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Neuroscience Letters 231 (1997) 135–138

NEUROSCIENCE LETTERS

Remacemide hydrochloride reduces cortical lesion volume following brain trauma in the rat

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Received 11 April 1997; received in revised form 10 July 1997; accepted 10 July 1997

Abstract

We evaluated the therapeutic effects of remacemide hydrochloride, an *N*-methyl-d-aspartate (NMDA) receptor-associated ionophore blocker with sodium channel blocking activity, on cortical lesion volume and memory dysfunction following parasagittal fluid-percussion brain injury in the anesthetized rat. We found that intravenous (i.v.) administration 15 min following injury of remacemide hydrochloride at both 25 and 10 mg/kg significantly reduced posttraumatic cortical lesion volume ($P < 0.05$), measured at 48 h postinjury using a tetrazolium salt tissue staining technique. However, neither of these doses nor the dosing regimen of 25 mg/kg i.v. 15 min postinjury plus a subcutaneous infusion over 24 h of 20 mg/kg remacemide hydrochloride improved posttraumatic memory function determined by a Morris water maze paradigm. © 1997 Elsevier Science Ireland Ltd.

Keywords: Brain trauma; Remacemide hydrochloride; Lesion volume; Memory; Neuroprotection; 2,3,5-Triphenyltetrazolium chloride

Due to substantial evidence that excitatory amino acid (EAA) toxicity plays a major role in the pathophysiology of traumatic brain injury (TBI), many pharmacologic modulators of EAA receptors have been evaluated in experimental models of brain trauma (for review see [19]). Blockade of the *N*-methyl-d-aspartate (NMDA) receptor-associated ionophore has been one of the most extensively pursued therapeutic strategies in experimental TBI. Efficacy of this class of compounds has been demonstrated through evaluation of posttraumatic cognitive and neuromotor function, neurochemical outcome, and physiologic status. However, in contrast to many studies employing models of experimental focal cerebral ischemia, surprisingly few brain trauma studies have investigated potential beneficial effects of treatment by evaluating changes in lesion volume [5,22]. Moreover, tetrazolium salt (2,3,5-triphenyltetrazolium chloride; TTC) staining techniques that are commonly utilized to determine neuroprotection in models of focal ischemia [2–4,15] have not been previously applied in experimental brain injury to evaluate pharmacologic efficacy.

To address these issues, we have recently developed a sensitive TTC staining technique for models of brain trauma to measure posttraumatic cortical lesion volume [16]. In the present study, we used this technique to evaluate potential neuroprotective effects of the NMDA receptor-associated ionophore blocker, remacemide hydrochloride ((±)-2-amino-*N*-(1-methyl-1,2-diphenylethyl)acetamide hydrochloride) following experimental brain trauma in the rat. To investigate potential correlations between functional outcome and neuroprotection we also evaluated the effects of remacemide hydrochloride treatment on posttraumatic memory dysfunction.

Remacemide hydrochloride was chosen for this study in light of a recent report of its ability to reduce lesion volume in a cat model of focal cerebral ischemia [1]. The activity of this compound may be partly attributable to its in vivo desglycinated metabolite (1,2-diphenyl-2-propylamine; AR-C12495AA) [13,14], which is a low affinity inhibitor of MK-801 binding to the NMDA receptor ionophore site and sodium channel blocker. An attribute of many low affinity blockers of the opened NMDA ionophore, such as remacemide hydrochloride, is their reduced neuronal toxicity in comparison to high affinity compounds such as MK-801 [13].

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0304-3940/97/$17.00
PII S0304-3940(97)00551-X

Evaluation of posttraumatic memory function was performed using a modification of the Morris water maze paradigm [9,21], which has been extensively utilized to evaluate pharmacologic efficacy on cognitive dysfunction following experimental brain injury [19]. Briefly, male Sprague–Dawley rats (350–400 g, $n = 53$) were trained in the maze to locate a submerged hidden platform using external visual cues. Each animal was given 20 training trials over 2 days. Two and a half hours after the final trial all animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and subjected to left parasagittal fluid-percussion (FP) brain injury of moderate severity or sham (surgery without injury) treatment (as previously described in detail) [8]. Fifteen minutes following injury the animals randomly received intravenous (i.v.) administration (femoral vein) of remacemide hydrochloride 10 mg/kg ($n = 15$), 25 mg/kg ($n = 12$) or saline ($n = 14$). Sham animals ($n = 12$) received saline. The doses of remacemide hydrochloride utilized in this study were shown to be efficacious in previous studies using different models of central nervous system (CNS) injury [1,13,23].

Forty-two hours postinjury these animals were tested in the water maze for memory retention of the platform seeking task. The platform was removed, and the animals were given a 1 min test period while a computerized video tracking system (Omnitech Electronics, Inc.) recorded their swimming patterns. Memory scores were derived by determining the number of seconds spent in zones ranked according to their proximity to the platform site, as previously described in detail [20,21].

Following memory evaluation, at 48 h postinjury, all animals received an overdose of sodium pentobarbital (200 mg/kg, i.p.), and their brains were rapidly removed and stained with TTC for determination of cortical lesion volume as previously described [16]. Briefly, each brain was cut into 1 mm coronal sections which were immersed in 0.2 M phosphate buffer with 2% TTC, washed with 0.2 M phosphate buffer and stored in 10% neutral buffered formalin. Imaging and lesion volume analysis was performed by an independent investigator blind to the treatment. Digitized, monochrome images of the rostral side of each section (7–10 sections/animal) were captured and stored using a Dage-MTI CCD72 video camera and M1-MCID imaging system software (Imaging Research, St. Catherines, Ontario, Canada). For each section, lesion area was determined by utilizing a standardized imaging scheme which allows for demarcation between red tissue, pink tissue, and white tissue. In each coronal brain section the area of white and/or pink tissue (lesion area) was calculated and multiplied by 1 mm for single section lesion volume and added to volumes from serial sections for determination of total lesion volume. The lesion volume determined using this technique has been shown to be very similar to lesion volumes evaluated using microscopic examination of hematoxylin and eosin stained sections.

Following results from the first memory evaluation, a second study was performed, whereby another group of animals ($n = 30$) was examined in the water maze using the identical protocol. At 15 min postinjury 12 animals received remacemide hydrochloride 25 mg/kg i.v. and subcutaneous implantation of an osmotic minipump (Alza Scientific Products) containing an additional 20 mg/kg remacemide hydrochloride to be infused over 24 h. Eleven brain injured animals received saline with the same dosing regimen (i.v. and via 24 h minipump). Seven animals served as sham controls. No lesion volume analysis was performed on these animals.

All animal procedures were approved by the University of Pennsylvania Animal Care and Use Committee, and we carefully adhered to the animal welfare guidelines set forth in the Guide for the Care and Use of Laboratory Animals, U.S. Department of Health and Human Services Publication 85-23.

All statistics were performed using a two-way analysis of variance (ANOVA). The mean values for each group were then compared by *t*-tests with a Bonferroni correction.

A profound memory dysfunction (retrograde amnesia) was induced in all brain injured animals ($P < 0.001$; Fig.

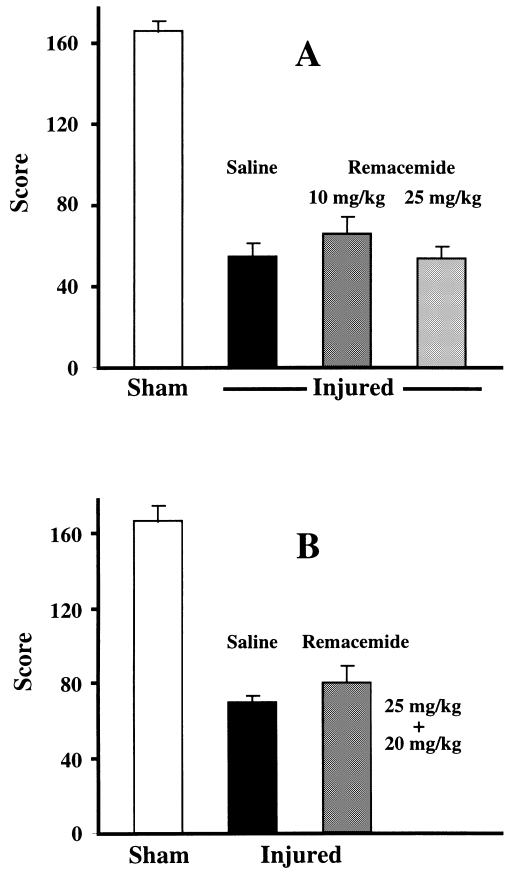

Fig. 1. Histograms representing mean ± SEM of the memory scores of sham and FP brain injured animals. Two dosing regimens were evaluated in (A) and (B), respectively. A significant difference in memory scores is observed between sham animals and all groups of injured animals ($P < 0.001$). There is no significant difference found between injury groups.

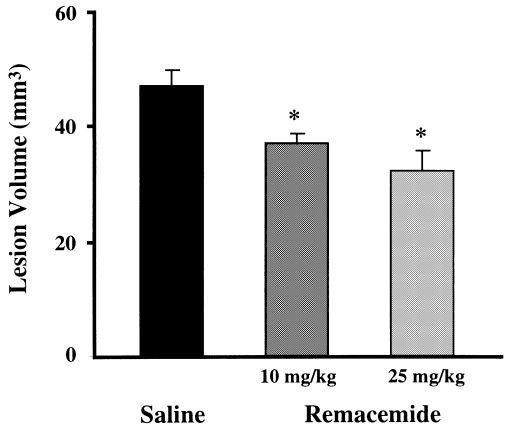

Fig. 2. Histogram representing cortical lesion volume of FP brain injured animals. *$P < 0.05$ compared to saline treated animals.

1) consistent with that observed previously in this model [20,21]. There was no apparent improvement in posttraumatic memory function 2 days following injury in the animals receiving either 10 or 25 mg/kg remacemide hydrochloride (Fig. 1A). Although it was initially thought that slow infusion of 20 mg/kg remacemide hydrochloride added to the 25 mg/kg acutely administered dose may convey some additional benefit by inhibiting both acute and prolonged EAA toxicity, no significant effect on posttraumatic memory function was observed (Fig. 1B). However, a significant reduction in cortical lesion volume was observed following treatment with either remacemide hydrochloride 10 or 25 mg/kg ($P < 0.05$). There was no significant difference between these two remacemide hydrochloride treated groups (Fig. 2). TTC stained sections demonstrating that the reduction in lesion in animals treated with remacemide hydrochloride occurred in both the rostral-caudal extent of the lesion, as well as in the coronal diameter (Fig. 3). Nonetheless, it is important to note that the TTC method did not reveal less overt subcortical injury previously seen in this model.

To our knowledge, this is the first report of a reduction in cortical lesion volume following treatment with an NMDA receptor ionophore blocker in a model of brain trauma. In addition, this is the first demonstration that the TTC staining method may be useful in determining pharmacologic efficacy following experimental TBI. Although no attenuation of posttraumatic memory dysfunction was observed, it is not clear whether this finding reflects a general inability of remacemide hydrochloride to affect cognitive outcome or if the dosing regimen was not ideal. Though not observed in this study, relatively dramatic enhancement of posttraumatic memory function has been previously observed following treatment with several EAA receptor antagonists using the same method described here [19]. The differences in apparent efficacy on the two outcome measures in the present study underscores the need for multiple evaluation parameters to determine various aspects of potential therapeusis. Compared to the assessment of posttraumatic cognitive function, evaluation of neurologic motor function may appear to have been a more appropriate outcome measure to be linked with the examination of cortical lesion volume in the present study. However, the early timepoint necessary for TTC evaluation (48 h postinjury), negated our ability to properly assess neurologic motor outcome, which, based on previous studies, is most reliable at 1 and 2 weeks following injury [8].

While some of the tissue injury in the cortex following FP brain trauma may be due to primary mechanical damage, recent evidence suggests that an EAA toxicity may contribute to the extent of the lesion. Intracerebral microdialysis analysis of traumatized brains has demonstrated a marked and acute increase in the concentration of excitatory amino acids [6,7,10,12]. Also, a recent study using routine histopathologic staining and light microscope analysis demonstrated that an NMDA receptor polyamine site antagonist, eliprodil, reduced cortical lesion volume in a model of FP brain injury [22]. Moreover, posttraumatic neurologic motor function, presumed to reflect cortical integrity has been shown to be greatly improved following administration of EAA receptor antagonists [19]. Taken together with these data, the results from the present study add support to the premise that there is potentially reversible EAA mediated damage to cortical tissue following trauma. In addition our results help confirm the promise of NMDA receptor antagonists as neuroprotective agents following trauma.

Previously, the NMDA receptor-associated ionophore blockers MK-801, ketamine and PCP have all been shown to be efficacious in models of brain trauma using a variety of outcome measures [19]. However, these compounds have also been shown to have psychotomimetic effects, produce pathologic changes (vacuole formation) in neurons, and induce the expression of heat shock protein [11,18], causing debate concerning the potential therapeutic utility verses possible adverse affects. Accordingly, there has been interest in developing pharmacologic agents that may exert similar protection as this class of compounds, but without the

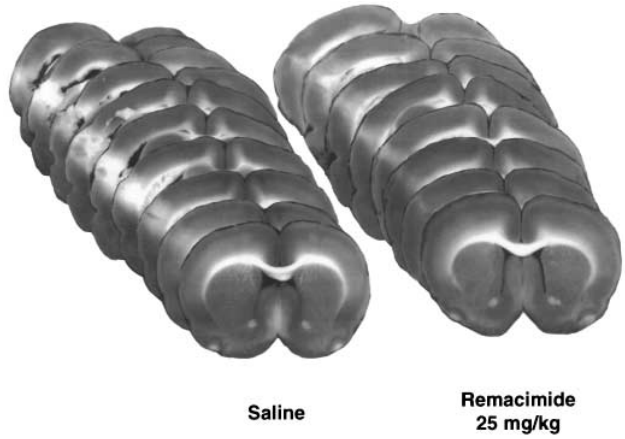

Fig. 3. Representative 1 mm coronal brain sections from FP brain injured animals demonstrating rostral-caudal extent of cortical lesion (white region) in the left parietotemporal region demarcated by the absence of TTC staining.

same extent of toxicity. It has been observed that low affinity blockers of the NMDA receptor ionophore generally exert less toxic effects than high affinity blockers, yet maintain neuroprotective attributes [17]. Remacemide hydrochloride has previously been shown to be neuroprotective in models of mouse hypoxia, cat ischemia, rat ischemia, and rat subarachnoid hemorrhage [1,13,23]. However, remacemide hydrochloride does not disrupt memory processes or produce substantial neuronal pathology at neuroprotective doses in animals, much in contrast with high affinity NMDA receptor ionophore blockers [13]. In addition, in humans, remacemide hydrochloride has been shown to be well tolerated and does not appear to have untoward psychotomimetic or general cognitive effects as have been seen with high affinity NMDA receptor blockers [13].

The results from the present study support previous findings of the neuroprotective abilities of remacemide hydrochloride in models of CNS injury, and are suggestive that this compound may have utility in the treatment of TBI.

We would like to thank Jeanne Marks for her excellent preparation of this manuscript. This work was supported, in part, by a grant from Astra Charnwood, Loughborough Leics., UK and from NIH grants AG12527, NS08803, and NS26818.

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