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Tissue Distribution of Two NMDA Receptor Antagonists, [^{3}H]CGS 19755 and [^{3}H]MK-801, After Intrathecal Injection in Mice

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NÄSSTRÖM, J., É. BÖÖ, M. STÅHLBERG AND O.-G. BERGE. *Tissue distribution of two NMDA receptor antagonists, [^{3}H]CGS 19755 and [^{3}H]MK-801, after intrathecal injection in mice.* PHARMACOL BIOCHEM BEHAV 44(1) 9–15, 1993.—The tissue distribution of [^{3}H]*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) and [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (MK-801) was investigated after a single IT injection into lumbar spinal cord of mice. The level of radioactivity was analyzed in the lumbar, thoracic, and cervical spinal cord, brainstem, frontal cortex, liver, lungs, kidneys, stomach, intestine, spleen, heart, and blood from 5 min up to 6 h after injection. Within the CNS, [^{3}H]CGS 19755 redistributed slowly from the site of injection toward the brainstem and cortex, peaking in the cortex 3–4 h after IT injection. At no time, however, did the relative level per gram of tissue in the frontal cortex exceed 10% of the relative level in the lumbar region of the spinal cord. The highest peripheral level of [^{3}H]CGS 19755 was found in the kidneys. [^{3}H]MK-801 redistributed rapidly from the spinal cord injection site to the peripheral organs. The highest peripheral levels of [^{3}H]MK-801 were found in the lungs and liver, where the radioactivity peaked at 10 and 30–60 min, respectively, after injection. The relative levels of [^{3}H]CGS 19755 were consistently higher in CNS tissues (except for the first 15 min in the frontal cortex) and blood than the corresponding levels of [^{3}H]MK-801. The opposite relationship was true in the liver, lungs, kidneys, stomach, intestine, spleen, and heart. The effect on the response latency in the hot-plate test was quantified in the same animals immediately prior to sacrifice for the distribution study. For the first hour after injection, the effect of [^{3}H]CGS 19755 in the hot-plate test followed the temporal distribution of the antagonist to the lumbar region of the spinal cord. [^{3}H]MK-801 did not produce any substantial effect in the hot-plate test even at a dose 100 times greater than the effective equimolar dose of [^{3}H]CGS 19755. As discussed, the difference in the distribution to the spinal cord of [^{3}H]MK-801 and [^{3}H]CGS 19755 does not appear to explain the lack of effect of MK-801 in the hot-plate test.

Distribution Intrathecal NMDA receptor antagonist Hot plate

BEHAVIORAL, biochemical, and electrophysiological studies have suggested that excitatory amino acids (EAAs) participate in nociceptive neurotransmission in the spinal cord (2,5,19,21,23–26). Although both competitive and noncompetitive NMDA receptors have been implicated in nociceptive mechanisms, this has not been a consistent finding. For example, Nässtrom et al. reported that competitive, but not noncompetitive, NMDA receptor antagonists increased response latency in the hot-plate test following IT administration in mice (21). In this article, we address this question of lack of effect of the noncompetitive NMDA receptor antagonists in the mouse hot-plate test.

cis-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) is a highly selective and potent competitive antagonist at the NMDA receptor (16), while (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (MK-801) is a potent noncompetitive NMDA receptor antagonist acting at the ion channel (27). Apart from modulating the NMDA receptor complex in two mechanistically different ways, these two antagonists also differ physicochemically. Thus, CGS 19755 is highly hydrophilic because of its many ionic groups, while MK-801 is lipophilic. In this regard, it is interesting that intrathecally administered lipophilic opioids redistribute faster from the spinal cord than do hydrophilic opioids (10).

The aim of this study was to investigate the temporal and spatial distribution of [^{3}H]CGS 19755 and [^{3}H]MK-801 in mice and examine the effect of both these substances in the hot-plate test. In this way, we hoped to ascertain whether or not the previously reported lack of effect of MK-801 (21) could be explained by its tissue distribution. As reported here, however, differences in spinal cord distribution of these two substances do not appear to provide the explanation. A distribution study would also reveal from which tissues potential side effects might be expected with these types of substances.

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METHOD

Chemicals

[^{3}H]CGS 19755 (specific radioactivity 1972.1 GBq/mmol; in 10 mmol potassium phosphate, pH 3) and [^{3}H]MK-801 (specific radioactivity 1065.6 GBq/mmol; in ethanol) were purchased from New England Nuclear (NEN; Du Pont Scandinavia AB, Stockholm, Sweden). Unlabeled MK-801 was purchased from Research Biochemicals Inc. (Natick, MA) and CGS 19755 was synthesized at Astra Pain Control (Södertälje, Sweden). Unlabeled CGS 19755 was added to the shipping solution of [^{3}H]CGS 19755 to a final concentration of 0.32 nmol/5 μl. The shipping solution of [^{3}H]MK-801 was diluted 1 : 10 with 0.9% saline to a final concentration of 17.4 pM/5 μl before IT injection. Unlabeled MK-801 was dissolved in 0.9% saline.

Animals

All experimental procedures involving animals were approved by the regional ethical committee for animal experiments and performed according to the guidelines recommended by the International Association for the Study of Pain (29). Mice (ALAB outbred albino males, NMRI origin, ALAB, Sollentuna, Sweden; weight range 20–26 g) were housed for 1 week prior to drug injections in animal rooms that were thermostatically maintained at 20 ± 0.2°C and with a 12 L : 12 D light schedule. They were given free access to food and water.

Lumbar Puncture

The procedure for IT administration of drugs was based upon the technique described by Hylden and Wilcox (11). Thus, a 30-ga cannula was inserted between the L5 and L6 vertebrae at least 30 min after making an incision through the skin over the lumbar spine. Mice were not anesthetized during these procedures. All drugs were injected in a volume of 5 μl.

Hot-Plate Test

Mice were tested without habituation. The hot-plate test (28) was performed on an electrically heated and thermostatically controlled aluminum surface set to a temperature of 58 ± 0.2°C. Animals were confined to the plate by a transparent observation chamber (34 × 37 cm). The latency to a response consisting of shaking or repeated kicking of the hind legs was measured. A cut-off of 20 s was used to avoid tissue damage. Each animal was tested once, just prior to sacrifice.

Analysis of Tissue Radioactivity

Animals were sacrificed by decapitation at 5, 10, 15, 30 min, 1, 2, 3, 4, or 6 h after IT injection (only up to 2 h for [^{3}H]MK-801). The tissues from each set of animals were weighed and frozen (−20°C) until the radioactivity was analyzed within 4 days. The liver was homogenized in physiological saline with a Polytron homogenizer (Kinematica GmbH, Littau, Switzerland), while cooling on ice. Aliquots of the liver homogenate, samples of blood, and the tissues were mixed with cellulose powder and combusted in a sample oxidizer (Packard Oxidizer 306, Packard Instruments Co., Downers Grove, IL). The condensed [^{3}H]H_2O from the oxidizer was mixed with Carbosorb-Permafluor (9 : 15) and the radioactivity was measured by liquid scintillation analysis in a Tri-Carb 460C (Packard) with internal standardization.

Calculations

The relative level of radioactivity per g of blood or tissue was calculated using the following equation:

$$\text{Relative level/g} = \frac{\text{dpm in sample} \cdot 100}{\text{dpm administered} \cdot \text{weight of sample (g)}}.$$

Due to technical difficulties inherent in injections directly into the intrathecal space, a certain failure rate is to be expected. Therefore, animals in which all CNS tissues (i.e., lumbar, thoracic, and cervical spinal cord, brainstem, and frontal cortex) deviated by more than 2 SD from the mean value were excluded. This resulted in data from 4 of the 60 [^{3}H]CGS 19755 and 2 of the 36 [^{3}H]MK-801 animals being excluded.

RESULTS

The highest relative level/g of both [^{3}H]CGS 19755 and [^{3}H]MK-801 was found in the lumbar spinal cord (Fig. 1). With the exception of the first 15 min in the frontal cortex, the relative levels/g of [^{3}H]CGS 19755 were consistently higher in the CNS tissues and blood than those of [^{3}H]MK-801 (Figs. 1–4).

Tissue Distribution of [^{3}H]CGS 19755

The level of [^{3}H]CGS 19755 increased in the lumbar region of the spinal cord for the first 15 min after the IT injection and then decreased rapidly until leveling out at 60 min. From 180 min after injection, the level decreased slowly with a terminal half-life of approximately 3.2 h (Fig. 1). The level of [^{3}H]CGS 19755 in the lumbar spinal cord remained the highest of all tissues from 5 min until the end of the experiment at 6 h after IT injection. Rather than redistributing to any great extent to the periphery, [^{3}H]CGS 19755 redistributed slowly toward the brain, with high levels also being found in both the thoracic and cervical spinal cord soon after injection (Fig. 1). The radioactivity peaked in the cortex 3–4 h after the IT injection (Fig. 2). At no time did the relative level/g in the frontal cortex exceed 10% of that in the lumbar region of the spinal cord. The levels of [^{3}H]CGS 19755 found in the brainstem, frontal cortex, kidneys, and blood were lower than spinal cord levels (Figs. 2–4). Even lower levels of [^{3}H]CGS 19755 were found in the liver, lungs, heart, stomach, intestine, and spleen (Figs. 3 and 4; data not shown for heart, stomach, intestine, and spleen). The highest peripheral level of [^{3}H]CGS 19755 was found in the kidneys (Fig. 3).

Tissue Distribution of [^{3}H]MK-801

Unlike [^{3}H]CGS 19755, [^{3}H]MK-801 redistributed rapidly from the site of injection, at the lumbar spinal cord, to the periphery. The level of [^{3}H]MK-801 in the lumbar and thoracic spinal cord was relatively high for only the first 15 min after injection (Fig. 1). From 30 min after injection, the lumbar spinal cord level decreased with a terminal half-life of approximately 0.5 h. The highest level of [^{3}H]MK-801 outside the CNS 5 min after injection was found in the lungs (Fig. 4). The level of [^{3}H]MK-801 increased in the lungs for the first 10 min, after which it decreased rapidly. From 30 min after the IT injection until the end of the experiment (at 2 h), the highest overall level of [^{3}H]MK-801 was found in the liver (Fig. 3). Lower levels of [^{3}H]MK-801 were found in the stomach, intestine, spleen, kidneys, spinal cord, brainstem, frontal cortex, and heart (Figs. 1–3; data not shown for stomach, intes-

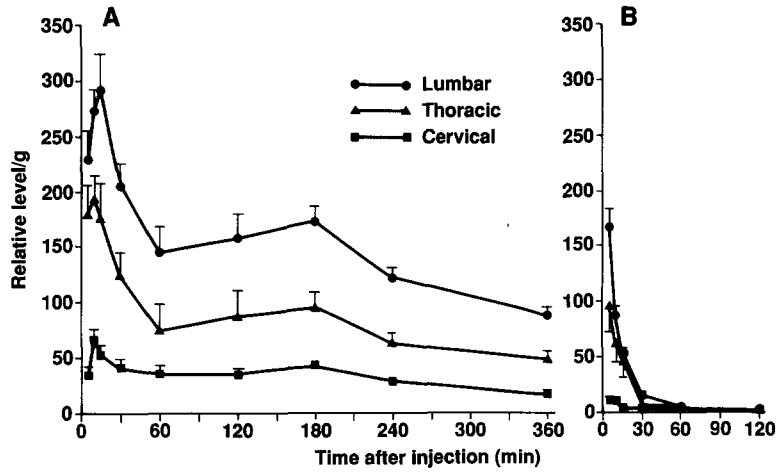

FIG. 1. Relative level/g (proportion of total radioactivity per g of tissue) of [^{3}H]*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) (A) and [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) (B) in the lumbar, thoracic, and cervical spinal cord. Values represent mean ± SEM of between 5 and 12 animals per time point.

tine, and spleen). Only the blood contained a consistently low level of [^{3}H]MK-801 (Fig. 4).

Behavioral Effect in the Hot-Plate Test

For the first hour after injection, the effect of 0.32 nmol [^{3}H]CGS 19755 on response latency in the hot-plate test followed the temporal distribution of [^{3}H]CGS 19755 in the lumbar region of the spinal cord. The hot-plate response latency increased for the first 15 min, after which it first decreased rapidly until 60 min, and then somewhat more slowly until 240 min after IT injection (Fig. 5). IT injection of 17.4 pmol [^{3}H]MK-801 did not produce any effect in the hot-plate test. IT injection of MK-801 in a dose equimolar to [^{3}H]CGS 19755 (= 0.33 nmol), as well as 10 and 100 times this dose, also failed to produce any substantial effect in the hot-plate test (Fig. 5).

Variation in the Levels of Radioactivity

In approximately 16% of IT injections with [^{3}H]CGS 19755, a much higher than average level of radioactivity was found in the kidneys. A similar phenomenon was seen after IT injection of [^{3}H]MK-801. In this case, however, the increased levels were found in the lungs and/or liver in approximately 12% of animals. The lumbar spinal cord level was below average in both these cases. It should be noted that mice with these higher than average peripheral levels of

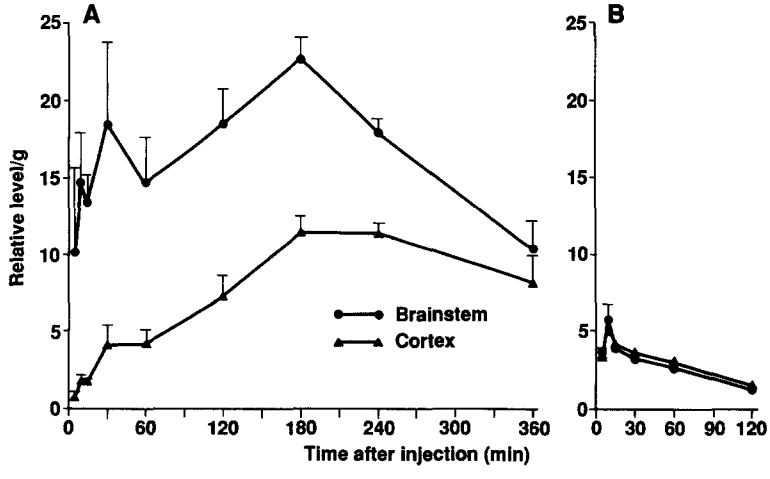

FIG. 2. Relative level/g (proportion of total radioactivity per g of tissue) of [^{3}H]*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) (A) and [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) (B) in the brainstem and frontal cortex. Values represent mean ± SEM of between 5 and 12 animals per time point.

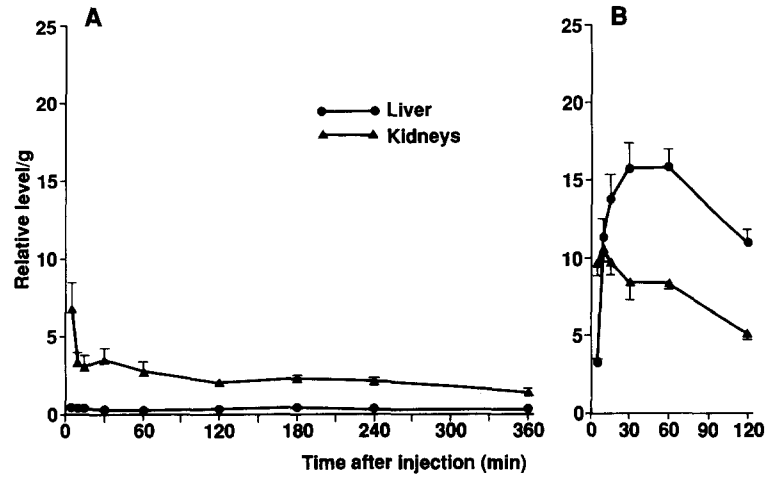

FIG. 3. Relative level/g (proportion of total radioactivity per g of tissue) of [^{3}H]*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) (A) and [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) (B) in the liver and kidneys. Values represent mean ± SEM of between 5 and 12 animals per time point.

[^{3}H]CGS 19755 and [^{3}H]MK-801 were not among mice excluded from the study according to the criteria outlined in the Method section.

DISCUSSION

The present results demonstrate that the lipophilic noncompetitive NMDA receptor antagonist [^{3}H]MK-801 disappears more rapidly from the spinal cord than the hydrophilic competitive NMDA receptor antagonist [^{3}H]CGS 19755. These findings agree with those shown previously following IT administration of lipo- and hydrophilic opioids (10).

It might be argued that, as a result of metabolism, measurement of tissue radioactivity does not necessarily reflect the presence of the originally administered compound. However, pharmacokinetic principles (4) would suggest that the tissue radioactivity recovered from the CNS structures throughout this experiment and from the rest of the tissues directly after IT injection does reflect [^{3}H]CGS 19755 and [^{3}H]MK-801. Metabolites may account for some of the radioactivity recovered from peripheral tissues later in the experiment, especially in the case of [^{3}H]MK-801 from the liver, kidneys, and lungs.

In the lumbar spinal cord, the level of [^{3}H]MK-801 decreased rapidly between 5 and 30 min following IT injection, whereas the level of [^{3}H]CGS 19755 increased for the first 15 min before decreasing. Despite this difference, the lack of

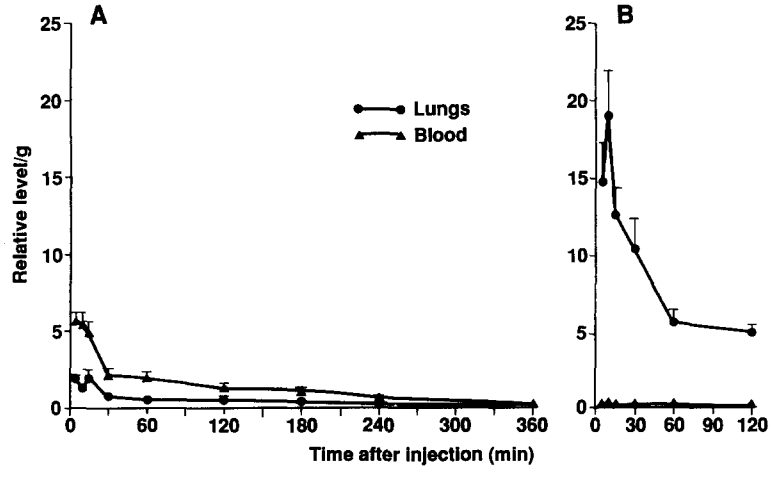

FIG. 4. Relative level/g (proportion of total radioactivity per g of tissue) of [^{3}H]*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) (A) and [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) (B) in the lungs and blood. Values represent mean ± SEM of between 5 and 12 animals per time point.

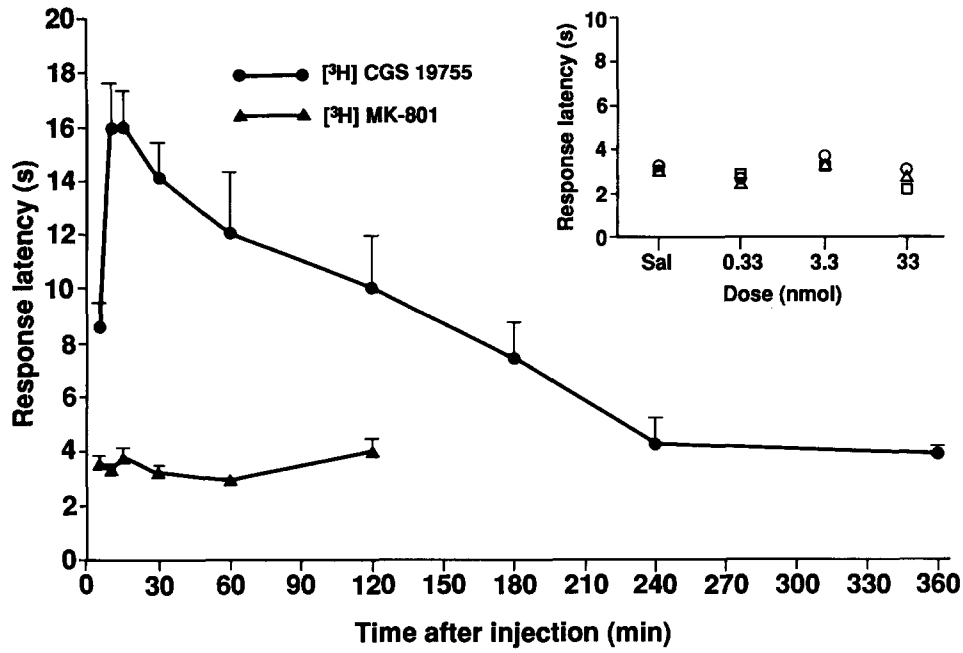

FIG. 5. Effect of 0.32 nmol [^{3}H]*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) and 17.4 pmol [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) on the response latency in the hot-plate test. Values represent mean ± SEM, n = 5–12 animals per time point. Inset shows hot-plate effect at 5 (○), 10 (△), and 15 (□) min for saline (Sal), 0.33 nM, 3.3 nM and 33 nM MK-801.

effect of MK-801 in the hot-plate test does not appear to be explained by the difference in lumbar spinal cord levels of MK-801 and CGS 19755 for the following reasons. MK-801, even at 10 and 100 times the equimolar effective dose of [^{3}H]CGS 19755, failed to produce any hot-plate effect in the first 15 min after injection. Such high doses would be expected to increase the hot-plate response latency considering that MK-801 and CGS 19755 have comparable affinities for their respective binding sites (16,27). Further, the relative level/g of [^{3}H]CGS 19755 from 5 to 15 min was only between 1.5 and 5 times that of [^{3}H]MK-801. Thus, a dose of MK-801 that was equimolar to the effective dose (for the hot-plate test) of [^{3}H]CGS 19755 would be expected to produce absolute lumbar spinal cord levels for the first 15 min that were approximately 20–70% of those associated with [^{3}H]CGS 19755. However, even after IT injection of MK-801 in a dose that was 100 times greater than this we still failed to observe an antinociceptive response.

There are several possible explanations for the difference in the hot-plate effect between CGS 19755 and MK-801. The rapid redistribution of [^{3}H]MK-801 to the brain during the first 10 min after IT injection could have produced supraspinal effects of MK-801 that counteracted its spinal effect. At the time that the supraspinal levels decreased, the remaining spinal levels may then have been too low to produce any effect. This is in line with the observation that the response latency in the hot-plate test for [^{3}H]CGS 19755 decreased between 1 and 3 h after injection. The lumbar spinal cord level of [^{3}H]CGS 19755 was relatively constant at about half its peak value during this period, while the brainstem and cortical levels were increasing steadily. It has been suggested that both the anesthetic agent ketamine, which also antagonizes NMDA, and NMDA agonists can activate supraspinal nociceptive and antinociceptive systems (12–14,22). Thus, it may be important to consider both spinal and supraspinal sites of action in the hot-plate test after IT injection of NMDA receptor antagonists.

Another explanation could be a mismatch between NMDA receptors and binding sites for noncompetitive NMDA receptor antagonists in the spinal cord, as has been suggested to exist in the cerebellum (18). There are indications of this possibility in the literature (1,9,20), although to our knowledge the localization in the spinal cord of binding sites for both competitive and noncompetitive NMDA receptor antagonists has not yet been investigated in the same study. However, as both competitive and noncompetitive NMDA receptor antagonists block NMDA-induced depolarization and C-fiber-induced wind-up of dorsal horn neurons (3,6–8), this suggestion seems unlikely.

A third possibility is that noncompetitive NMDA receptor antagonists may need a long application time to produce sufficient channel blockade. In one study, it was shown that the maximum NMDA blocking effect in cortical tissue occurred after 2 h of continuous perfusion with MK-801 (27). Agonist activation of the NMDA receptor complex is another important parameter that influences channel blockade. It has been shown that repeated agonist application progressively augmented the ketamine blockade of NMDA-induced currents [i.e., use-dependent block; (17)]. In the present study, however, the levels of MK-801 in lumbar spinal cord would almost certainly have been inadequate for such prolonged exposure due to its rapid redistribution to the brain and, more particularly, to the periphery. Similarly, the hot-plate stimulus may have been too short and weak to induce use-dependent chan-

nel blockade. Clearly, further investigations are needed to ascertain why competitive and noncompetitive NMDA receptor antagonists exert such different effects in the hot-plate test.

[^{3}H]CGS 19755 spread rostrally from the lumbar spinal cord, peaking in the cortex 3–4 h after the lumbar IT injection. This suggests that psychotomimetic side effects may not occur for several hours following IT injection of such hydrophilic NMDA receptor antagonists. This is in line with a human case study where the hydrophilic competitive NMDA receptor antagonist 3-(2-carboxypiperazin-4-*yl*)-propyl-1-phosphonic acid (CPP) induced psychotomimetic ketamine-like side effects 4 h after the last in a series of IT injections (15). Adequate dosing may, on the other hand, minimize or eliminate psychotomimetic side effects because our results show that only a fraction of the dose reaches the frontal cortex. The lipophilic noncompetitive NMDA receptor antagonists may, on the other hand, produce peripheral side effects. Certainly, the high levels of [^{3}H]MK-801 in the lungs and liver raise such a possibility.

Finally, this distribution study revealed that the technique of injecting directly into the IT space in mice leads to substantial variation between animals. This was more obvious for the hydrophilic compound [^{3}H]CGS 19755, which does not penetrate the dura readily, than for [^{3}H]MK-801. In approximately 16% of animals injected with [^{3}H]CGS 19755, there were indications that a substantial fraction of the dose was injected outside the IT space (i.e., epidurally). Thus, a lower than average effect was seen in the hot-plate test in these animals, in accordance with the lower than average levels of [^{3}H]CGS 19755 in the lumbar spinal cord and much higher than average levels in the kidneys. This problem with the injection technique must account for some of the variation in the results.

It can be concluded that the lipophilic noncompetitive NMDA receptor antagonist [^{3}H]MK-801 is rapidly and primarily redistributed to the periphery, while the hydrophilic competitive NMDA receptor antagonist [^{3}H]CGS 19755 remains largely confined within the CNS. However, this difference in distribution within the spinal cord does not appear to explain the lack of effect of MK-801 in the hot-plate test.

REFERENCES

1. Aanonsen, L. M.; Seybold, V. S. Phencyclidine and sigma receptors in rat spinal cord: Binding characterization and quantitative autoradiography. Synapse 4:1–10; 1989.
2. Aanonsen, L. M.; Wilcox, G. L. Nociceptive action of excitatory amino acids in the mouse: Effects of spinally administered opioids, phencyclidine and sigma agonists. J. Pharmacol. Exp. Ther. 243:9–19; 1987.
3. Anis, N. A.; Berry, S. C.; Burton, N. R.; Lodge, D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurons by *N*-methyl-aspartate. Br. J. Pharmacol. 79:565–575; 1983.
4. Benet, L. Z.; Mitchell, J. R.; Sheiner, L. B. Pharmacokinetics: The dynamics of drug absorption, distribution and elimination. In: Goodman Gilman, A.; Rall, T. W.; Nies, A. S.; Taylor, P., eds. The pharmacological basis of therapeutics. New York: Pergamon Press: 1990:3–32.
5. Cahusac, P. M.; Evans, R. H.; Hill, R. G.; Rodriquez, R. E.; Smith, D. A. The behavioural effects of an *N*-methylaspartate receptor antagonist following application to the lumbar spinal cord of conscious rats. Neuropharmacology 23:719–724; 1984.
6. Davies, J.; Watkins, J. C. Role of excitatory amino acid receptors in mono- and polysynaptic excitation in the cat spinal cord. Exp. Brain Res. 49:280–290; 1983.
7. Davies, S. N.; Lodge, D. Evidence for involvement of *N*-methylaspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat. Brain Res. 424:402–406; 1987.
8. Dickenson, A. H.; Sullivan, A. F. Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. Neuropharmacology 26:1235–1238; 1987.
9. Greenamyre, J. T.; Young, A. B.; Penney, J. B. Quantitative autoradiographic distribution of L-[^{3}H]glutamate-binding sites in rat central nervous system. J. Neurosci. 4:2133–2144; 1984.
10. Gustafsson, L. L.; Post, C.; Edvardsen, B.; Ramsay, C. H. Distribution of morphine and meperidine after intrathecal administration in rat and mouse. Anesthesiology 63:483–489; 1985.
11. Hylden, J. L.; Wilcox, G. L. Intrathecal morphine in mice: A new technique. Eur. J. Pharmacol. 67:313–316; 1980.
12. Jensen, T. S.; Yaksh, T. L. Spinal monoamine and opiate systems partly mediate the antinociceptive effects produced by glutamate at brainstem sites. Brain Res. 321:287–297; 1984.
13. Jensen, T. S.; Yaksh, T. L. Comparison of the antinociceptive effect of morphine and glutamate at coincidental sites in the periaqueductal gray and medial medulla in rats. Brain Res. 476:1–9; 1989.
14. Jensen, T. S.; Yaksh, T. L. The antinociceptive activity of excitatory amino acids in the rat brainstem: An anatomical and pharmacological analysis. Brain Res. 569:255–267; 1992.
15. Kristensen, J. D.; Svensson, B.; Gordh, T., Jr. The NMDA receptor antagonist CPP abolishes neurogenic "wind-up pain" after intrathecal administration in humans. Pain (in press).
16. Lehmann, J.; Hutchison, A. J.; McPherson, S. E.; Mondadori, C.; Schmutz, M.; Sinton, C. M.; Tsai, C.; Murphy, D. E.; Steel, D. J.; Williams, M.; Cheney, D. L.; Wood, P. L. CGS 19755, a selective and competitive *N*-methyl-D-aspartate-type excitatory amino acid receptor antagonist. J. Pharmacol. Exp. Ther. 246: 65–75; 1988.
17. MacDonald, J. F.; Miljkovic, Z.; Pennefather, P. Use-dependent block of excitatory amino acid currents in cultured neurons by ketamine. J. Neurophysiol. 58:251–266; 1987.
18. Maragos, W. F.; Penney, J. B.; Young, A. B. Anatomic correlation of NMDA and ^{3}H-TCP-labeled receptors in rat brain. J. Neurosci. 8:493–501; 1988.
19. Miller, K. E.; Clements, J. R.; Larson, A. A.; Beitz, A. J. Organization of glutamate-like immunoreactivity in rat superficial dorsal horn: Light and electron microscopic observations. Synapse 2:28–36; 1988.
20. Monaghan, D. T.; Cotman, C. W. Distribution of *N*-methyl-D-aspartate-sensitive L-[^{3}H]glutamate-binding sites in rat brain. J. Neurosci. 5:2909–2919; 1985.
21. Näsström, J.; Karlsson, U.; Post, C. Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice. Eur. J. Pharmacol. 212:21–29; 1992.
22. Pekoe, G. M.; Smith, D. J. The involvement of opiate and monoaminergic neuronal systems in the analgesic effects of ketamine. Pain 12:57–73; 1982.
23. Raigorodsky, G.; Urca, G. Intrathecal *N*-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems. Brain Res. 422:158–162; 1987.
24. Schneider, S. P.; Perl, E. R. Comparison of primary afferent and glutamate excitation of neurons in the mammalian spinal dorsal horn. J. Neurosci. 8:2062–2073; 1988.
25. Schouenborg, J.; Sjölund, B. H. First-order nociceptive synapses in rat dorsal horn are blocked by an amino acid antagonist. Brain Res. 379:394–398; 1986.
26. Skilling, S. R.; Smullin, D. H.; Beitz, A. J.; Larson, A. A. Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. J. Neurochem. 51:127–132; 1988.
27. Wong, E. H.; Kemp, J. A.; Priestley, T.; Knight, A. R.; Wood-

ruff, G. N.; Iversen, L. L. The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist. Proc. Natl. Acad. Sci. USA 83: 7104–7108; 1986.
28. Woolfe, G.; MacDonald, A. D. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J. Pharmacol. Exp. Ther. 80:300–307; 1944.
29. Zimmermann, M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109–110; 1983.