wild-running fits), leading to tonic extension of the limbs, and eventually

Correspondence: P.P. De Deyn, Laboratory of Neurochemistry and Behavior, Born-Bunge Foundation, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium.

*Visiting from Beijing Medical University, Beijing, People's Republic of China.

death [24]. Such are also the characteristics of GSA-induced convulsions [8].

This study is meant to shed some light upon the possible involvement of excitatory amino acid receptors in GSA-induced behavioral effects. Male and female Swiss mice (30–40 g) were used. Animals were housed under standard conditions (12–12 h light–dark cycle, constant room temperature and humidity), with free access to food and water. GSA, L-glutamate (mono-Na salt), NMDA and kainate as well as carbamazepine, diazepam, phenobarbital, Na valproate, D(-)-2-amino-5-phosphonovalerate (APV), ketamine and kynurenate were purchased from Sigma Chemical, St Louis, MO. CGP 37849 [DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoate] was a kind gift from Ciba-Geigy Belgium (M. Geelhand, Medical Director). For i.c.v. administration, all drugs were dissolved in saline and injected in a volume of 5 μl into the left lateral brain ventricle according to an earlier described technique [9]; 50 μl of a 1% lidocaine solution was used as a local anaesthetic. Phenobarbital, Na valproate, CGP 37849 and ketamine were dissolved in saline, carbamazepine and diazepam were suspended in 30% polyethylene glycol; all were injected i.p. in a volume of 10 ml/kg body wt, 40 min before i.c.v. injection of GSA, except diazepam and carbamazepine (20 min), Na valproate (1 h) and CGP 37849 (2 h). CD_{50} (dose inducing clonic convulsions in 50% of the animals) and ED_{50} (effective dose in 50% of the animals) values and their 95% confidence limits were determined with Weil's method [26] and significance of differences between CD_{50} values according to Litchfield and Wilcoxon [15]. In the potentiation experiments, the CD_3 dose (maximal dose inducing no convulsions in control animals) of GSA (3.125 μg or 17.8 nmol/mouse) was co-injected i.c.v. with the convulsants tested; in the experiments with the anti-epileptic drugs and with the NMDA antagonists, i.c.v. injection of the CD_{97} dose (minimal dose-inducing clonic convulsions in all control animals) of GSA (12.5 μg or 71.3 nmol/mouse) succeeded i.p. drug injection, except for APV which was co-injected with GSA. Immediately after their i.c.v. injection, the animals were placed in individual cylindrical plastic cages for the assessment of epileptiform activity within a 30-min observation period using criteria described earlier [9].

Although a slight potentiation of L-glutamate- and kainate-induced convulsions did occur, co-injection of the CD_3 dose of GSA did not decrease the CD_{50} of either substance significantly (Table I). However, co-injection of the CD_3 dose of GSA did result in nine-fold decrease of the CD_{50} of NMDA. Thus, like with the other succinate derivative quinolinate [24], GSA-induced convulsions seem to be related to NMDA-type excitatory amino acid receptors exclusively.

TABLE I

POTENTIATING EFFECTS OF GSA UPON EXCITATORY AMINO ACID-INDUCED CONVULSIONS

The table gives CD_{50} dose inducing clonic convulsions in 50% of the animals and 95% confidence interval between parentheses) of i.c.v. administered L-glutamate, kainate and NMDA, with and without co-injection of CD_3 (maximal dose inducing no convulsions in control animals, $N > 30$) GSA and potency ratios (ratio of CD_{50} without GSA on CD_{50} with GSA co-injection). Only, the CD_{50} of *N*-methyl-D-aspartate is significantly decreased by GSA co-injection (***$P < 0.001$).

Convulsant	CD_{50} without GSA	CD_{50} with GSA	Potency Ratio
L-glutamate	1150 (590–2250) nmol	680 (350–1340) nmol	1.68
Kainate	330 (250–430) pmol	210 (160–290) pmol	1.57
NMDA	450 (350 580) pmol	50 (32–80) pmol***	9.00

Accordingly, both competitive NMDA receptor antagonists, like APV and CGP 37849, and noncompetitive NMDA receptor antagonists, like ketamine [2], blocked clonic convulsions, tonic extension and lethality induced by CD_{97} i.c.v. injection of GSA dose dependently (Table II). Kynurenate, an antagonist at the NMDA receptor-associated glycine-binding site [2], did not attenuate GSA-induced effects (Table II) nor did D-serine, an agonist at this site [2], potentiate convulsions induced by GSA (data not shown). Kynurenate has also been shown to be ineffective against NMDA-induced convulsions [13] although it did block convulsions induced by quinolinate [10].

On the other hand, treatment with the anti-epileptic drugs carbamazepine, diazepam, phenobarbital and Na valproate did not protect the animals against the clonic convulsions induced by CD_{97} i.c.v. injection of GSA (Table III). All four drugs did attenuate, however, GSA-induced convulsions since tonic extension and lethality were effectively blocked by anti-epileptic treatment. The mechanisms of action of the anti-epileptic drugs which were used here are not primarily related to the suppression of excitatory amino acid receptor function but rather to either enhancement of GABAergic inhibitory processes or to modulation of voltage-dependent ionic channels [23]. Tonic convulsions induced by i.c.v. administered NMDA have also been shown to be sensitive to treatment with both NMDA antagonists as with anti-epileptic drugs, like diazepam, phenobarbital or Na valproate [18]. However, Koek and Colpaert [13] demonstrated that the clonic convulsions induced by i.c.v. NMDA are indeed selectively blocked by competitive as well as by noncompetitive NMDA antagonists but not by carbamazepine, diazepam, Na valproate or other anti-epileptic drugs.

TABLE II

EFFECTS OF COMPETITIVE AND NONCOMPETITIVE NMDA ANTAGONISTS UPON CD_{97} (MINIMAL DOSE-INDUCING CLONIC CONVULSIONS IN ALL CONTROL ANIMALS) GSA-INDUCED CONVULSIONS AND LETHALITY

Data are represented as percentages of total number of animals tested (n = 5 for each dose). Antagonists were either co-injected with the i.c.v. GSA injection or injected i.p. before GSA administration. ED_{50} (effective dose 50% of the animals, and 95% confidence interval between parentheses) is calculated for protective effect on GSA-induced clonic convulsions, except for kynurenate which has no effect on the number of animals displaying convulsions. In control experiments, all of the animals receiving i.c.v. CD_{97} GSA injection displayed clonic convulsions, ~ 40% displayed tonic extension of the limbs and ~ 40% of them died (n > 30). APV, 2-amino-5-phosphonovalerate.

Antagonist	Dose and Route	Clonic	Tonic	Lethal	ED_{50}
APV	3.125 nmol, i.c.v.	100	40	40	
	6.25 nmol, i.c.v.	80	20	20	10.2 (6.6–15.7) nmol
	12.5 nmol, i.c.v.	40	0	40	
	25 nmol, i.c.v.	0	0	0	
CGP 37849	2.5 mg/kg i.p.	80	60	40	
	5 mg/kg, i.p.	80	0	0	7.7 (4.4–13.5) mg/kg
	10 mg/kg, i.p.	40	0	0	
	20 mg/kg, i.p.	0	0	0	
KETAMINE	10 mg/kg, i.p.	80	40	40	
	20 mg/kg, i.p.	60	0	0	21.8 (11.7–40.8) mg/kg
	40 mg/kg, i.p.	20	0	0	
	80 mg/kg, i.p.	0	0	0	
KYNURENATE	3.125 nmol, i.c.v.	80	40	40	
	6.25 nmol, i.c.v.	80	60	60	
	12.5 nmol, i.c.v.	100	80	80	

Thus, several lines of inference lead us to propose a direct excitatory action of GSA and, in particular, the involvement of NMDA receptors in the pathogenesis of GSA-induced generalized convulsions. Meldrum [17] pointed out that several abnormalities in amino acid metabolism may manifest epileptic phenomena through altered excitatory neurotransmission. For example, myoclonus and seizures in hyperglycinaemia might result from agonist action at the NMDA receptor-associated glycine-binding site, which is supported by the preliminary evidence that ketamine has therapeutic action in this syndrome. Similarly, the greatly increased GSA levels in patients with renal insufficiency [6] may contribute to the epileptic symptoms of these patients through enhanced NMDA receptor activation. However, the therapeutic action of NMDA antagonists in renal insufficiency patients still needs to be determined.

TABLE III

EFFECTS OF ANTICONVULSANT DRUGS UPON CD_{97} (MINIMAL DOSE-INDUCING CLONIC CONVULSIONS IN ALL CONTROL ANIMALS) GSA-INDUCED CONVULSIONS AND LETHALITY

All data are represented as percentages of total number of animals tested (n = 5 for each dose). I.p. drug injection preceded GSA injection. In control experiments, all of the animals receiving i.c.v. CD_{97} GSA injection displayed clonic convulsions, ~ 40% displayed tonic extension of the limbs and ~ 40% of them died (n > 30).

Anticonvulsant	Dose and Route	Clonic	Tonic	Lethal
Carbamazepine	40 mg/kg, i.p.	100	0	0
Diazepam	4 mg/kg, i.p.	100	0	0
	20 mg/kg, i.p.	80	0	0
Phenobarbital	40 mg/kg, i.p.	80	0	0
	80 mg/kg, i.p.	80	0	0
Valproate	125 mg/kg, i.p.	100	40	20
	250 mg/kg, i.p.	100	0	0
	500 mg/kg, i.p.	100	0	0

As discussed above, and in addition to the pharmacological data, GSA not only seems to meet the structural requirements of excitatory amino acids in general [12] but more in particular those of NMDA receptor ligands [20]. An agonist action of GSA at the NMDA receptor is not, however, in keeping with previous findings which were rather in line with antagonist actions of GSA at the NMDA receptor [7,22] although Reynolds and Rothermund [22] also reported some excitatory effects of GSA on the NMDA receptor functions they studied. As we have recently reported, the CD_{50} of GSA after its i.p. administration corresponds with a brain concentration of 56 nmol/g tissue [9]. The inhibitory effects of GSA upon [^{3}H]dizocilpine-binding and upon NMDA-induced increases in intracellular Ca [22], as well as on excitatory synaptic neurotransmission in rat hippocampal slices [7], appeared at much higher concentrations. To account for these different effects, we propose the excitatory effects of GSA to take place around and above 50 μM while

inhibitory effects would appear at higher concentrations. This could have important pathophysiological consequences since in patients with renal insufficiency, GSA concentrations of > 30 μM were measured in cerebrospinal fluid [6]. It remains to be determined, however, whether such a biphasic effect could be due to differential binding of GSA at different sites on the NMDA receptor in a similar manner to polyamines which act at a stimulatory polyamine site on the NMDA receptor at low concentrations (~30 μM) but at an inhibitory site at higher concentrations [4]. Agonist actions at NMDA receptors together with actions at other receptors (e.g., GABA receptors) could account for the excitant activity of GSA in vivo.

We acknowledge the expert technical assistance of F. Franck. Financial support was obtained from Antwerp University UIA, Born-Bunge Foundation, OCMW Medical Research Foundation, and Belgian Foundation of Scientific Research NFWO. R. D'Hooge holds a Flemish Community IWONL Scholarship (No. 920012).

1 Catterall, W.A., Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes, Annu. Rev. Pharmacol. Toxicol., 20 (1980) 15–43.
2 Collingridge, G.L. and Lester, R.A.J., Excitatory amino acid receptors in the vertebrate central nervous system, Pharmacol. Rev., 40 (1989) 143–210.
3 Curtis, D.R., Duggan, A.W., Felix, D., Johnston, A.R., Tebecis, A.K. and Watkins, J.C., Excitation of mammalian central neurons by acidic amino acids, Brain Res., 41 (1972) 283–301.
4 Daniell, L.C., Alteration of general anesthetic potency by agonists and antagonists of the polyamine binding site of the *N*-methyl-D-aspartate receptor, J. Pharmacol. Exp. Ther., 261 (1992) 304–310.
5 De Deyn, P.P. and Macdonald, R.L., Guanidino compounds that are increased in cerebrospinal fluid and brain of uremic patients inhibit GABA and glycine responses on mouse neurons in cell culture, Ann. Neurol., 28 (1990) 627–633.
6 De Deyn, P.P., Marescau, B., Cuykens, J.J., Van Gorp, L., Lowenthal, A. and De Potter, W.P., Guanidino compounds in serum and cerebrospinal fluid of non-dialysed patients with renal insufficiency, Clin. Chim. Acta, 167 (1987) 81–88.
7 D'Hooge, R., Manil, J., Colin, F., Diltoer, M., Nagels, G., Vervaeck, M. and De Deyn, P.P., Guanidinosuccinic acid affects excitatory postsynaptic response in rat hippocampal slices. In P.P. De Deyn et al. (Eds.), Guanidino Compounds in Biology and Medicine, John Libbey and Co., London, 1992, pp. 395–402.
8 D'Hooge, R., Pei, Y.Q., Manil, J. and De Deyn, P.P., The uremic guanidino compound guanidinosuccinic acid induces behavioral convulsions and concomitant epileptiform electrocorticographic discharges in mice, Brain Res., 598 (1992) 316–320.
9 D'Hooge, R., Pei, Y.Q., Marescau, B. and De Deyn, P.P., Convulsive action and toxicity of uremic guanidino compounds: behavioral assessment and relation to brain concentration in adult mice, J. Neurol. Sci., 112 (1992) 96–105.
10 Foster, A.C., Vezzani, A., French, E.D. and Schwarcz, R., Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid, Neurosci. Lett., 48 (1984) 273–278.
11 Jinnai, D., Sawai, A. and Mori, A., γ-Guanidinobutyric acid as a convulsive substance, Nature (London), 212 (1966) 617.
12 Kizer, J.S., Nemeroff, C.R. and Youngblood, W.W., Neurotoxic amino acids and structurally related analogs, Pharmacol. Rev., 29 (1978) 301–318.
13 Koek, W. and Colpaert, F.C., Selective blockade of *N*-methyl-D-aspartate (NMDA)-induced convulsions by NMDA antagonists and putative glycine antagonists: Relationship with phencyclidine-like behavioral effects, J. Pharmacol. Exp. Ther., 252 (1990) 349–357.
14 Lapin, I.P., Convulsant action of intracerebroventricularly administered *l*-kynurenine sulphate, quinolinic acid and other derivatives of succinic acid, and effects of amino acids: structure–activity relationships, Neuropharmacology, 21 (1982) 1227–1233.
15 Litchfield, J.T. and Wilcoxon, F., A simplified method for evaluating dose–effect experiments, J. Pharmacol. Exp. Ther., 96 (1949) 99–113.
16 Marescau, B., Deshmukh, D.R., Kockx, M., Possemiers, I., Qureshi, I.A., Wiechert, P. and De Deyn, P.P., Guanidino compounds in serum, urine, liver, kidney, and brain of man and some ureotelic animals, Metabolism, 41 (1992) 526–532.
17 Meldrum, B.S., Excitatory amino acids in epilepsy and potential novel therapies, Epilepsy Res., 12 (1992) 189–196.
18 Moreau, J.L., Pieri, L. and Prud'hon, B., Convulsions induced by centrally administered NMDA in mice: effects of NMDA antagonists, benzodiazepines, minor tranquilizers and anticonvulsants, Br. J. Pharmacol., 98 (1989) 1050–1054.
19 Mori, A., Biochemistry and neurotoxicology of guanidino compounds: history and recent advances, Pav. J. Biol. Sci., 22 (1987) 85–94.
20 Olverman, H.J. and Watkins, J.C., NMDA agonists and competitive antagonists. In J.C. Watkins and G.L. Collingridge (Eds.), The NMDA Receptor, IRL Press, Oxford, 1989, pp. 19–36.
21 Raskin, N.H., Neurological aspects of renal failure. In M.J. Aminoff (Ed.), Neurology and General Medicine, Churchill Livingstone, New York, 1989, pp. 231–246.
22 Reynolds, I.J. and Rothermund, K., Multiple modes of NMDA receptor regulation by guanidines. In P.P. De Deyn et al. (Eds.), Guanidino Compounds in Biology and Medicine, John Libbey and Co., London, 1992, pp. 441–448.
23 Rogawski, M.A. and Porter, R.J., Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds, Pharmacol. Rev., 42 (1990) 223–286.
24 Stone, T.W. and Connick, J.H., Quinolinic acid and other kynurenines in the central nervous system, Neuroscience, 15 (1985) 597–617.
25 Vogel, S.M., Watanabe, S., Yeh, J.Z., Farley, J.M. and Narahashi, T., Current-dependent block of endplate channels by guanidine derivatives, J. Gen. Physiol., 83 (1984) 901–918.
26 Weil, C.S., Tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions in their use, Biometrics, 8 (1952) 249–263.