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N-Methyl-D-aspartate Antagonist Activity of α- and β-Sulfallorphans[1]

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ABSTRACT

Resolved equatorial (α) and axial (β) forms of *S*-allylmorphinans, α-sulfallorphan and β-sulfallorphan, were tested for their ability to compete with the binding of phencyclidine and *sigma* receptor ligands to mouse brain membranes and to antagonize N-methyl-D-aspartate (NMDA)-induced convulsions in mice. α- and β-sulfallorphans displayed distinct binding affinities for phencyclidine and *sigma* sites, inhibiting the binding of [^{3}H]-(5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine ([^{3}H]MK-801) with K_i values of 2.32 and 0.13 μM and that of [^{3}H](+)-pentazocine with K_i values of 1.97 and 1.61 μM, respectively. Intracerebroventricular administration of these compounds in mice caused dose-dependent inhibitions of NMDA-induced convulsions, but did not affect convulsions induced by (*R*,*S*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainic acid and bicuculline. α- and β-sulfallorphans blocked the convulsive activity of NMDA (1 nmol/mouse; intracerebroventricular) with ED_{50} values of 0.48 and 0.015 nmol/mouse, as compared with 0.55, 0.039 and 0.013 nmol/mouse for dextrorphan, MK-801 and (±)3-(2-carboxypiperazine-4yl)propyl-1-proprionic acid, respectively. The structurally related compound, dextrallorphan, significantly but less potently blocked NMDA-induced convulsions (ED_{50}, 2.68 nmol/mouse). At the protective doses, α- and β-sulfallorphans markedly reduced NMDA- and AMPA-induced mortality without inducing locomotion and falling behavior. These results indicate that α- and β-sulfallorphans are potent and selective NMDA antagonists devoid of motor side effects at protective doses.

Excitatory amino acids receptors can be classified into at least three types according to their specific affinity for selective agonists, namely NMDA, AMPA and kainic acid (Monaghan *et al.*, 1989). These receptors are coupled to cation channels that open in response to agonist stimulation which causes the depolarization of the target cell. The NMDA receptor channel complex is endowed with several special features that distinguish it from AMPA or kainate receptor channels. These features include a sensitivity to blockade by a physiological concentration of Mg^{++}, a high permeability to Ca^{++} and a requirement of glycine as positive allosteric regulator (Rogawski and Porter, 1990). Animal studies indicate that the NMDA receptor is involved in several physiological phenomena including developmental plasticity (Tsumoto *et al.*, 1987; Rauschecker and Hahn, 1987; Kleinschecker *et al.*, 1987; Cline *et al.*, 1987), learning and memory processes (Collingridge and Bliss, 1987; Morris *et al.*, 1986), sensory transmission (Spierra and Davis, 1988; Kemp and Sillito, 1982; Salt, 1986) and the control of respiration (Foutz *et al.*, 1988) and blood pressure (Kubo and Kihara, 1988). Major advances that were recently achieved in our understanding of the role of excitatory amino acid receptors in the etiology of neuropathophysiological conditions provide new potential therapeutic approaches. Overstimulation of these sites induce excitotoxic effects manifested by various cellular events that include the excessive entry of Ca^{++}, the activation of lipases and phospholipases, the production of free radicals and ultimately neuronal cell death (Olney, 1990). Thus, excitatory amino acids have been suggested to participate in the pathophysiology of some neurological disorders that include cerebral ischemia (Meldrum, 1985; Rothman and Olney, 1987; Herrling, 1989), hypoglycemia (Weiloch, 1985), epilepsy (Croucher *et al.*, 1982; Meldrum *et al.*, 1989; Rogawski and Porter, 1990), anxiogenesis (Stephens *et al.*, 1986), motor-neuron diseases (Spencer *et al.*, 1986) and olivopontocerebellar atrophy (Plaitakis, 1984). The possibility of providing a rational therapy for these conditions has prompted a search for specific NMDA antagonists with potential neuroprotective activities (Watkins and Olverman, 1987; Watkins *et al.*, 1990; Rogawski and Porter, 1990).

Competitive and noncompetitive NMDA receptor antagonists offer protection against convulsions and neuronal cell

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ABBREVIATIONS: α-sulfallorphan, 3-hydroxy-17-deaza-17-α-(allylthia)morphinan; β-sulfallorphan, 3-hydroxy-17-deaza-17-β-(allylthia)morphinan; NMDA, N-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-proprionate; PCP, phencyclidine; MK-801, (+)5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate; CPP, (±)3-(2-carboxypiperazine-4yl)propyl-1-proprionic acid.

death in different animal models (Collingridge and Lester, 1989; Rogawski and Porter, 1990). Despite their high anticonvulsant properties, all the NMDA antagonists have the unfortunate problem of causing neurological side effects on motor performance and memory function at doses similar to or close to those that provide significant protection (Morris *et al.*, 1986, 1989; Löscher *et al.*, 1988; Tricklebank *et al.*, 1989). In rats and mice, NMDA antagonists produce PCP-like behavioral effects (Bernard and Bennett, 1986; Koek *et al.*, 1987; Liebman *et al.*, 1987; Compton *et al.*, 1987; Iversen *et al.*, 1988; Koek and Colpaert, 1990). At present, it is unknown whether these or other side effects will limit the usefulness of NMDA receptor antagonists in the treatment of different neurological disorders. Therefore, there is a need to develop NMDA antagonists devoid of motor or toxic side effects at therapeutic doses. Such compounds may prove useful for the prevention of brain cell damage mainly in cases of cerebral ischaemia (Holden, 1993).

Morphinans are opiate compounds with chemical structures related to those of levorphanol, dextrorphan and dextromethorphan. These drugs were clinically introduced in the 60s for their analgesic and antitussive properties (Benson *et al.*, 1953). They do not produce neurological side effects at therapeutic doses. Renewed interest in these drugs derives from the recent findings regarding the anticonvulsant and neuroprotective antiischemic effects of dextrorphan and dextromethorphan in different experimental tests with laboratory animals (Ferkany *et al.*, 1988; Moreau *et al.*, 1989; Steinberg *et al.*, 1989; Tortella and Musacchio, 1986). The anticonvulsant and neuroprotective effects of these drugs were ascribed to their glutamate-antagonist properties (Steinberg *et al.*, 1989). Dextrorphan and dextromethorphan counteract, in a noncompetitive way, the excitatory properties of N-methyl-D-aspartate, both *in vitro* and *in vivo* (Church *et al.*, 1985; Choi, 1987; Goldberg *et al.*, 1987). Recently, we have demonstrated that the resolved axial (β) form of *S*-allylmorphinan (β-sulfallorphan) has no opioid activity while the equatorial (α) form of *S*-allylmorphinan (α-sulfallorphan) has opioid antagonist activity (Lemaire *et al.*, 1994). In the present study, the NMDA receptor antagonist activity and motor effects (locomotion and falling behavior) of α- and β-sulfallorphans were assessed and compared with those of prototypic NMDA antagonists.

Materials and Methods

Drugs. The axial and equatorial forms of *S*-allylmorphinan (α-sulfallorphan and β-sulfallorphan) were synthesized in the laboratory of Dr. Bernard Belleau, Biochem Pharma, Laval (Belleau *et al.*, 1985; 1986). The various conformers were separated by high-performance liquid chromatography (Belleau *et al.*, 1985). NMDA and kainic acid were purchased from Sigma Chemical Co., St. Louis, MO. AMPA and (+)bicuculline were products of Research Biochemical Incorporated, Natick, MA. CPP and MK-801 were obtained from Tocris Neuramin, Essex, England. [^{3}H]MK-801 (22.3 Ci/mmol) and [^{3}H](+)pentazocine (35 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Levorphanol, dextrorphan, dextromethorphan, levallorphan and dextrallorphan were products of Hoffman La Roche, Ltd. (Vaudreuil, Quebec).

Animals. Male Swiss Webster [(SW)fBR] mice (20–25 g; Canadian Breeding Farm, St-Constant, Quebec) were housed five per cage in a room with controlled temperature (22 ± 2°C), humidity and artificial light (6:30 A.M. to 7:00 P.M.). The animals had free access to food and water and were used after a minimum of 4 days of acclimation to housing conditions.

Radioligand binding. The mouse brain membranes were prepared as described previously (Rogers and Lemaire, 1993). Mice were sacrificed by decapitation, and their brains were homogenized with a glass Teflon homogenizer in 10 volumes (w/v) of ice-cold 5 mM Tris-HCl buffer (pH 7.4; buffer A). The homogenate was centrifuged at 27,000 × *g* for 30 min at 4°C. The pellet was resuspended in buffer A, incubated at 37°C for 30 min and centrifuged at the same speed. The resulting pellet was resuspended in buffer A containing 0.3 M KCl and stirred at 4°C for 60 min (Lee *et al.*, 1982). The suspension was recentrifuged at 27,000 × *g* for 30 min at 4°C, and the pellet was washed twice in 10 volumes of buffer A. The final membrane pellet was resuspended in buffer A at a concentration of 1.2 mg protein/ml and frozen at −80°C. Proteins were measured by the method of Lowry *et al.* (1951) with bovine serum albumin diluted in the same milieu as membrane samples as standard. [^{3}H]MK-801 and [^{3}H](+)-pentazocine were used to monitor the interaction of α- and β-sulfallorphan with PCP and *sigma* receptors, respectively (Shukla *et al.*, 1992; Bowen *et al.*, 1993). Binding assays were performed in buffer A at room temperature (22°C) for 30 min with a 2-ml aliquot of membrane preparation (1 mg protein) in presence of [^{3}H]MK-801 (5 nM) and increasing concentrations (10^{-9}–10^{-5} M) of α- or β-sulfallorphan. Incubations were terminated by filtration under reduced pressure through GF934AH Whatman filters pretreated with 0.05% polyethylenimine. Filters were washed with 4 × 3 ml aliquots of ice-cold buffer A, placed in liquid scintillation vials along with 10 ml Ecolume (ICN Biochemical Inc., Mississauga, Ontario, Canada) and counted in a Beckman scintillation counter. Nonspecific binding of [^{3}H]MK-801 was determined in the presence of 10 μM MK-801. Specific binding was defined as the difference between the radiolabel bound in the presence and absence of MK-801. The concentration of α- or β-sulfallorphan that produced 50% inhibition of [^{3}H]MK-801 binding (IC_{50}) was derived by use of the nonlinear least-square computer fitting program CDATA (EMF Software, Knoxville, TN). K_i values were calculated by the method of Cheng and Prusoff (1973). The effect of α- and β-sulfallorphan on the *sigma* receptor was also monitored, with the *sigma* receptor ligand [^{3}H](+)-pentazocine (5 nM). The binding experiments were performed as described above, and the nonspecific binding was determined in the presence of 10 μM haloperidol.

Anticonvulsive activity. Mice were coinjected i.c.v. with various doses of α-sulfallorphan (0.2–1.0 nmol, as indicated) or β-sulfallorphan (0.05–0.4 nmol, as indicated) and increasing doses of the convulsant compounds, NMDA (0.25–2.0 nmol), AMPA (0.25–5 nmol), kainic acid (0.25–0.75 nmol) or bicuculline (1–10 nmol) in a total volume of 10 μl of saline. Control experiments were conducted in the absence of the morphinan derivative. The animals were observed for 30 min for the signs of convulsions and death. The convulsive response to different convulsants began within 5 min and was characterized. The following responses were noted during the observation period: 1) mild myoclonus (moderate jerky movement of one or two limbs); 2) whole body clonus (dramatic and violent movements involving all the limbs and the body leading to loss of the righting reflex); 3) clonic-tonic seizures consisting of the following successive components: wild running characterized by episodes of running with explosive jumps, clonus and finally tonus characterized by extreme rigidity of the whole body. Groups of 15 animals were injected with increasing doses of an analeptic (NMDA, AMPA, kainate or bicuculline) in the presence or abscence of morphinan derivatives, as indicated. The animals were scored as showing seizure activity when one or more of the three responses mentioned above were present, and the number of animals showing these behavioral signs of convulsions in each group was recorded. The doses of different convulsants which, alone and in combination with increasing doses of α- or β-sulfallorphan, produced convulsions (CD_{50}) and lethality (LD_{50}) in 50% of animals with 95% confidence limits (95% CL) and potency ratios with 95% CL were calculated by the method of Litchfield and

Wilcoxon by procedure 47 of the computer program of Tallarida and Murray (1987).

Similarly in other sets of experiments increasing doses of α-sulfallorphan, β-sulfallorphan, dextrorphan, dextromethorphan, dextrallorphan, MK-801 and CPP were coadministered with NMDA (1 nmol/mouse i.c.v.), and the number of animals showing signs of convulsions at different dose levels in each treatment group was recorded. The doses of the NMDA-antagonists which produced protection in 50% of animals (ED_{50}) with 95% CL and potency ratios with 95% CL were calculated by procedure 47 of the computer program of Tallarida and Murray (1987).

Locomotion and falling behavior. In this set of experiments, locomotion and falling behavior were assessed according to a modificatiion of the procedure of Koek and Colpaert (1990). Mice were placed individually in observation cages for a 60-min habituation period. Thereafter, animals were injected with increasing doses of α-sulfallorphan, β-sulfallorphan, dextromethorphan, MK-801 or CPP (10 μl/mouse i.c.v.) and observed for 30 min after the injection. For each mouse, the presence of locomotion (locomotion with all four legs moving for at least 15 sec) and falling (falling from a rearing or standing position backward or to the side) was assessed. Each dose was tested in 10 mice, and the proportion of mice showing a particular behavior was determined.

The statistical significance of drug-induced changes in the occurrence of a particular behavior was tested by means of the method of Fray *et al.* (1980) and also described by Koek and Colpaert (1990). For each drug and each behavior, the data were arranged in a contingency table with two columns (the number of mice out of 10 showing the behavior and the number not showing the behavior) and one row for each drug dose tested (including the corresponding vehicle control). The statistic 2I, distributed as χ^2 with $(i - 1)(j - 1)$dF, was calculated as: $2I = 2\Sigma_{ij}[N_{ij}\cdot\ln(N_{ij}/E_{ij})]$, where N_{ij} is the observed cell frequency, E_{ij} is the expected cell frequency (row total·column total/grand total), and i and j are row and column numbers, respectively. If 2I was statistically significant, possible dose dependency was tested by using the following method of planned contrasts. A 2×2 cell was constructed with the number of mice showing and not showing a particular behavior after the vehicle control and after the lowest drug dose, and 2I was again calculated. If the lowest drug dose and the vehicle control were significantly different, the next higher drug dose was compared with the lowest dose with use of the 2I statistic. If the lowest drug dose did not differ significantly from the vehicle control, the next higher dose was compared with the combined results obtained with the lowest drug dose and the vehicle control. This process was repeated until the highest drug dose was reached. In this manner, adjacent doses could be grouped in such a way that there were no significant differences between members of a group, but adjacent groups were significantly different at the 5% level. Worked examples of the procedure are given by Fray *et al.* (1980) and Iwamoto (1984).

Results

PCP and *sigma* receptor binding activity of α- and β-sulfallorphans. α- and β-sulfallorphans were monitored for their ability to displace the binding of specific PCP ([^{3}H]MK-801) and *sigma* ([^{3}H](+)-pentazocine) receptor ligands to membrane preparations of mouse brain (fig. 1). α-Sulfallorphan was slightly more potent in inhibiting the binding of [^{3}H](+)-pentazocine (K_i, 1.97 ± 0.16 μM; Hill coefficient, 0.85) than that of [^{3}H]MK-801 (K_i, 2.32 ± 0.04 μM; Hill coefficient, 0.98) (fig. 1A). On the other hand, β-sulfallorphan was much more potent in competing with the binding of [^{3}H]MK-801 (K_i, 0.13 ± 0.01 μM; Hill coefficient, 0.97) than that of [^{3}H](+)-pentazocine (K_i, 1.61 ± 0.11 μM; Hill coefficient, 0.61) (fig. 1B). The ratios between the *sigma*/PCP K_i values were 0.85 and 12.4 for α- and β-sulfallorphans, respectively. Thus, both drugs showed similar affinities for the *sigma* site, whereas β-sulfallorphan displayed a significantly higher affinity for the PCP site than α-sulfallorphan ($P \leq .05$).

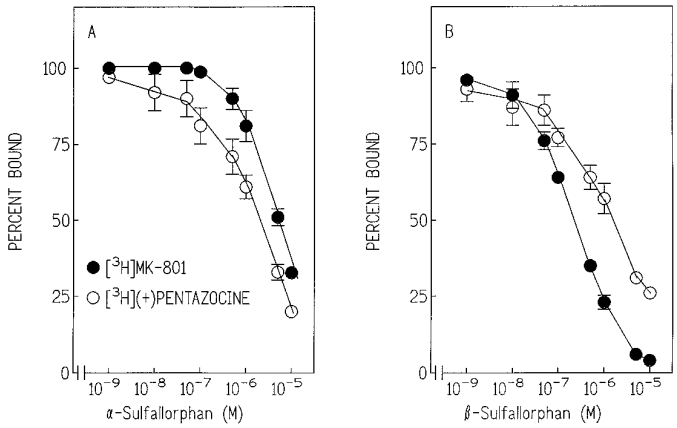

Fig. 1. Effect of increasing concentrations of α-sulfallorphan (A) and β-sulfallorphan (B) on the binding of [^{3}H]MK-801 (closed circle) and [^{3}H](+)-pentazocine (open circle) to mouse brain membranes.

Anticonvulsive activity. NMDA, AMPA, kainic acid and bicuculline dose-dependently induced rapid and short-lasting convulsions in mice. α-Sulfallorphan (1.0 nmol i.c.v.) or β-sulfallorphan (0.4 nmol i.c.v.) alone did not produce any behavioral change. Coadministration of α-sulfallorphan (0.2–1.0 nmol/mouse; table 1) or β-sulfallorphan (0.05–0.4 nmol/mouse; table 2) with NMDA blocked its convulsive activity with a corresponding increase in the CD_{50} and a significant change in the potency ratio. The anticonvulsive activities of α- and β-sulfallorphans were also dose dependent (fig. 2, A and B). On the other hand, α-sulfallorphan (1 nmol/mouse) and β-sulfallorphan (0.4 nmol/mouse) did not display any protection against AMPA-, kainic acid- and bicuculline-induced convulsions (tables 1 and 2, respectively). NMDA- and AMPA-induced mortality was either significantly reduced or totally blocked by α-sulfallorphan (1 nmol/mouse; table 1) and β-sulfallorphan (0.05–0.4 nmol/mouse; table 2), respectively.

The abilities of α- and β-sulfallorphans to antagonize NMDA (1 nmol)-induced convulsions were compared between each other and with those of other NMDA antagonists (fig. 3; table 3). Among the various compounds tested, β-sulfallorphan was the most potent blocker of NMDA-induced convulsions, being 32 times as potent as α-sulfallorphan with an ED_{50} of 0.015 nmol/mouse as compared with 0.48 nmol/mouse for α-sulfallorphan (table 3). Interestingly, α-sulfallorphan had itself a potency that was comparable with that of dextrorphan (ED_{50} of 0.48 as compared with 0.55 nmol/mouse for dextrorphan) and 10 times higher than that of dextromethorphan (ED_{50} of 4.87 nmol/mouse). β-Sulfallorphan was 166 times as potent as its structurally related compound, dextrallorphan (ED_{50} of 2.68 nmol/mouse). Finally, the noncompetitive NMDA antagonist, MK-801, was 2.6 times less potent than β-sulfallorphan, and the competitive NMDA antagonist, CPP, displayed a comparable activity (potency ratios of 0.38 and 1.15, respectively). However, the marked locomotor effects of these two latter compounds impaired the measurement of their anticonvulsive activity at supramaximal doses (fig. 3).

TABLE 1
Effect of α-sulfallorphan on NMDA-, AMPA-, kainic acid- and bicuculline-induced convulsions and mortality in mice

Convulsant	α-Sulfallorphan	Convulsions		Mortality	
		CD_{50} (95% CL)[a]	Potency ratio (95% CL)	LD_{50} (95% CL)	Potency ratio (95% CL)
	nmol/mouse	*nmol/mouse*		*nmol/mouse*	
NMDA	0	0.62 (0.55–0.71)	1	1.52 (1.33–1.75)	1
	0.2	0.81 (0.63–1.03)	0.77 (0.58–1.01)	1.69 (1.21–2.36)	0.90 (0.63–1.29)
	0.5	1.05 (0.85–1.29)	0.59 (0.47–0.76)*	1.58 (1.24–2.02)	0.96 (0.73–1.28)
	1.0	1.50 (1.15–1.98)	0.41 (0.31–0.56)*	2.56 (1.79–3.67)	0.59 (0.40–0.87)*
AMPA	0	0.34 (0.22–0.51)	1	2.51 (1.68–3.74)	1
	1	0.26 (0.13–0.49)	1.31 (0.61–2.86)	5.24 (3.16–8.69)	0.48 (0.25–0.91)*
Kainic acid	0	0.40 (0.32–0.49)	1	–[b]	NA[c]
	1	0.39 (0.29–0.51)	1.03 (0.73–1.46)	–[b]	NA[c]
Bicuculline	0	2.48 (1.60–3.84)	1	13.40 (5.49–32.70)	1
	1	4.44 (2.63–7.50)	0.56 (0.28–1.10)	12.69 (4.84–33.25)	1.06 (0.28–3.92)

[a] CL, confidence limit.
[b] No mortality was observed.
[c] NA, not applicable.
* $P < .05$.

TABLE 2
Effect of β-sulfallorphan on NMDA-, AMPA-, kainic acid- and bicuculline-induced convulsions and mortality in mice

Convulsant	β-Sulfallorphan	Convulsions		Mortality	
		CD_{50} (95% CL)[a]	Potency ratio (95% CL)	LD_{50} (95% CL)	Potency ratio (95% CL)
	nmol/mouse	*nmol/mouse*		*nmol/mouse*	
NMDA	0	0.62 (0.55–0.71)	1	1.52 (1.33–1.75)	–
	0.05	0.95 (0.81–1.11)	0.65 (0.53–0.80)*	[b]	–
	0.10	1.33 (1.14–1.57)	0.47 (0.38–0.57)*	[b]	–
	0.20	1.63 (1.39–1.92)	0.38 (0.31–0.47)*	[b]	–
	0.40	2.34 (1.86–2.93)	0.26 (0.20–0.34)*	[b]	–
AMPA	0	0.34 (0.22–0.51)	1	2.51 (1.68–3.74)	–
	0.40	0.29 (0.14–0.58)	1.16 (0.52–2.61)	[c]	–
Kainic acid	0	0.40 (0.32–0.49)	1	[b]	NA[d]
	0.40	0.39 (0.30–0.50)	1.03 (0.74–1.44)	[b]	NA[d]
Bicuculline	0	2.48 (1.60–3.84)	1	13.40 (5.49–32.70)	–
	0.40	3.12 (1.91–5.10)	0.79 (0.41–1.53)	[b]	–

[a] CL, confidence limit.
[b] No mortality was observed.
[c] Against 5 nmol of AMPA, one mouse died of 15 tested.
[d] NA, not applicable.
* $P < .05$.

Locomotion and falling behavior. α-Sulfallorphan did not produce locomotion and falling at doses ranging between 1.25 and 20 nmol (i.c.v.; fig. 4). β-Sulfallorphan produced locomotion in 20 to 100% of tested mice at doses (≥1.25 nmol/mouse) exceeding its anticonvulsive ED_{50} value (0.015 nmol/mouse) by factors of 83 or more (fig. 5; table 4). These high doses of β-sulfallorphan did not produce significant falling (0–10% mice at the tested dose range). Other NMDA antagonists produced both locomotion and falling behavior at protective doses. MK-801 (0.125–2.0 nmol i.c.v.) produced significant increases in locomotion and falling behavior in 30 to 100% and 0 to 50% of mice, respectively (fig. 6). The minimum dose of MK-801 producing significant falling behavior (1.5 nmol i.c.v.) was higher than that (0.25 nmol i.c.v.) producing significant locomotion (table 4). A comparison between the ratios of the lowest effective dose showing locomotion and the dose showing protection against NMDA-induced convulsion in 50% of animals (ED_{50}) reveals values of 0.2, 6.4 and 1.9 for dextromethorphan, MK-801 and CPP, respectively, as compared with 83 for β-sulfallorphan (table 4).

Discussion

S-Allylmorphinans are morphinan derivatives that incorporate a cationic sulfuration instead of the nitrogen atom in position 17 of levallorphan. In these *S*-morphinan derivatives, the position of the allyl group is fixed in either equatorial (α) (*e.g.*, *S*-allyl-equatorial-morphinan: α-sulfallorphan) or axial (β) (*e.g.*, *S*-allyl-axial-morphinan: β-sulfallorphan) conformations; whereas the allyl substitution on the nitrogen atom at position 17 in levallorphan rotates freely either in the axial or equatorial positions not allowing the stable formation and separation of axial and equatorial conformers.

Morphinans are known to be stereoselective for their opioid and nonopioid activity (Collingridge and Lester, 1989; Murray and Leid, 1984; Mendelsohn *et al.*, 1984). At *mu* opioid receptors, levorphanol, a levorotatory isomer of 3-hydroxy-N-methylmorphinan, is approximately 4,000 times more potent than dextrorphan, a dextrorotatory isomer of the same molecule. On the other hand, dextrorphan affinity for [^{3}H]PCP binding sites is 2.3 times greater than that of levorphanol

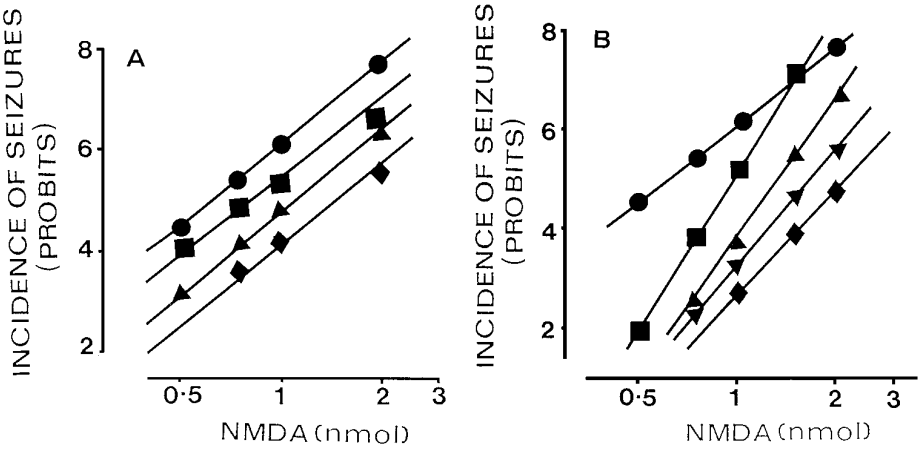

Fig. 2. (A) Probit-log dose regression curves for convulsions induced by NMDA (i.c.v.) in mice in the absence (closed circle) or presence of various doses (0.2, closed square; 0.5, closed triangle; 1.0, closed diamond, nmol/mouse) of α-sulfallorphan. (B) Probit-log dose regression curves for convulsions induced by NMDA (i.c.v.) in mice in the absence (closed circle) or presence of various doses (0.05, closed square; 0.10, closed triangle; 0.20, upside-down closed triangle; 0.40, closed diamond, nmol/mouse) of β-sulfallorphan.

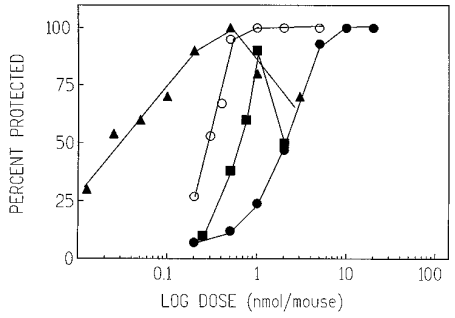

Fig. 3. Log-dose protection curves of α-sulfallorphan (closed circle), β-sulfallorphan (open circle), MK-801 (closed square) and CPP (closed triangle) against convulsions induced by NMDA (1 nmol i.c.v.) in mice.

(Murray and Leid, 1984). Previous studies indicated that the axial and equatorial orientation of the allyl group in sulfallorphan is also important for its opioid activity. In the *mu* receptor binding assay, α-sulfallorphan was 60 times as potent as β-sulfallorphan (Lemaire *et al.*, 1994). The equatorial conformation of the allyl group in sulfallorphan conferred a strong opioid antagonist activity to the molecule, but the axial conformer was only a weak opioid antagonist (Lemaire *et al.*, 1994).

In the present study, we demonstrated that both α- and β-sulfallorphans inhibit the binding of the PCP receptor ligand, [^{3}H]MK-801, but β-sulfallorphan was 24 times as potent as α-sulfallorphan. The order of potency of the sulfallorphans on the PCP site paralleled the potency in the *in vivo* anticonvulsive assay (figs. 1 and 3). Noncompetitive antagonists of NMDA receptors, such as PCP and MK-801, are known to possess anticonvulsant properties (Chapman and Meldrum, 1989; Leander *et al.*, 1988; Clineschmidt *et al.*, 1982). The dextrarotatory opioids, dextrorphan, and its 3-methyl ether derivative, dextromethorphan, also exhibit anticonvulsant activity in several *in vivo* seizure models including NMDA-induced convulsions (Ferkany *et al.*, 1988; Leander *et al.*, 1988; Tortella *et al.*, 1988; Chapman and Meldrum, 1989; Roth *et al.*, 1992; Church *et al.*, 1985).

Our study indicates that axial and equatorial orientations of the allyl group in sulfallorphans are important for their NMDA antagonist activity. β-Sulfallorphan was 31 times more potent than α-sulfallorphan against NMDA (1 nmol)-induced convulsions. Moreover, β-sulfallorphan at all tested doses completely protected against NMDA-, AMPA- and bicuculline-induced mortality, whereas α-sulfallorphan produced a marked but not total protection against mortality induced by NMDA. It seems unlikely that effects at *sigma* receptors make a substantial contribution to the anticonvulsant and neuroprotective activities of sulfallorphans. The relative potency of sulfallorphans as anticonvulsants did not correspond to their potency in inhibiting the binding of the *sigma* receptor ligand, [^{3}H](+)pentazocine, to mouse brain membranes. Neither did the potency of other PCP/*sigma* ligands such as SKF 10,047, cyclazocine, pentazocine and dextromethorphan as anticonvulsants correlate with their affinity for the *sigma* binding sites (Aram *et al.*, 1989; Sircar *et al.*, 1986; Largent *et al.*, 1986). Pentazocine was weaker than cyclazocine in inhibiting the epileptiform activity of NMDA-evoked depolarization and the binding of PCP ligands, but the reverse was observed for their ability to inhibit the binding of *sigma* ligands (Aram *et al.*, 1989). On the other hand, *sigma* receptor ligands were shown to modulate NMDA receptor stimulation (Monnet *et al.*, 1992; 1990; Pontecorvo *et al.*, 1991). *Sigma* selective compounds such as ifenprodil (Contreras *et al.*, 1990), BMY 14802 and haloperidol produced some protection and greatly increased the anticonvulsant potency of MK-801 against NMDA-induced convulsions in mice (Pontecorvo *et al.*, 1991). Thus, the ability of morphinan derivatives to interfere with the *sigma* site may partly explain their protection against NMDA-induced convulsions. Interestingly, the lack of motor effect of α-sulfallorphan (fig. 4) and other morphinan derivatives (Tortella *et al.*, 1994) corresponds to increased binding selectivity of these compounds for the *sigma* receptor as compared with the PCP receptor.

The *mu* opioid receptor is not likely to be involved in the protective activity of sulfallorphans, because naloxone (a *mu* opioid antagonist) showed no protection against NMDA-induced convulsions in mice (Shukla and Lemaire, 1993). On the other hand, the *mu* antagonist activity of sulfallorphans (mainly α-sulfallorphan) may contribute in attenuating or annulling motor side effects of the morphinan derivatives (Lemaire *et al.*, 1994).

Increasing doses of MK-801 and CPP produced biphasic effects against NMDA-induced convulsions (*e.g.*, a dose-dependent protection at lower doses and a decrease in the protecting activity at supramaximal doses; fig. 3). Biphasic dose response curves were also obtained with MK-801 and NPC-12626 in mice in the experiments related to protection against hypoxic stress (Pontecorvo *et al.*, 1991). In contrast,

TABLE 3

Comparison of the anticonvulsant activity of α-sulfallorphan, β-sulfallorphan and various NMDA antagonists against NMDA (1 nmol/mouse)-induced convulsions in mice

Test compound	Dose Range	CD_{50} (95% CL)[a]	Potency ratio (95% CL)
	nmole/mouse	*nmol/mouse*	
β-Sulfallorphan	0.005–0.05	0.015 (0.009–0.024)	1
α-Sulfallorphan	0.25–6	0.48 (0.29–0.77)	0.032 (0.031–0.034)*
Dextrorphan	0.1–5	0.55 (0.39–0.78)	0.027 (0.023–0.031)*
Dextromethorphan	1–25	4.87 (2.60–9.12)	0.003 (0.002–0.003)*
Dextrallorphan	1–30	2.68 (1.66–4.33)	0.006 (0.005–0.006)*
MK-801	0.01–0.5	0.039 (0.024–0.065)	0.38 (0.3–0.37)*
CPP	0.005–0.05	0.013 (0.007–0.025)	1.15 (0.96–1.29)

[a] CL, confidence limit.
* $P < .05$.

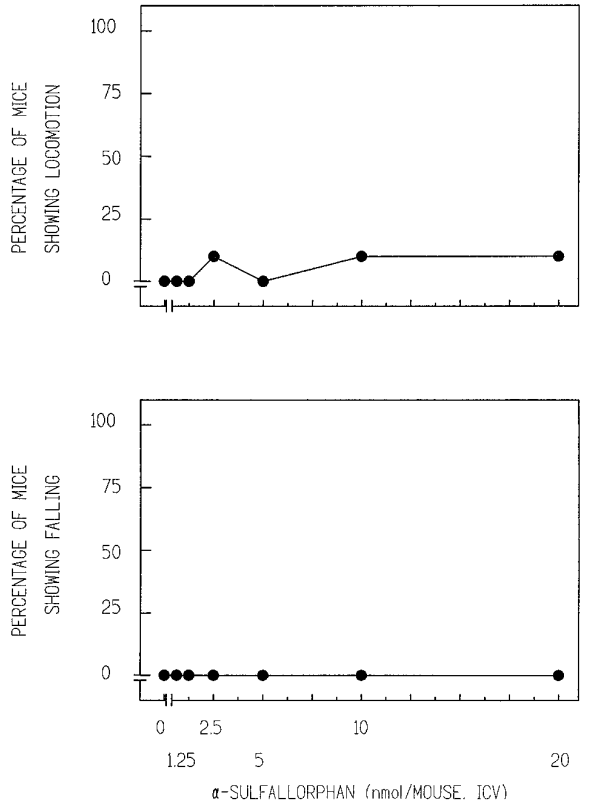

Fig. 4. Locomotion (A) and falling behavior (B) produced by i.c.v. administration of the indicated doses of α-sulfallorphan. These PCP-like grossly observable behavioral effects were measured as described under "Materials and Methods."

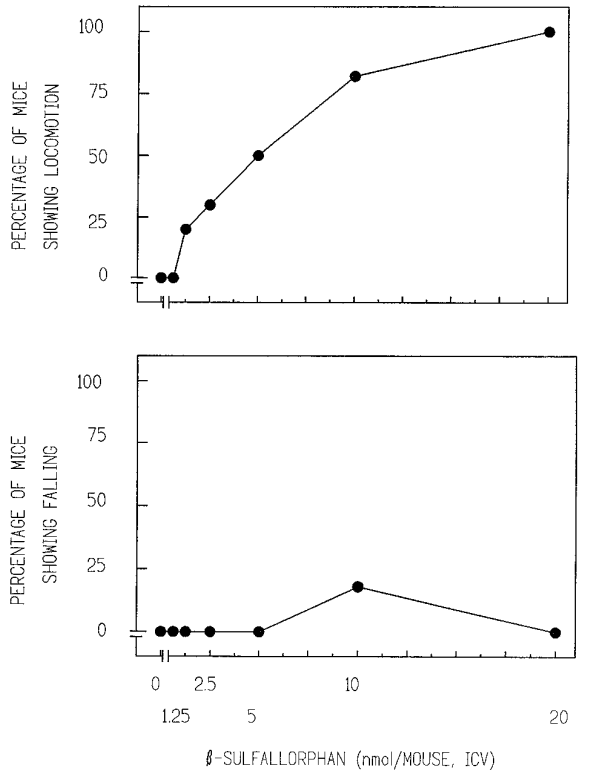

Fig. 5. Locomotion (A) and falling behavior (B) produced by i.c.v. administration of the indicated doses of β-sulfallorphan. These PCP-like grossly observable behavioral effects were measured as described under "Materials and Methods."

α- and β-sulfallorphans showed monophasic protection curves and remained fully protective at supramaximal doses (fig. 3), which suggested that they may be less toxic than the prototypic NMDA antagonists MK-801 and CPP.

Noncompetitive NMDA antagonists such as PCP and MK-801, and competitive antagonists such as AP7, AP5, CPP and CGS19755, produce PCP-like side effects in rodents. These include stereotypy characterized by increased locomotion, circling, head weaving and falling behavior. There is a good correlation between the relative potencies of these compounds in antagonizing NMDA-induced convulsions and producing PCP-like locomotion (Koek *et al.*, 1988; Tricklebank *et al.*, 1989; Koek and Colpaert, 1990). In contrast to competitive NMDA antagonists, PCP-like drugs generally antagonize convulsant or lethal effects of NMDA only at doses higher than those associated with motor disturbances or PCP-like stereotypy (Willetts *et al.*, 1990). Competitive antagonists penetrate the brain poorly and it has been suggested that this may limit their PCP-like effects (Tricklebank *et al.*, 1989; Koek and Colpaert, 1990; Koek *et al.*, 1988). In our study, i.c.v. administration of competitive (CPP) and noncompetitive (MK-801, dextromethorphan) NMDA antagonists showed overlaps between the doses producing an increase in locomotor activity and those producing protection against NMDA-induced convulsions (table 4). Such overlaps were not observed with the two thiamorphinan derivatives, α- and β-sulfallorphans.

There are some theoretical advantages of noncompetitive over competitive NMDA antagonists. The blockade of NMDA receptor channel by noncompetitive antagonists is voltage and use dependent (Davies *et al.*, 1988; McDonald and Nowak, 1990). Use dependency should enhance the efficacy to toxicity ratio of noncompetitive NMDA antagonists com-

TABLE 4
PCP-like behavioral effects (locomotion and falling) of α-sulfallorphan, β-sulfallorphan and various NMDA antagonists

	Percentage of Mice Showing Locomotion at Lowest Effective Dose[a]	Percentage of Mice Showing Falling at Lowest Effective Dose[a]	Ratio: Lowest Effective Dose Showing Locomotion/ED_{50}[b]
β-Sulfallorphan	20 (1.25)	Inactive[c]	83
α-Sulfallorphan	Inactive[c]	Inactive[c]	–
Dextromethorphan	30 (0.10)	27 (0.20)	0.20
MK-801	30 (0.25)	50 (1.50)	6.4
CPP	80 (0.025)	10 (0.0125)	1.9

[a] Number in parentheses indicates the lowest dose in nanomoles which produced a significant effect as compared with control.
[b] ED_{50} were derived from table 3.
[c] Not active in producing locomotion or falling behavior at the tested dose range (1.25–20 nmol/mouse i.c.v. for α- and β-sulfallorphans).

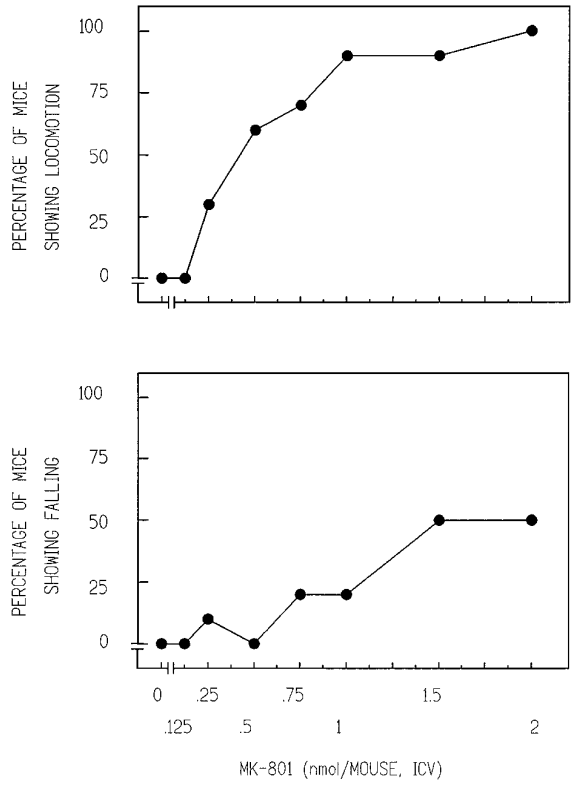

Fig. 6. Locomotion and falling behavior produced by i.c.v. administration of the indicated doses of MK-801. These PCP-like grossly observable behavioral effects were measured as described under "Materials and Methods."

pared with that of the competitive antagonists, because the block would be potentiated during strong stimulation of NMDA receptors, a condition that corresponds to excitotoxicity and disease-related nerve degeneration. Another theoretical advantage of noncompetitive NMDA blockers is the fact that their inhibitory effect cannot be overcome by high synaptic levels of endogenous transmitters (Rogawski and Porter, 1990). It is possible to design more specific noncompetitive NMDA antagonists because many of the toxic side effects of PCP, which are not shared by competitive NMDA antagonists, are probably unrelated to their interaction with the NMDA receptor (Rogawski *et al.*, 1989, 1990). In our study, β-sulfallorphan enhanced locomotor activity at doses 83 times higher than the ED_{50} dose for protection against NMDA-induced convulsions, whereas α-sulfallorphan did not produce any measurable motor activity at the tested dose range (up to 20 nmol/mouse i.c.v.). The lack of motor effect of this latter compound may depend on its poorer ability to bind to the PCP site and/or to its greater affinity for the *sigma* or *mu* sites.

Therefore, we may conclude that: 1) axial (β) and equatorial (α) *S*-allylmorphinans are potent NMDA receptor blockers; 2) β-sulfallorphan is more potent than α-sulfallorphan in blocking NMDA-induced convulsions, but it also displays some PCP-like side effects; 3) α-sulfallorphan possesses the same potency as dextrorphan in inhibiting NMDA-induced convulsions, and its lack of PCP-like side effects suggests that it may be used as a good working model to design effective neuroprotective agents devoid of motor side effects.

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