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Short communication

Alterations in plasma and brain amino acids after administration of the glycine/NMDA receptor partial agonist, D-cycloserine, to mice and rats

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Abstract

The NMDA/glycine receptor partial agonist, D-cycloserine, has recently been reported to exert anticonvulsant effects in different seizure models in mice and rats. In view of the high doses (> 100 mg/kg) needed for these effects, actions other than those mediated by the glycine site might be involved. In this respect, inhibition of pyridoxal phosphate-dependent enzymes involved in amino acid metabolism might play a role. In the present experiments, D-cycloserine was administered at an anticonvulsant dose (320 mg/kg) to mice and rats and levels of 11 amino acids, including several neurotransmitters, were determined in brain cortex and plasma at different times after administration. In addition, the concentration of D-cycloserine was determined in plasma and brain. Compared to peak concentrations of D-cycloserine in plasma, only about 20% of D-cycloserine appeared in the brain. The only marked alteration in brain amino acids was an increase in alanine levels, while amino acids acting as neurotransmitters were hardly altered. The data indicate that the anticonvulsant action of D-cycloserine is not secondary to changes in levels of amino acid neurotransmitters.

Keywords: GABA (γ-aminobutyric acid); Alanine; Glutamate; Epilepsy

1. Introduction

D-Cycloserine acts as a partial agonist at the strychnine-insensitive glycine site of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor, displaying about 40–70% of the efficacy of glycine (Kemp and Leeson, 1994). In low doses (about 10–20 mg/kg in rodents), D-cycloserine has been shown to exert cognition-enhancing effects, an effect attributed to its ability to potentiate NMDA receptor function (Kemp and Leeson, 1994). Higher doses (> 100 mg/kg) were reported to exert anticonvulsant activity against electrically or chemically induced seizures in rats and mice (Peterson, 1992; Peterson and Schwade, 1993; Löscher et al., 1994; Wlaz et al., 1994). These apparently opposing findings could be explained on the basis of a partial agonist action in that – depending on intrinsic efficacy and dose of the partial agonist and functional state of the NMDA receptor complex – such a drug could either increase or decrease the actions of glutamate at this receptor subtype (Emmett et al., 1991; Wlaz et al., 1994). However, the anticonvulsant effects found at high doses of D-cycloserine and other high efficacy partial agonists at the glycine site could also be due to actions unrelated to the NMDA receptor (Kemp and Leeson, 1994). For instance, in vitro at high concentrations D-cycloserine was found to affect pyridoxal phosphate-dependent enzymes involved in amino acid metabolism (Dengler et al., 1962; Fasella et al., 1978). For investigation of the biochemical in vivo effects of D-cycloserine at a dose recently found to exert marked anticonvulsant activity in mice and rats (Löscher et al., 1994; Wlaz et al., 1994), we determined the concentrations of various amino acids, including several inhibitory and excitatory amino acids, in brain cortex of these species after administration of D-cycloserine. Furthermore, amino acids were determined in plasma.

2. Materials and methods

All experiments were carried out in age-matched female Wistar rats (body weight 200–250 g) and male NMRI mice (body weight 28–32 g), using 6 animals per

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group. Doses and pretreatment times for D-cycloserine (Sigma, Munich, Germany) were chosen on the basis of previous experiments with seizure models in mice and rats (Löscher et al., 1994; Wlaz et al., 1994). In both species, a dose of 320 mg/kg D-cycloserine (dissolved in saline) was injected i.p. and amino acids were determined after 15 and 60 min. One additional experiment in mice was done with a dose of 5 mg/kg D-cycloserine and pretreatment times of 15 and 60 min. Together with drug-treated groups of animals, 6 animals were i.p. injected with saline and were used as controls for neurochemical measurements.

For amino acid determinations in brain cortex and plasma, the animals were decapitated, blood was collected in tubes containing EDTA, and the brain was rapidly removed, shortly immersed in liquid nitrogen, and then dissected within 4 min on a cold plate at −10°C. Following dissection, samples from frontal cortex were rapidly weighed and homogenized with an Ultra-Turrax in 2 ml of ice-cold 80% ethanol. Further processing of homogenates and determination of amino acids by high performance liquid chromatography (HPLC) have been described elsewhere (Löscher et al., 1991, 1993). The following 11 amino acids were determined after derivatization with *o*-phthaldialdehyde/2-mercaptoethanol by HPLC analysis with fluorescence detection: aspartate, glutamate, asparagine, serine, glutamine, glycine, threonine, arginine, taurine, alanine, and γ-aminobutyric acid (GABA). All amino acid levels in brain tissue are given in μmol/g tissue (wet weight). For determination of amino acids in plasma, the blood samples were immediately centrifuged at 4°C at 2000 × *g* for 7 min and plasma was stored at −80°C until analysis of the amino acids described above. For

Table 1
Amino acids in plasma and brain cortex of rats after i.p. administration of D-cycloserine

Amino acid	Plasma (μmol/ml)			Brain cortex (μmol/g)		
	Control	15 min p.i.	60 min p.i.	Control	15 min p.i.	60 min p.i.
Aspartate	0.018 ± 0.003	0.026 ± 0.002 [c]	0.021 ± 0.004	3.84 ± 0.35	3.49 ± 0.21	3.81 ± 0.31
Glutamate	0.11 ± 0.012	0.13 ± 0.019 [a]	0.097 ± 0.017	13.95 ± 1.46	13.25 ± 1.29	15.52 ± 0.56 [a]
Asparagine	0.037 ± 0.0061	0.036 ± 0.0059	0.050 ± 0.0091 [b]	0.18 ± 0.04	0.14 ± 0.04	0.25 ± 0.03 [c]
Serine	0.25 ± 0.037	0.24 ± 0.022	0.28 ± 0.021	1.31 ± 0.1	1.2 ± 0.1	1.4 ± 0.11
Glutamine	0.68 ± 0.089	0.64 ± 0.051	0.74 ± 0.11	6.1 ± 0.45	5.86 ± 0.34	6.34 ± 0.29
Glycine	0.28 ± 0.056	0.30 ± 0.034	0.37 ± 0.056 [b]	0.98 ± 0.14	0.95 ± 0.14	1.06 ± 0.11
Threonine	0.41 ± 0.09	0.48 ± 0.072	0.49 ± 0.11	0.92 ± 0.17	0.8 ± 0.09	0.95 ± 0.05
Arginine	0.13 ± 0.034	0.13 ± 0.026	0.14 ± 0.033	0.14 ± 0.03	0.21 ± 0.04 [c]	0.16 ± 0.05
Taurine	0.14 ± 0.023	0.18 ± 0.050	0.16 ± 0.018	6.6 ± 0.28	6.13 ± 0.31 [b]	6.49 ± 0.33
D-Cycloserine	–	3.43 ± 0.2	2.19 ± 0.18	–	0.45 ± 0.29	0.8 ± 0.3
Alanine	0.28 ± 0.058	0.36 ± 0.031 [b]	0.36 ± 0.053	0.8 ± 0.12	1.0 ± 0.19	1.37 ± 0.21 [e]
GABA	0.0002 ± 0.00005	0.00018 ± 0.00005	0.00024 ± 0.00007	1.68 ± 0.19	1.62 ± 0.13	1.48 ± 0.26

Amino acids are shown in order of chromatographic appearance. Except for D-cycloserine, all data refer to the L-isomers of amino acids. Data are means ± S.D. for 6 animals per group. D-Cycloserine was administered at a dose of 320 mg/kg i.p. and amino acids were determined 15 and 60 min post-injection (p.i.). Significant differences between drug-treated groups and control are indicated as follows: [a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.002$; [e] $P < 0.001$.

Table 2
Amino acids in plasma and brain cortex of mice after i.p. administration of D-cycloserine

Amino acid	Plasma (μmol/ml)			Brain cortex (μmol/g)		
	Control	15 min p.i.	60 min p.i.	Control	15 min p.i.	60 min p.i.
Aspartate	0.025 ± 0.012	0.028 ± 0.013	0.038 ± 0.011	3.95 ± 0.5	3.92 ± 0.43	3.83 ± 0.27
Glutamate	0.068 ± 0.012	0.063 ± 0.01	0.089 ± 0.04	14.4 ± 0.7	13.84 ± 0.82	13.94 ± 0.94
Asparagine	0.035 ± 0.01	0.031 ± 0.0044	0.029 ± 0.0061	0.12 ± 0.03	0.11 ± 0.03	0.14 ± 0.03
Serine	0.12 ± 0.017	0.11 ± 0.013	0.12 ± 0.021	1.14 ± 0.15	1.03 ± 0.08	1.34 ± 0.09 [c]
Glutamine	0.47 ± 0.051	0.44 ± 0.048	0.34 ± 0.033 [e]	5.05 ± 0.46	4.43 ± 0.38 [b]	5.14 ± 0.98
Glycine	0.26 ± 0.04	0.22 ± 0.02 [a]	0.19 ± 0.024 [c]	0.84 ± 0.14	0.74 ± 0.08	1.01 ± 0.13 [a]
Threonine	0.21 ± 0.047	0.19 ± 0.057	0.14 ± 0.039 [c]	0.35 ± 0.09	0.41 ± 0.13	0.39 ± 0.1
Arginine	0.18 ± 0.034	0.14 ± 0.03 [b]	0.079 ± 0.034 [e]	0.17 ± 0.09	0.14 ± 0.1	0.17 ± 0.04
Taurine	0.38 ± 0.063	0.30 ± 0.047 [b]	0.37 ± 0.083	11.1 ± 0.49	10.38 ± 0.23 [d]	10.0 ± 0.85 [c]
D-Cycloserine	–	4.87 ± 0.33	1.45 ± 0.58	–	0.68 ± 0.28	0.86 ± 0.37
Alanine	0.34 ± 0.044	0.27 ± 0.024 [d]	0.24 ± 0.045 [d]	1.01 ± 0.29	1.35 ± 0.13 [c]	1.5 ± 0.22 [d]
GABA	n.d.	n.d.	n.d.	1.71 ± 0.16	1.72 ± 0.14	1.67 ± 0.2

Amino acids are shown in order of chromatographic appearance. Except for D-cycloserine, all data refer to the L-isomers of amino acids. Data are means ± S.D. for 6 animals per group; 'n.d.' signifies 'not detectable'. D-Cycloserine was administered at a dose of 320 mg/kg i.p. and amino acids were determined 15 and 60 min post-injection (p.i.). Significant differences between drug-treated groups and control are indicated as follows: [a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.002$; [e] $P < 0.001$.

HPLC analysis of plasma amino acids, 600 μl of 80% ethanol was added to 200 μl plasma. The sample was mixed, centrifuged (2000 × g, 20 min) and the supernatant was used for derivatization with *o*-phthaldialdehyde/2-mercaptoethanol and HPLC analysis of amino acids as described recently (Rundfeldt and Löscher, 1992). The low levels of GABA in rat plasma were determined by injecting less diluted samples (Rundfeldt and Löscher, 1992); in mouse plasma, GABA levels were below the detection limit of the assay. Since we were interested in also determining D-cycloserine in brain and plasma by the HPLC method used for determination of endogenous amino acids, the gradient program of the HPLC method had to be slightly modified to allow separation of the D-cycloserine peak, which appeared between the peaks of taurine and alanine. After development of optimal gradient conditions for separation, the linearity, sensitivity and reproducibility of the D-cycloserine analysis were demonstrated. Detection limits for D-cycloserine were 3 nmol/ml in the case of plasma and 60 nmol/g in the case of brain tissue. Reproducibility of the D-cycloserine analysis was proven by repeatedly (10 times) determining D-cycloserine in the same specimen (from animals treated with D-cycloserine), which yielded a coefficient of variation of 1% for plasma and 10% for brain, respectively.

The significance of differences in amino acid levels between drug-treated animals and the controls was calculated by means of Student's *t*-test (two-sided). In an additional in vitro experiment, the effect of D-cycloserine on the GABA synthesizing enzyme, glutamate decarboxylase, was determined in rat brain tissue as described previously (Löscher, 1980).

3. Results

As shown in Tables 1 and 2, D-cycloserine exerted only few marked effects on amino acids in the plasma and brain cortex of mice and rats after administration of 320 mg/kg. Among the neurotransmitter amino acids, levels of glutamate in cortex were slightly increased in rats but not mice, while cortical levels of aspartate were not changed in either species. Glycine levels in the cortex were slightly increased in mice but not rats, while GABA levels were not affected in both species. The only consistent changes in amino acid levels found in brain cortex of the two species were moderate decreases in taurine and marked increases in alanine. Most changes seen in brain cortex were not reflected in the plasma. Alanine levels were significantly increased in rat plasma but decreased in mouse plasma (Tables 1 and 2). After administration of 5 mg/kg D-cycloserine to mice, the only significant changes in brain amino acids seen were a 7% decrease in taurine and a 21% increase in serine levels, while the concentrations of other amino acids, including alanine, were not changed (not illustrated).

The levels of D-cycloserine in plasma decreased rapidly from 15 to 60 min after administration, demonstrating rapid elimination of this drug from plasma in both rats and mice (Tables 1 and 2). In contrast, the brain concentration of D-cycloserine increased from 15 to 60 min. However, D-cycloserine peak concentrations in brain were only about 20% of the peak concentrations in plasma, confirming results of previous experiments with D-cycloserine in rats (e.g., Crema and Berté, 1960). In these previous rat experiments, it was shown that peak brain levels of D-cycloserine in rats are reached 1 h after parenteral injection and decline thereafter with a half-life of about 2 h (Crema and Berté, 1960).

4. Discussion

As shown by the present data, the only marked effect of D-cycloserine on brain amino acids was an increase of alanine. Following 320 mg/kg D-cycloserine, alanine levels in brain cortex were increased by 50% in mice and 70% in rats. In bacteria, the bacteriostatic activity of D-cycloserine is thought to be related, at least in part, to disturbance of alanine metabolism (Iwainsky, 1988; Trnka et al., 1988). Bacteria are unique among living organisms in that they have an absolute requirement for the D-isomer of certain amino acids, specifically D-alanine and D-glutamate found in the peptidoglycan layer of almost all bacterial cell walls (Manning and Soper, 1978). D-Cycloserine largely inhibits the bacterial metabolism of D-alanine and glycine, whereas L-alanine metabolism and uptake are affected to a much lesser extent (Iwainsky, 1988; Trnka et al., 1988). The present data indicate that, at high doses, D-cycloserine interferes with metabolism of L-alanine in rodents. However, it is very unlikely that this effect is responsible for the anticonvulsant activity associated with high doses of D-cycloserine. L-Cycloserine, which is known to affect the metabolism of L-alanine much more potently than D-cycloserine in both bacteria and rodents (e.g. L-cycloserine potently blocks L-alanine:2-oxoglutarate aminotransferase in rodent brain and liver (Wood et al., 1978)), is clearly less potent than D-cycloserine in traditional models of epilepsy, such as the maximal electroshock seizure test (Peterson, 1992; Peterson and Schwade, 1993). Indeed, both the potency and spectrum of anticonvulsant activity of D-cycloserine and L-cycloserine differ markedly, strongly indicating that the two enantiomers of cycloserine act by different mechanisms (Peterson, 1992).

Based on in vitro experiments on glutamate decarboxylase inhibition by D-cycloserine, Dengler et al.

(1962) previously suggested that, at high doses, D-cycloserine might interfere with GABA synthesis. No GABA alterations were seen in the present in vivo experiments with D-cycloserine. Our in vitro experiments with D-cycloserine showed that glutamate decarboxylase was only moderately inhibited by D-cycloserine, the IC_{50} being higher than 5 mM (not illustrated). The in vivo determinations of D-cycloserine in brain tissue demonstrated that such concentrations are not reached after administration of a high, anticonvulsant dose of D-cycloserine. In contrast to D-cycloserine, the L-enantiomer of cycloserine is a potent inhibitor of glutamate decarboxylase and the GABA degrading enzyme, GABA aminotransferase (Metcalf et al., 1978). As its effect is more pronounced on GABA aminotransferase than on glutamate decarboxylase, L-cycloserine increases brain GABA content following its administration to animals (Wood et al., 1978; Polc et al., 1986). No such GABA increase was observed with D-cycloserine in mice and rats, making it highly unlikely that anticonvulsant effects of this drug are mediated by the GABA system.

In conclusion, the present data indicate that, except for alanine, D-cycloserine does not exert any marked effect on the levels of brain amino acids, including several amino acids with neurotransmitter function in the brain. The lack of any marked effect of D-cycloserine on brain amino acid neurotransmitter levels is comparable with recent data on NMDA receptor antagonists (Löscher et al., 1991). The data thus support previous suggestions that anticonvulsant effects of D-cycloserine observed after administration of high doses relate to a specific action on the glycine site of the NMDA receptor (Peterson, 1992; Löscher et al., 1994; Wlaz et al., 1994) and are not secondary to changes in the levels of amino acid neurotransmitters. In line with this view, the anticonvulsant effects of D-cycloserine in rodents could be antagonised by glycine site antagonists (Peterson, 1992; Löscher et al., 1994). However, in view of the increasing use of D-cycloserine as a glycine/NMDA receptor partial agonist, the present findings demand that the disturbance in brain L-alanine metabolism must give some cause for concern, particularly with regard to chronic treatment. Furthermore, the lack of alterations in whole tissue levels of various amino acids in response to D-cycloserine does not exclude that this drug causes subtle changes in amino acid turnover and/or release. This possibility deserves further experimental evaluation, including the use of in vivo brain microdialysis.

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