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Anticonvulsant activity of ester- and amide-type lipid conjugates of glycine and *N*-benzyloxycarbonylglycine

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Abstract

The anticonvulsant activity of ester- and amide-type lipid conjugates of glycine and *N*-benzyloxycarbonylglycine (Z-glycine) was evaluated in the maximal electroshock (MES) test and the strychnine test. Regardless of the type of the conjugates the Z-glycine derivatives were always more potent than the corresponding glycine derivatives. The amide lipids proved to be more active than the ester derivatives. While the lipid carriers with straight chain fatty acids that did not contain glycine or Z-glycine did not show anticonvulsant activity in either of the tests applied, lipid carriers containing aromatic or aliphatic rings in the acids displayed activity although lower than the conjugates containing glycine or Z-glycine. The lipid conjugates did not display significant binding to the strychnine-sensitive glycine receptor or to the strychnine-insensitive NMDA receptor.

Keywords: Anticonvulsant; Lipid conjugates; Glycine; *N*-Benzyloxycarbonylglycine; Glycine receptor; NMDA receptor

1. Introduction

Glycine mediates central nervous system effects via interaction with two different receptors (Aprison, 1990Betz, 1992; Kemp and Leeson, 1992), governing opposite responses of excitatory and inhibitory neurotransmission. Both, the strychnine-sensitive glycine receptor and the strychnine-insensitive glycine site of the NMDA receptor complex are implicated in the control of seizures. Agonists of the strychnine-sensitive receptor such as β-alanine or taurine, exhibit anticonvulsant activity, while agonists of the strychnine-insensitive receptor act as co-agonists of the excitatory amino acids glutamate and aspartate, and can be considered pro-convulsants.

Glycine exhibits modest anticonvulsant activity in several seizure-induced models (Lapin, 1981Toth et al., 1983; Seiler and Sarhan, 1984Halsey et al., 1989) including the 3-mercaptopropionic acid test and the strychnine test, while it is inactive in the maximal electroshock seizure (MES) test and the pentylenetetrazol sc(Met) test. On the other hand, co-administration of glycine potentiates the action of different anticonvulsants (Peterson et al., 1990Peterson, 1991). Glycine itself does not readily cross the blood–brain barrier due to its zwitterionic character and the absence of an active transport mechanism. Consequently, pharmacological effects upon systemic administration can only be observed with heroic amounts of glycine (10–40 mmol/kg). Recently, we reported that the *N*-benzyloxycarbonylglycine (Z-glycine) was far more active than glycine in the strychnine and 3-mercaptopropionic acid tests. Moreover, the lipophilic derivative of glycine exhibited anticonvulsant activity in the MES test after i.p. administration to mice comparable to milacemide (Lambert et al., 1994).

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Jacob and co-workers reported a 10 to 100 fold increase of the brain uptake of GABA after administration of mixed triglycerides in which one or two fatty acids had been replaced by GABA (Jacob et al., 1985Jacob et al., 1987, 1990). This 'pseudo-triglyceride' approach was employed for numerous drugs and has been the subject of a recent review (Lambert et al., 1995a). However, the short plasma half-life of the triglycerides (Goncalves, 1990) limits the potential of these glycerides as CNS vectors. To overcome this drawback we have developed amide isosters of triglycerides in which the ester bonds in position 1 and 3 have been replaced by an amide bond in order to increase the metabolic stability. Using this approach, improved delivery of the anticonvulsant drug valproic acid and the endopeptidase inhibitor acetylthiorphan could be demonstrated (Mergen et al., 1991aMergen et al., 1991b; Lambert et al., 1995b).

The present study was conducted in order to evaluate the anticonvulsant activity of ester- and amide-type lipid conjugates of glycine and Z-glycine. Preliminary results of the seizure-antagonizing activity of the 2-*N*-benzyloxycarbonylglycinate-1,3-*N,N'*-dipalmitoyldiaminopropan-2-ol in the MES test have been reported (Mergen et al., 1991a).

2. Experimental procedures

2.1. Chemistry

Melting point determination: Kofler melting point apparatus, uncorrected. NMR, Varian Gemini 200 (TMS); ^{1}H-, 200 MHz; ^{13}C-, 50 MHz; solvent, $CDCl_3$ with TMS as internal standard. DCI-MS, Varian MAT 44S, source temperature 100°C, ammonia. FAB-MS, Kratos MS-80. 1,3-Dipalmitoylglycerol (**8**) was prepared according to Bentley and McCrae (1970), 1,2-dipalmitoylglycerol (**9**) according to Howe and Malkin (1951). The compounds **1a–h**, **2b**, **2e** and **3b**, **3d** and **3e** were prepared as described (Mergen et al., 1991b).

2.1.1. 2-(1,3-Dipalmitoylglycerol)-N-benzyloxycarbonyl-2-aminoacetic acid ester (4)

2.3 g of freshly distilled oxalyl chloride in 20 ml of dry diethyl ether were slowly added to a slurry of 3.2 g (15 mmol) Z-glycine in 30 ml of dry diethyl ether containing 5 drops of DMF under nitrogen at 0°C. After 30 min Z-glycine had been dissolved and the resulting mixture was stirred for additional 3 h at 0°C. The excess of oxalyl chloride and the solvent were evaporated under reduced pressure. The resulting yellowish oil was dissolved in 20 ml of dry CH_2Cl_2 and slowly added to a solution of 6.0 g (10.5 mmol) 1,3-dipalmitoylglycerol (**8**) and 2.1 g (21 mmol) triethylamine in 100 ml dry CH_2Cl_2 under nitrogen at 0°C. The mixture was stirred for 2 h at 0°C, overnight at room temperature, and poured into ice/0.5 M HCl. The organic layer was washed with 0.5 M HCl, water, 10% $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated under reduced pressure. Column chromatography and crystallization from 2-propanol afforded 5.5 g of **4** (7.24 mmol; 68.6% based on 1,3-dipalmitoylglycerol), m.p. 56°C. $C_{45}H_{77}NO_8$ (760.08): Calc. C 71.10, H 10.21, N 1.84; found C 71.61, H 10.79, N 1.86.

^{1}H-NMR: δ=7.35 (s, 5 H, aromat. H), 5.47 (t, J=5.6 Hz, 1 H, NH), 5.32–5.24 (m, 1 H, CH), 5.13 (s, 2 H, CH_2-C_6H_5), 4.34 and 4.14 (dd each, J=4.2/12.0 Hz, and 5.9/12.0 Hz, 2 H each, CH_2-CH-CH_2), 4.00 (d, J=5.6 Hz, 2 H, CH_2-NH), 2.31 (t, J=7.2 Hz, 4 H, 2×CH_2-CH_2-CO), 1.63–1.54 (m, 4 H, CH_2-CH_2-CO), 1.37–1.19 (m, 48 H, fatty acid CH_2), 0.88 (t, J=6.2 Hz, 6 H, 2×CH_3).

^{13}C-NMR: δ=173.3 (CO), 169.3 (CO), 156.2 (CO), 136.3 (C), 128.6 (CH), 128.2 (CH), 128.1 (CH), 70.6 (CH), 67.2 (CH_2), 61.8 (CH_2), 42.8 (CH_2), 34.0 (CH_2), 32.0 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 24.9 (CH_2), 22.7 (CH_2), 14.1 (CH_3).

DCI-MS: m/z=777 (100%, $[M+NH_4]^+$).

2.1.2. 2-(1,3-Dipalmitoylglycerol)-2-aminoacetic acid ester (5)

5.0 g (6.6 mmol) of **4** in 100 ml CH_2Cl_2/2-propanol (5:1, v,v) were hydrogenated over 500 mg Pd/C 10% for 3 h. After removal of the catalyst by filtration the solvent was evaporated under reduced pressure. Column chromatography and crystallization

from n-hexane yielded 2.5 g of the base (4.0 mmol, 60.7%) which was converted to the hydrochloride (**5**) by treatment with etherial HCl. m.p. base 58–59°C; m.p. hydrochloride 105–106°C. $C_{37}H_{71}NO_6 \cdot HCl$ (662.41): Calc. C 67.08, H 10.96, N 2.11; found C 67.17, H 11.05, N 2.18.

^{1}H-NMR: δ=5.31–5.26 (m, 1 H, CH), 4.34 and 4.15 (dd each, J=4.3/12.0 Hz and 5.8/12.0 Hz, 2 H each, CH_2-CH-CH_2), 4.11 (s, 2 H, CH_2-NH_2), 2.32 (t, J=7.2 Hz, 4 H, 2×CH_2-CH_2-CO), 1.64–1.54 (m, 6 H, 2×CH_2-CH_2-CO and NH_2), 1.37–1.19 (m, 48 H, fatty acid CH_2), 0.88 (t, J=6.5 Hz, 6 H, 2×CH_3).

^{13}C-NMR: δ=174.1 (CO), 173.2 (CO), 69.9 (CH), 61.9 (CH_2), 44.0 (CH_2), 34.0 (CH_2), 31.9 CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 24.9 (CH_2), 22.7 (CH_2), 14.1 (CH_3).

DCI-MS: m/z=626 (100%, $[M+H]^+$).

2.1.3. 1-(2,3-Dipalmititoylglycerol)-N-benzyloxycarbonyl-2-aminoacetic acid ester (6)

6 was prepared from 3.2 g (15 mmol) Z-glycine and 5.7 g (10 mmol) of 1,2-dipalmitoylglycerol (**9**) as described for **4**. Yield: 6.7 g (8.9 mmol, 89.5% based on 1,2-dipalmitoylglycerol); m.p. 63°C. $C_{45}H_{77}NO_8$ (760.08): Calc. C 71.10, H 10.21 N 1.84; found C 71.50, H 10.66, N 1.76.

^{1}H-NMR: δ=7.35 (s, 5 H, aromat. H), 5.33–5.22 (m, 2 H, CH and NH), 4.42–4.09 (m, CH_2-CH_2-CO), 1.64–1.54 (m, 4 H, 2×CH_2-CH_2-CO), 1.46–1.14 (m, 48 H, fatty acid CH_2), 0.88 (t, J=6.2 Hz, 6 H, 2×CH_3).

^{13}C-NMR: δ=173.2 (CO), 172.9 (CO), 169.6 (CO), 156.3 (CO), 136.2 (C), 128.5 (CH), 128.2 (CH), 128.1 (CH), 68.6 (CH), 67.2 (CH_2), 63.3 (CH_2), 61.9 (CH_2), 42.7 (CH_2), 34.2 (CH_2), 34.0 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 24.9 (CH_2), 22.7 (CH_2), 14.1 (CH_3).

DCI-MS: m/z=777 (100%, $[M+NH_4]^+$).

2.1.4. 1-(2,3-Dipalmitoylglycerol)-2-aminoacetic acid ester (7)

1.0 g of **6** (1.3 mmol) in 50 ml dioxane was hydrogenated over 100 mg 10% Pd/C for 3 h. Work-up and conversion to the hydrochloride by treatment with etherial HCl gave 93 mg analytically pure **7** (0.14 mmol, 10.8%), m.p. 156–158°C. $C_{37}H_{71}NO_6 \cdot HCl$ (662.41): Calc. C 67.08, H 10.96, N 2.11; found C 67.24, H 11.21, N 2.06.

^{1}H-NMR: δ=5.31–5.25 (m, 1 H, CH), 4.41–4.10 (m, 4 H, CH_2-CH-CH_2), 3.45 (s, 2 H, CH_2-NH_2), 2.32 (t, J=7.5 Hz, 2 H, CH_2-CH_2-CO), 2.31 (t, J=7.4 Hz; 2 H,CH_2-CH_2-CO), 179–153 (m, 6 H, 2×CH_2-CH_2-CO and NH_2), 1.40–1.17 (m, 48 H, fatty acid CH_2), 0.88 (t, J=6.4 Hz, 6 H, 2×CH_3).

^{13}C-NMR: δ=173.9 (CO), 173.2 (CO), 172.9 (CO), 68.8 (CH), 62.8 (CH_2), 62.0 (CH_2), 43.9 (CH_2), 34.2 (CH_2), 34.1 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 24.9 (CH_2), 22.7 (CH_2), 14.1 (CH3).

DCI-MS: m/z=626 (100%, $[M+H]^+$).

2.2. Pharmacology

The reference compounds were obtained from the following sources: phenytoin from Sigma (Deisenhofen, Germany), milacemide from Continental Pharma (Mont-Saint-Guibert, Belgium) valpromide (2-propylpentanamide) and sodium valproate (2-propylpentanoate) from Sanofi (Brussels, Belgium).

2.2.1. Electrically-induced seizures (MES test)

Male OF1 mice (Iffa-Credo, Les Oncins, France) weighing 18–30 g were housed in colony cages in a 12-h light–dark cycle with free access to commercial rodent chow and water. During the experiment the animals were only allowed free access to water. Maximal electroshock seizures were induced by delivering an electrical stimulus of 50 mA for 0.2 s via corneal electrodes. Blockade of the tonic extension of the hind limbs was considered as protection against seizures (Swinyard et al., 1952). The compounds were administered in DMSO. ED_{50} values

were calculated according to Litchfield and Wilcoxon (1949).

2.2.2. Chemically-induced seizures

Male NMRI mice, 18–30 g, were obtained from the animal breeding facility at the Catholic University of Louvain, and housed as described above. 1.2 mg/kg of strychnine was injected s.c. The animals were observed for 30 min after the administration of the convulsant. The time of the seizure onset was recorded (Lapin, 1981).

2.2.3. Radioligand binding assays

Male Wistar rats were killed by decapitation. Cerebral cortex and spinal cord were carefully removed and conserved at least 24 h at −80°C. Protein concentration in homogenates was determined using the Coomassie blue method (Bio-Rad Laboratories, Brussels, Belgium). Stock solutions of the tested compounds were prepared in absolute ethanol. Following filtration, the filters were placed in plastic scintillation vials, 7 ml Aqualuma (Lumac, Landgraaf, Netherlands) was added and after vigorous agitation counted in a Pharmacia Wallac 1410 scintillation counter (Uppsala, Sweden). All assays were performed in triplicate.

2.2.4. Strychnine-insensitive glycine binding site associated to the NMDA-receptor binding

Buffy-coat membranes from rat cerebral cortex were prepared according to Canton et al. (1992) with minor modifications. Membranes (0.4 mg protein/ml, final volume 1 ml) were incubated for 30 min at 4°C with [^{3}H]5,7-dichlorokynurenic acid (New England Nuclear, spec. act. 614.2 GBq/mmol, 16.6 Ci/mmol, final concentration 20 nM) in 50 mM HEPES/KOH buffer, pH 7.5. Nonspecific binding was determined with 1 mM glycine. Following the incubation, the homogenate was filtered through Whatman GF/C filters, treated with 0.5% polyethylenimine and rinsed three times with 4 ml of ice-cold 50 mM HEPES/KOH buffer containing 10 mM $MgSO_4$.

2.2.5. Strychnine-sensitive glycine receptor binding

Binding experiments using membranes from rat spinal cord were performed according to Marvizon et al. (1986). The membrane preparations (0.3 mg protein/ml; final volume 1 ml) were incubated in 50 mM sodium phosphate buffer (pH 7.4) with [^{3}H]strychnine (New England Nuclear, spec. act. 906.5 GBq/mmol, 24.5 Ci/mmol, final concentration 2 nM) for 15 min at 4°C. Non-specific binding was determined with 10 mM unlabelled strychnine. Following the incubation, the homogenate was filtered through Whatman GF/B glass filters and rinsed three times with 4 ml ice-cold 0.15 M NaCl.

3. Results

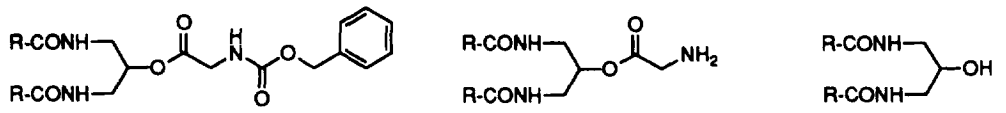
3.1. Chemistry

The amide lipid conjugates of Z-glycine (**1a–h**) and glycine (**2b** and **2e**), as well as the vector entities **3b**, **3d** and **3e** summarized in Fig. 1 were prepared as described previously (Mergen et al., 1991aMergen et al., 1991b; Lambert et al., 1995b). The synthesis of the isomeric ester lipid conjugates of Z-glycine (**4** and **6**), as well as the conjugates of glycine (**5** and **7**) was achieved by standard procedures as outlined in Fig. 2. In contrast to the glycine-2-triglyceride (**5**), the corresponding 1-glyceride (**7**) proved to be unstable during purification and storage even at low temperatures. The compound was stable as the hydrochloride salt.

3.2. Pharmacological evaluation

The anticonvulsant activity of ester and amide lipid conjugates of glycine and Z-glycine, as well as the vector entities which did not contain a glycine derivative were evaluated in the MES test. Table 1 summarizes the initial screening of the compounds 30 min and 3 h after the i.p. administration of the drugs dissolved in DMSO to mice. Regardless of the type of the conjugate (ester or amide) compounds containing Z-glycine exhibited a higher anticonvulsant activity than the corresponding glycine derivatives. Moreover, the amide lipids **1a–h**, **2b** and **2e** were more effective antagonizing MES-induced seizures than the ester glycerides **4–7**. The shift of the glycine residue from position 2 to position 1 in the mixed triglycerides resulted in a decrease of the anticonvulsant activity. The median effective doses (ED_{50}) of the most active amide derivatives in comparison to standard anticonvulsant drugs have been summarized in Table 2.

Fig. 3 shows a comparison of the time-courses of



	R	acid
a	C_9H_{19}	decanoic acid
b	$C_{15}H_{31}$	palmitic acid
c	$C_{18}H_{35}$	oleic acid
d	C_6H_5	benzoic acid
e	C_6H_5-CH_2-CH_2	phenylpropanoic acid
f	C_6H_{11}-CH_2	cyclohexylacetic acid
g	C_6H_{11}-CH_2-CH_2	cyclohexylpropanoic acid
h		amantoic acid

Fig. 1. Structures of Z-glycine amide lipids, glycine amide lipids and amide lipid carriers.

the anticonvulsant activity of Z-glycine and amide lipid conjugates **1b** and **1d** in the MES test. All three compounds exhibit a maximum of the activity around 3–4 h.

The anticonvulsant activity of the lipidic carrier molecules **3b**, **3d**, **3e**, **8**, and 1,3-dibenzoylglycerol that did not contain glycine or Z-glycine were also evaluated in the MES test (Table 3). The ester lipids were inactive at the concentrations tested as was the dipalmitoylamide derivative **3b**. In contrast, amide lipid derivatives containing aromatic side chains such as 1,3-dibenzoylaminopropan-2-ol (**3d**), 1,3-phenylpropanoylaminopropan-2-ol (**3e**) displayed considerable activity especially 30 min after the administration.

Compounds **1b** and **1d** were compared to glycine and the corresponding amide vectors **3b** and **3d** in their effect on strychnine-induced convulsions (Table 4). Both conjugates were more active than Z-glycine. As shown for electrically induced seizures 1,3-dibenzoylaminopropan-2-ol **3d** also displayed anticonvulsant activity while 1,3-dipalmitoylaminopropan-2-ol (**3b**) was inactive. **3d** was more active after 30 min than after 3 h.

In contrast to glycine, neither the conjugates nor the carrier molecules displayed significant binding to the strychnine-sensitive glycine receptor or to the strychnine-insensitive glycine site of the NMDA receptor complex in concentrations up to 10 mM (Table 5). Z-glycine exhibited a 15% displacement of labelled strychnine from the glycine receptor at a concentration of 1 mM.

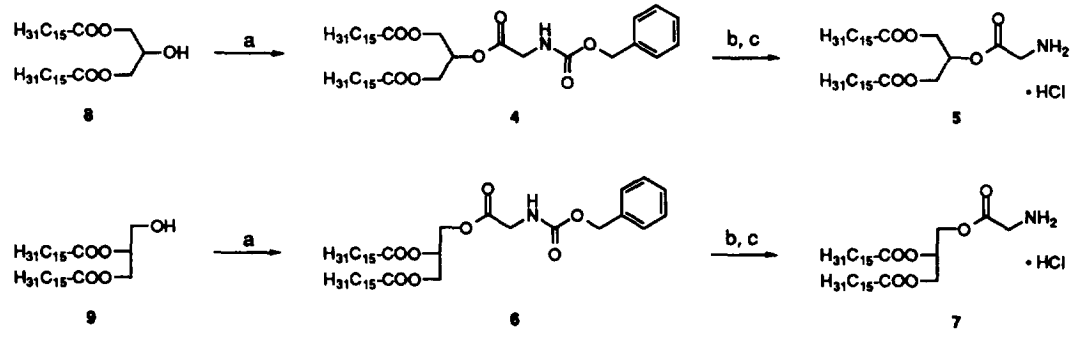


Fig. 2. Synthesis of ester lipid conjugates of glycine and Z-glycine. (a) Z-glycine chloride, Et_3N; (b) Pd/C, H2; (c) etherial HCl.

Table 1
MES activity of the glycine, Z-glycine and the ester and amide conjugates after i.p. administration in DMSO in mice

Compound	Dose (mmol/kg)]	MES activity (%)	
		30 min	3 h
Control (DMSO)	–	0	0
Glycine	10	0	0
Z-glycine	0.4	25	100
Ester lipid conjugates			
4	0.5	0	60
6	0.5	0	40
5	0.8	0	0
7	0.8	0	0
Amide lipid conjugates			
1a	0.4	0	100
1b	0.4	88	100
1c	0.4	83	100
1d	0.4	71	100
1f	0.4	12.5	37.5
1g	0.4	25	100
1h	0.4	25	57
2b	0.4	50	33
2e	0.4	67	60

The values are expressed as percent of the animals protected against seizures (n=4–8).

4. Discussion

The comparison of the anticonvulsant activity of ester- and amide-type lipid conjugates of glycine or Z-glycine revealed that the amide derivatives are generally more active than the esters. This may be attributed to the increased metabolic stability of the amide bonds compared to ester bonds and is consistent with previous results obtained for lipid-conju-

Table 2
Median effective dose (ED_{50}) of the compounds after i.p. administration in DMSO in mice

Compound	30 min	3 h
1b	0.139 (0.084–0.229)	0.068 (0.034–0.137)
1c	0.230 (0.108–0.492)	0.048 (0.018–0.126)
1d	0.104 (0.059–0.181)	0.036 (0.015–0.089)
1g	>0.4	0.043 (0.023–0.082)
2e	0.282 (0.174–0.454)	0.120 (0.086–0.197)
3b	>0.4	>0.4
3d	0.120 (0.049–0.295)	>0.4
Z-glycine	>0.4	0.270 (0.195–0.373)
Milacemide	>0.4	0.210 (0.070–0.290)
Phenytoin	0.014 (0.010–0.019)	0.012 (0.007–0.021)
Valpromide	0.104 (0.82–0.124)	0.080 (0.042–0.150)
Sodium valproate	0.305 (0.153–0.606)	0.560 (0.340–0.890)

ED_{50} values were calculated from 5–7 doses (n=5–8 animals per dose) according to Litchfield and Wilcoxon (1949). The values are expressed in mmol/kg; 95% confidence intervals are given in parentheses.

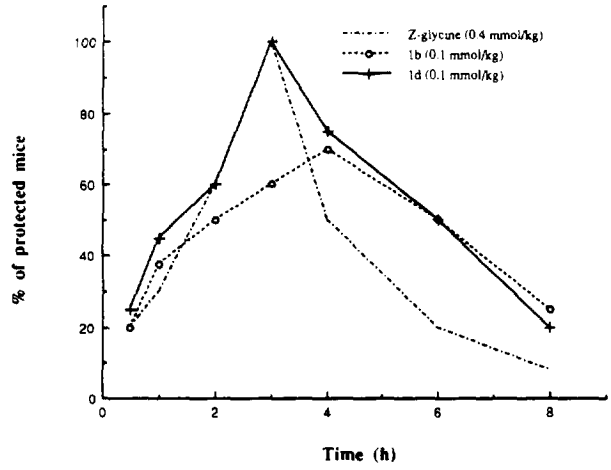


Fig. 3. Time-course of the MES activity of Z-glycine (0.4 mmol/kg, i.p.) and Z-glycine amide lipids **1b** and **1d** (0.1 mmol/kg, i.p.).

Table 3
MES activity of the ester and amide carrier moieties after i.p. administration in DMSO in mice

Compound	Dose (mmol/kg)	MES activity (%)	
		30 min	3h
Control (DMSO)	–	0	0
Amide lipid moieties			
3b	0.4	0	0
3d	0.4	100	0
3e	0.4	100	50
Ester lipid moieties			
8	0.4	0	0
1,3-dibenzoylglycerol	0.4	0	0

The values are expressed as percent of the animals protected against seizures (n=4–8).

Table 4
Determination of the activity against strychnine-induced seizures after i.p. administration of Z-glycine, the amide Z-glycine conjugates (**1b** and **1d**) and the amide lipid carriers (**3b** and **3d**) in DMSO

Compound	Dose	Latency (%)	
	(mmol/kg)	30 min	3 h
Control (DMSO)	–	100±10	100±7
Z-glycine	0.4	90±7	120±11
	1	150±27[b]	142±9[c]
1b	0.4	179±19[c]	164±10[c]
1d	0.4	202±34[c]	133±16[b]
3b	0.4	102±11	96±11
3d	0.4	374±65[c]	92±11
Palmitic acid	1	110±12	94±11
Benzoic acid	1	94±8	96±8
Valpromide	0.4	133±16[a]	120±8[a]

The results are expressed as percentages of the mean control latency±SEM (%) of the onset of the seizures. Statistical comparison was performed using Student's t-test: [a] $P<0.1$; [b] $P<0.05$; [c] $P<0.01$.

Table 5
Determination of the binding of Z-glycine, the lipid conjugates (**1b**, **1d**, **2b** and **2d**) and the carrier moieties (**3b** and **3d**) to the strychnine-sensitive glycine receptor (with [^{3}H]strychnine) and to the strychnine-insensitive glycine binding site of the NMDA receptor (with [^{3}H]dichlorokynurenic acid)

Compound	[^{3}H]Strychnine	[^{3}H]Dichlorokynurenic acid
Glycine	28±9 μM[b]	0.75±0.03 μM[a]
D-Alanine	n.d.	1.9±0.11 μM
Strychnine	0.015±0.004 μM	No displacement at 100 μM
Z-glycine	15% displacement at 1 mM	No displacement at 10 mM
1b	No displacement at 10 mM	No displacement at 10 mM
1d	No displacement at 10 mM	No displacement at 10 mM
2b	No displacement at 10 mM	No displacement at 10 mM
2d	No displacement at 10 mM	No displacement at 10 mM
3b	No displacement at 10 mM	No displacement at 10 mM
3d	No displacement at 10 mM	No displacement at 10 mM

The results are expressed as IC_{50} values±SE ($n=3$).
[a] Literature value: $IC_{50}=0.71\pm0.04$ μM (Canton et al., 1992).
[b] Literature value: $IC_{50}=32\pm6$ μM (Marvizon et al., 1986).

gates of valproic acid (Mergen et al., 1991aMergen et al., 1991b).

The introduction of the *N*-benzyloxycarbonyl group greatly enhanced the anticonvulsant activity, regardless of the type of compound. Thus, based on the ED_{50} the activity of **2b** was roughly doubled by *N*-derivatization to give **1b**. These results are in accordance with the previous anticonvulsant evaluation of glycine derivatives. Milacemide (Van Dorsser et al., 1983) and the *N*-linoleylglycine (Vamvakides, 1986) were found more active than glycine while glycine methyl ester, glycine ethyl ester, glycine *tert*-butyl ester and glycine dodecanoic ester did not exhibit any significant improvement (Toth et al., 1983Sarhan et al., 1984). The MES activity of the Z-glycine lipid conjugates upon i.p. administration is in the range of the activity of valpromide.

The amide derivatives may be divided into two groups. The first group includes compounds with linear fatty acids. While these Z-glycine–lipid conjugates (**1a–c**) effectively antagonized electrically or chemically induced seizures, the lipid moiety **3b** containing no glycine was devoid of anticonvulsant activity. Moreover, neither the conjugate **1b**, nor the respective carrier **3b** bind to CNS-glycine receptors. Therefore, the data suggest that lipid conjugates with linear fatty acids act as prodrugs of glycine.

The second group of amide derivatives includes compounds containing acids with aromatic or cyclic aliphatic rings. In this series the lipid moieties without Z-glycine also displayed anticonvulsant activity as shown for **3d** and **3e**. Neither the Z-glycine containing conjugates nor the lipid carriers bind to the CNS glycine receptors. However, both derivatives have been shown to possess muscle relaxant activity in isolated guinea-pig ileum (Lambert et al., 1993). Thus, a muscle-relaxant component might contribute to the seizure antagonizing activity of these conjugates although an intrinsic anticonvulsant activity of the compounds cannot be ruled out. The 1,3-diaminopropan-2-ol structure appeared to be necessary for the seizure-antagonizing effect of **3d** because the correponding 1,3-dibenzoylglycerol was inactive.

In conclusion, the results indicate that coupling of glycine or Z-glycine to amide-type lipids can enhance the anticonvulsant activity of the compounds. Conjugates containing straight chain fatty acids may be regarded as prodrugs of glycine. An additional muscle relaxing effect might contribute to the activity of derivatives of acids with aromatic or aliphatic rings. The ability of the lipid conjugates to cross the blood–brain barrier, as well as further experiments for the elucidation of the pharmacological mechanism of the amide lipids are currently under investigation.

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