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ONTOGENETIC DIFFERENCES IN CONVULSIVE ACTION AND CEREBRAL UPTAKE OF UREMIC GUANIDINO COMPOUNDS IN JUVENILE MICE

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Abstract—Guanidinosuccinate (GSA) and methylguanidine (MG) are endogenous, convulsant guanidino compounds which have been shown to be greatly increased in uremic patients. In the present study, we have investigated the age-related differences in convulsive action and cerebral uptake of these compounds in juvenile mice of 7, 14 and 21 days old. An age-dependent decrease was apparent in the severity of the GSA- and MG-induced convulsions and toxicity. Mean latency for the appearance of clonic convulsions increased with increasing age. Two hours following the i.p. injection of GSA or MG in a dose of 250 mg/kg, the resulting brain concentration decreased with increasing age of the animals. This effect was more pronounced in the case of MG. Neither for GSA, nor for MG was this age-dependent effect apparent after 30 min. GSA and MG serum as well as brain concentrations were lower in 21-day-old mice than in 7-day-old ones. However, the brain/serum concentration ratios of GSA and of MG were significantly lower in 21-day-old mice than in 7-day-old ones, indicating that at least part of the difference in brain level can be explained by higher permeability of the immature blood–brain barrier to these uremic guanidino compounds. In addition, brain/serum ratios of GSA in mice of 7 days old and in mice of 21 days old were significantly lower than the ratios of MG in these age groups, indicative of lower overall blood–brain barrier permeability to GSA than to MG. The observed differences in seizure incidence can be due to differences in brain level of the injected compounds (functional immaturity of the blood–brain barrier) as well as to differences in susceptibility of developing mice.

Guanidino compounds might contribute to the epileptic phenomena displayed by renal insufficiency patients. Indeed, serum and cerebrospinal fluid levels of guanidinosuccinate (GSA) and methylguanidine (MG) are greatly increased in these patients (De Deyn *et al.*, 1987), and both of these uremic guanidino compounds have been shown to induce clonic and tonic convulsions, and concomitant epileptiform electrographic discharges in adult laboratory animals (D'Hooge *et al.*, 1992a; Matsumoto *et al.*, 1976). Of the uremic guanidino compounds tested, GSA and MG were by far the most potent both in the induction of behavioral convulsions (D'Hooge *et al.*, 1992b), as well as in the possibly underlying antagonism of inhibitory GABA and glycine neurotransmission (De Deyn *et al.*, 1990, 1992). Due to differences in drug absorption, metabolism, blood–brain barrier permeability, and susceptibility of the immature brain, the convulsive action of a number of convulsants is much higher in young animals than in older ones (see Moshé, 1987). It is not known, however, whether this is also the case for uremic guanidino compounds like GSA or MG. In the present study, we have investigated the age-related differences in convulsive action and cerebral uptake of these compounds in juvenile mice of 7, 14 and 21 days old.

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EXPERIMENTAL PROCEDURES

Swiss mice from the same strain were bred in our animal colony and maintained under standard environmentally con-

trolled conditions. Guanidinosuccinic acid and methylguanidine hydrochloride were purchased from Sigma Chemical Co. (St Louis, U.S.A.); all other chemicals were obtained from Merck (Darmstadt, Germany) and were of analytical grade. Smooth suspensions of GSA in 30% polyethylene glycol solution were made with an agate mortar; methylguanidine hydrochloride dissolved in the polyethylene glycol solution. Each animal received 0.1 ml suspension or solution per 10 g body weight by i.p. injection. After injection, behavior was observed for either 30 min or 2 h during which time the animals were individually maintained in plastic beakers at 26°C to avoid hypothermia. Observers were well acquainted with the typical behavior of sham injected mice under these conditions. Although behavioral expression of drug-induced seizures is related to age (see Moshé, 1987; Mares, 1991), crude standardized differentiation between normal behavior, clonic convulsions (hyperactive swimming or crawling movements, rolling, and head tremor in 7-day-old mice; pop-corn jumps, running fits, forelimb clonus, rolling, etc. in older ones), and tonic extension of the limbs was possible across the three age groups. After the observation period, the animals were decapitated and brains were removed; in some mice, blood was collected as well. Procedures of tissue homogenization and preprocessing were described earlier (Marescau *et al.*, 1986). Tissue guanidino compound concentration was determined by cation exchange chromatography and fluorescence ninhydrin detection according to Marescau *et al.* (1992). Guanidino compound tissue concentrations were expressed in nmol per g wet brain tissue or ml serum. Significance of differences between mean values were determined by Student's *t*-test. Mean brain concentration in mice of 7, 14 and 21 days old injected with polyethylene glycol solution: below detection limits to 0.15 nmol/g tissue (GSA), 0.40–2.64 nmol/g tissue (MG).

RESULTS

Two hours following the i.p. injection of GSA or MG in a dose of 250 mg/kg, the resulting brain concentration decreased with increasing age of the animals (Fig. 1). This effect was more pronounced in the case of MG. Neither for GSA, nor MG was this age-dependent effect apparent after 30 min. In the case of GSA [Fig. 1(a)], brain concentration 2 h after injection in mice of 7 days old was 2.5 times higher than that after 30 min. While in mice of 21 days old, GSA brain concentration after 2 h was 1.75 times higher than that after 30 min.

MG, on the other hand, showed a different profile [Fig. 1(b)]. In mice of 7 days old, MG brain concentration 2 h after injection was more than 2 times higher than that after 30 min. In mice of 14 days old there was no significant difference between MG brain concentration after 30 min and that after 2 h, while in 21-day-old animals MG brain concentration after 2 h was significantly lower than that after 30 min.

In parallel with decreasing brain concentration, an age-dependent decrease was apparent in the severity of the GSA- and MG-induced convulsions and toxicity (Table 1). Whereas mice of 21 days old did not display any behavioral effect after a 250 mg/kg injection of GSA, the same dose did induce severe convulsions in mice of 7 days old. After a 250 mg/kg i.p. injection of GSA, all of the 7-day-old mice displayed clonic and tonic convulsions, and 3 out of 5 even died from the effects of the GSA application. Mice of 14 days old had an intermediate response, 4 out of 5 displayed clonic convulsions, one displayed tonic extension of the limbs, and none died. Clonic convulsions appeared in 7-day-old mice 52.0 ± 1.2 min after GSA injection, but in 14-day-old mice after 75.0 ± 2.0 min (mean latencies $\pm$ SEM).

After a 250 mg/kg injection of MG (data not shown), all injected animals of the three age groups displayed clonic convulsions. Mean latency for the appearance of clonic convulsions increased with increasing age. Mice of 7 days old displayed clonic convulsions 7.2 ± 1.6 min after MG injection, 250 mg/kg; 14-day-old mice after 18.8 ± 3.0 min; 21-day-old mice after 28.4 ± 1.6 min (all latencies are means $\pm$ SEM). A smaller dose of 125 mg/kg induced clonic convulsions in all of the 7- and 14-day-old mice but in none of the 21-day-old animals. Tonic extension was not induced by either dose of MG, nor did any of the animals die.

To investigate whether the age-related differences in convulsive activity and brain concentration of the uremic guanidino compounds tested could be due to differences in blood–brain barrier permeability, brain/serum concentration ratio 2 h after i.p. injection of the compounds was determined in 7- and 21-day-old mice (Table 2). Both for GSA as for MG, serum as well as brain concentration of the injected compounds were lower in 21-day-old mice than in 7-day-old ones. However, as the brain/serum concentration ratios of GSA ($P \leqslant 0.05$) and of MG ($P \leqslant 0.001$) were significantly lower in 21-day-old mice than in 7-day-old ones, serum concentration of the injected compounds did not decrease with increasing age quite to the same extent as their brain concentration did. In addition, brain/serum ratios of GSA in mice of 7 days old ($P \leqslant 0.01$) and in mice of 21 days old ($P \leqslant 0.001$) were significantly lower than the ratios of MG in these age groups. The age-dependent decrease in brain/serum ratio was more pronounced in the case of GSA than in the case of MG.

DISCUSSION

Monoguanidines like GSA and MG are generated as a result of protein and amino acid metabolism. Renal failure leads to the accumulation of these and

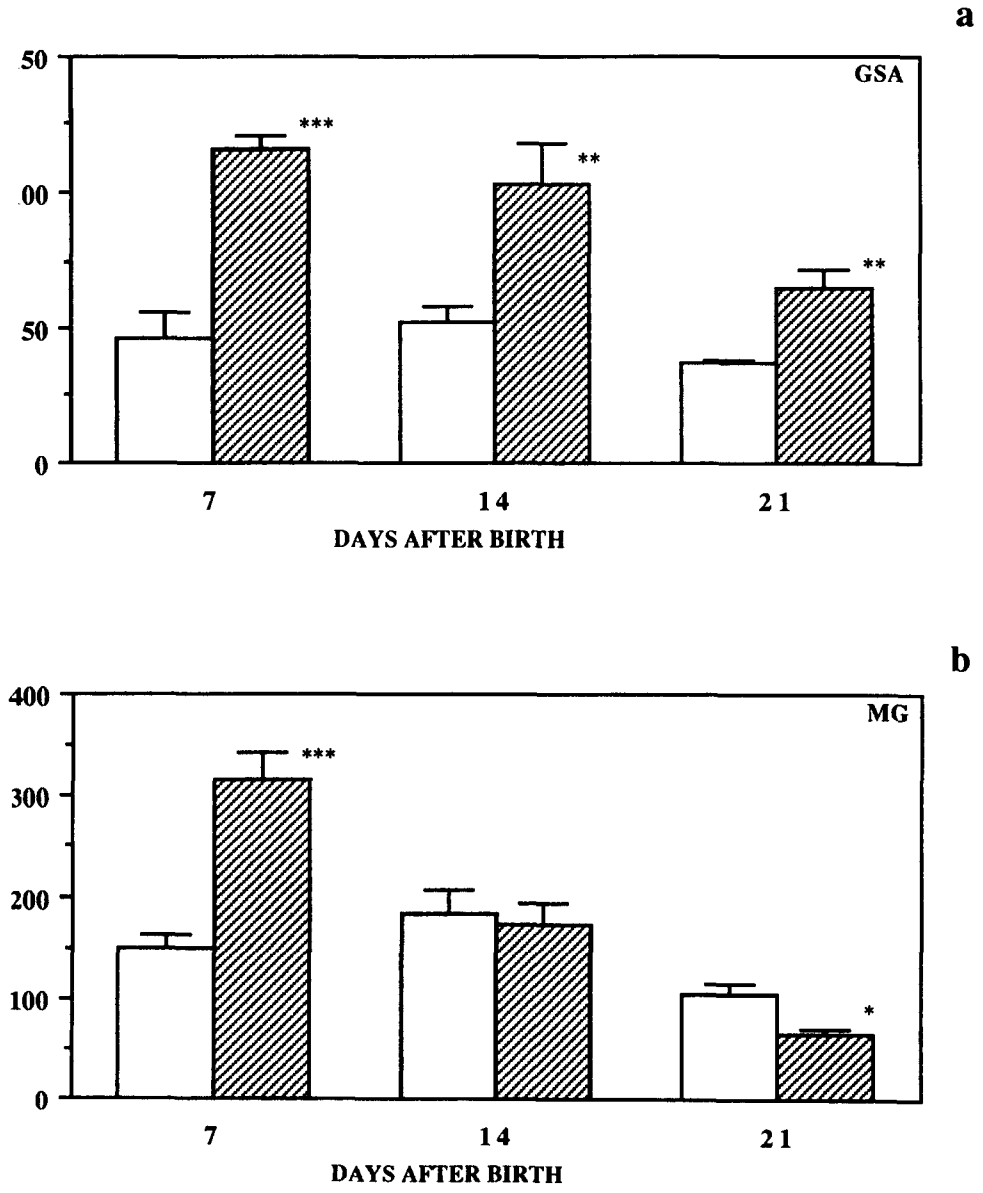

Fig. 1. Brain concentration of (a) guanidinosuccinate and (b) methylguanidine following i.p. injection of 250 mg/kg of either one of these uremic guanidino compounds. In mice of 7, 14 and 21 days old, brain concentrations were determined 30 min (open bars) and 2 h (hatched bars) after injection. Asterisks indicate the significance of the difference between 30-min and 2-h groups: $*P \leqslant 0.05$, $**P \leqslant 0.01$, $***P \leqslant 0.001$.

Table 1. Age-dependent decrease in severity of GSA- and MG-induced behavioral response

	Days after birth		
	7	14	21
250 *mg GSA/kg*			
C/T/D	5/5/3	4/1/0	0/0/0
Brain concentration	116 ± 5	103 ± 14	65 ± 6
125 *mg MG/kg*			
C/T/D	5/0/0	5/0/0	0/0/0
brain concentration	94 ± 5	81 ± 7	32 ± 2

For groups of 7-, 14- and 21-day-old mice the number of animals is shown that displayed clonic convulsions (C), tonic extension of the limbs (T), or died (D) following i.p. injection of either GSA or MG. Each age group consisted of 5 mice that were observed for 2 h; brain concentration (nmol/g brain tissue) of the applied compounds at the end of the observation period is shown as well (mean ± SEM).

Table 2. Comparison of brain/serum concentration ratio of GSA and MG in 7- and 21-day-old mice

	Days after birth	
	7	21
GSA		
Serum	677	482
Brain	58	13
Brain/serum	0.085 ± 0.019	0.027 ± 0.005
MG		
Serum	360	286
Brain	96	32
Brain/serum	0.265 ± 0.009	0.112 ± 0.001

In groups of three 7- and 21-day-old mice, serum (in nmol/ml) and brain concentration (in nmol/g brain tissue), 2 h after i.p. injection of 125 mg/kg of either GSA or MG, have been used to calculate brain/serum concentration ratio (mean ± SEM).

other metabolic end products in the body of subjects suffering from this disorder, and accordingly, guanidino compounds have been found to be greatly increased in biological fluids of uremic patients (De Deyn *et al.*, 1987). Epileptic symptomatology is a prominent neurological manifestation of uremia (Fraser, 1992), and guanidino compounds have been proposed to contribute to this significantly (De Deyn *et al.*, 1992). Like many other guanidino compounds (see Mori, 1987), GSA (D'Hooge *et al.*, 1992a) as well as MG (Matsumoto *et al.*, 1976) have been shown to induce convulsions and concomitant epileptiform electrographic discharges experimentally, but epileptogenic potency of GSA appeared to be higher than that of MG (De Deyn *et al.*, 1992; D'Hooge *et al.*, 1992b).

It has been demonstrated that the convulsive dose of a number of convulsants is lower early in life (see Moshé, 1987). For example, Peñafiel *et al.* (1991) reported an inverse relationship between the convulsive action of monosodium glutamate and the degree of development of their rats. Kainic acid, a powerful exogenous glutamate analogue, induced behaviors similar to those termed 'clonic' in the present report, and extension of the limbs, in rat pups in their first week of life (Cherubini *et al.*, 1983). Tremblay *et al.* (1984) found that in older rats higher doses of kainic acid than in younger ones were necessary to induce clonic and tonic convulsions. Mares and Velisek (1992) found a high sensitivity in immature rats to the convulsive action of *N*-methyl-D-asparate. Here it is reported that this appears to be the case for uremic guanidino compounds like GSA and MG as well. Indeed, doses of GSA or MG which did not induce convulsions or lethality in 21-day-old mice did so in 7-day-old animals.

The brain concentration of the compounds 2 h after their injection was higher in mice of 7 days old than in mice of 21 days old. After 30 min, brain concentration of the compounds was not significantly related to the age of the animals. In the study of Peñafiel *et al.* (1991), rate of increase and maximum

level reached in brain glutamate following systemic administration depended on the age of the injected rats. The authors related the reduction in seizure incidence with age to decreased permeability of the blood–brain barrier to glutamate. The differences in the brain levels of the uremic guanidino compounds in the present study, both at 30 min and at 2 h, between mice of different age groups are indicative of ontogenetic differences in the uptake kinetics of these compounds. Seta *et al.* (1972) measured brain and blood concentrations of 10 different amino acids following i.p. injection, and found the increase in brain concentration, corresponding with a particular increase in plasma concentration, to be greater in newborn than in adult mice for each of the amino acids. We found this to be the case for the two guanidino compounds in this study also. This could mean that these uremic toxins cross the blood–brain barrier in young mice more easily than in older ones.

Seta *et al.* (1972) also found that cerebral uptakes of glycine and aspartate were smaller than those of the other compounds, and it might be interesting to note that the cerebral uptake of GSA, a structural analogue of aspartate, is low as well, significantly lower still than that of MG (cf. the much higher brain/serum concentration ratio of MG in both 7-day-old and 21-day-old mice). It remains to be determined whether brain levels of GSA may be controlled by the same processes which control the levels of structurally related amino acids like aspartate or glutamate.

The ontogenetic differences in brain level of the compounds that were observed 2 h following injection, could be caused by immaturity of the blood–brain barrier in the youngest mice. Indeed, functional maturation of the blood–brain barrier occurs in mice between 12 and 24 days of life and is characterized by gradual appearance of a negatively charged layer on the endothelium of the cerebral microvasculature (Vorbrodt *et al.*, 1986; 1990). Evidently, 7-day-old mice possess a functionally immature blood–brain barrier, whereas the barrier in 21-day-old animals approaches maturation. The higher blood–brain barrier permeability to uremic guanidino compounds in 7-day-old mice could be explained by this functional immaturity. In addition, permeability of blood–brain barrier to GSA appears to be lower than that to MG in both 7- and 21-day-old animals.

Moshé (1987) argued that the lower convulsive dose of convulsants in young animals can be due to either differences in drug absorption, metabolism, blood–brain barrier permeability, or nervous system susceptibility. Since serum levels of GSA and MG after injection are higher in mice of 7 days old than in those of 21 days old, absorption of the injected compounds appears to be age-related. However, as the higher brain/serum concentration ratios of GSA and MG in 7-day-old mice indicate, at least part of the difference in brain level can be explained by higher permeability of the immature blood–brain barrier to these uremic guanidino compounds. Therefore, the observed differences in seizure incidence could be due either to differences in brain level of the injected compounds or to differences in susceptibility of developing mice. Following the injection of MG (250 mg/kg), mice of all three age groups displayed convulsions within the first half hour. During this time, however, no age-dependent difference in MG brain level was observed. Thus, as a dose of MG of 125 mg/kg induced convulsions in mice of 7 and 14 days old but not in 21-day-old animals, this ontogenetic difference in seizure incidence must have been due to lower susceptibility of the nervous substrate of young mice to the convulsive action of this compound. In the case of GSA, convulsions appeared somewhat between 1 and 2 h after injection of this compound. Here, the observed difference in seizure incidence could be due to lower susceptibility as well as to higher brain level.

In conclusion, we have investigated the age-related differences in convulsive action and cerebral uptake of the two most important uremic guanidino compounds in juvenile mice of 7, 14 and 21 days old. It was found that seizure incidence as well as brain levels of the compounds following their i.p. injection were higher in young than in older mice. Observed age-related differences in brain uptake kinetics, and seizure incidence and latency are proposed to be due, at least partly, to functional immaturity of the blood–brain barrier system. However, our results also seem to indicate that the nervous tissue of immature mice is more susceptible to the toxic effects of uremic guanidino compounds. The concentration of GSA measured in the brains of the animals is very similar to those measured in the brains of uremic patients, while the other brain (MG) and serum (GSA and MG) concentrations are much higher than those in patients (De Deyn, 1989; De Deyn *et al.*, 1987). Nevertheless, since guanidino compounds have been found to be greatly increased in brain and biological fluids of uremic patients, these results could have important consequences regarding the human condition. They should prompt more detailed research along the lines proposed in this paper (e.g. sensitivity of the human fetus or infant to these endogenous toxins might be higher than that of the adult).

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REFERENCES

Cherubini E., de Feo M. R., Mecarelli O. and Ricci G. F. (1983) Behavioral and electrographic patterns induced by systemic administration of kainic acid in developing rats. *Dev. Brain Res.* **9**, 69–77.

De Deyn P. P. (1989) *Analytical studies and pathophysiological importance of guanidino compounds in uremia and hyperargininemia.* Dissertation, University of Antwerp, Antwerp, Belgium.

De Deyn P. P. and Macdonald R. L. (1990) 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**, 627–633.

De Deyn P. P., D'Hooge R., Pei Y. Q., Marescau B. and Macdonald R. L. (1992) Possible mechanisms of action of guanidino compounds as candidate convulsants in uraemia and hyperargininaemia. In: *Guanidino Compounds in Biology and Medicine* (De Deyn P. P., Marescau B., Stalon V. and Qureshi I. A., eds), pp. 409–417. John Libbey, London, U.K.

De Deyn P. P., Marescau B., Cuykens J. J., Van Gorp L., Lowenthal A. and De Potter W. P. (1987) Guanidino compounds in serum and cerebrospinal fluid of non-dialysed patients with renal insufficiency. *Clin. Chim. Acta* **167**, 81–88.

D'Hooge R., Pei Y. Q., Manil J. and De Deyn P. P. (1992a) The uremic guanidino compound guanidinosuccinic acid induces behavioral convulsions and concomitant epileptiform electrocorticographic discharges in mice. *Brain Res.* **598**, 316–320.

D'Hooge R., Pei Y. Q., Marescau B. and De Deyn P. P. (1992b) Convulsive action and toxicity of uremic guanidino compounds: behavioral assessment and relation to brain concentration in adult mice. *J. Neurol. Sci.* **112**, 96–105.

Fraser C. L. (1992) Neurologic manifestations of the uremic state. In: *Metabolic Brain Dysfunction in Systemic Disorders* (Arieff A. I. and Griggs R. C., eds), pp. 139–166. Little, Brown, Boston, U.S.A.

Mares P. (1991) Epileptic phenomena in the immature brain. *Arch. Int. Physiol. Biochim. Biophys.* **99**, A135–136.

Mares P. and Velisek, L. (1992) *N*-methyl-D-aspartate (NMDA)-induced seizures in developing rats. *Dev. Brain Res.* **65**, 185–189.

Marescau B., De Deyn P. P., Wiechert P. Van Gorp L. and Lowenthal A. (1986) Comparative study of guanidino compounds in serum and brain of mouse, rat, rabbit, and man. *J. Neurochem.* **46**, 717–720.

Marescau B., Deshmukh D. R., Kockx M., Possemiers I., Qureshi I. A., Wiechert P. and De Deyn P. P. (1992) Guanidino compounds in serum, urine, liver, kidney and brain of man and some ureotelic animals. *Metabolism* **41**, 526–532.

Matsumoto M., Kobayashi K., Kishikawa H. and Mori A. (1976) Convulsive activity of methylguanidine in cats and rabbits. *IRCS Med. Sci.* **4**, 65.

Mori A. (1987) Biochemistry and neurotoxicology of guanidino compounds: history and recent advances. *Par. J. Biol. Sci.* **22**, 85–94.

Moshé S. L. (1987) Epileptogenesis and the immature brain. *Epilepsia* **28**, S3–S15.

Peñafiel R., Cremades A., Monserrat F. and Puelles L. (1991) Monosodium glutamate-induced convulsions in rats: influence of route of administration, temperature and age. *Amino Acids* **1**, 81–89.

Seta K., Sershen H. and Lajtha A. (1972) Cerebral amino acid uptake *in vivo* in newborn mice. *Brain Res.* **47**, 415–425.

Tremblay E., Nitecka L., Berger M. L. and Ben-Ari Y. (1984) Maturation of kainic acid seizure-brain damage syndrome in the rat. I. Clinical, electrographic and metabolic observations. *Neuroscience* **13**, 1051–1072.

Vorbrodt A. W., Lossinsky A. S., Dobrogowska D. H. and Wisniewski H. M. (1986) Distribution of anionic sites and glycoconjugates on the endothelial surface of the developing blood–brain barrier. *Dev. Brain Res.* **29**, 69–79.

Vorbrodt A. W., Lossinsky A. S., Dobrogowska D. H. and Wisniewski H. M. (1990) Sequential appearance of anionic domains in the developing blood–brain barrier. *Dev. Brain Res.* **52**, 31–37.