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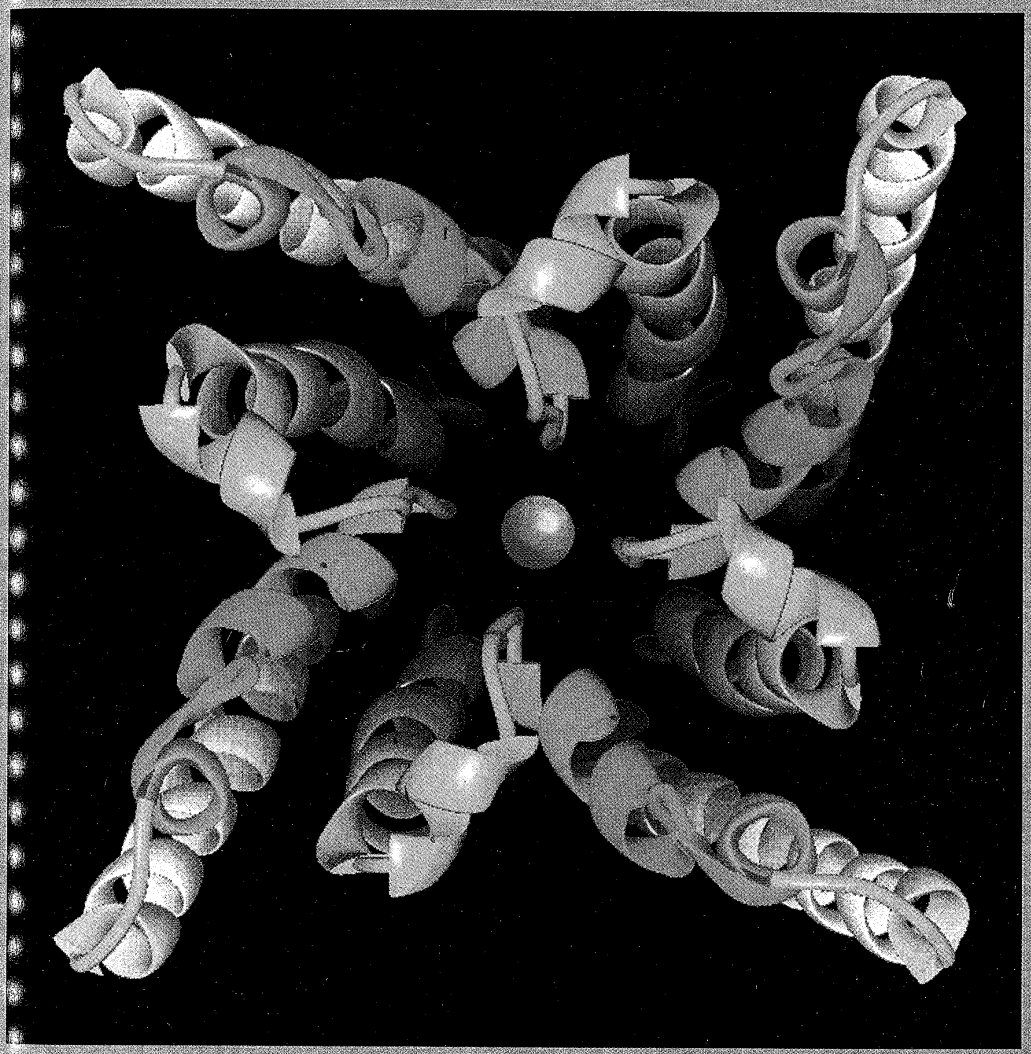

LOCALIZATION & EXPRESSION OF HmGluR1

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Cover—Structural model of the core portion of a Shaker potassium channel view down the axis of the pore from outside the cell. The channel has four identical subunits. The backbone of each subunit is colored according to the spectrum beginning with the N-terminal of S5 in red and ending at prolines in S6 in blue. The P or H5 region that forms the ion selective portion of the pore is postulated to span the outer half of the transmembrane region and be formed by an alpha helix (green) followed by an extended coil structure (cyan). Model by Guy, H.R. and Durell, S.R. (1996) *Biophysical J. Abstracts.*

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Anticonvulsant Activity of Novel Derivatives of 2- and 3-Piperidinecarboxylic Acid in Mice and Rats

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(Accepted 28 June 1996)

Summary—The relative ability of derivatives of 2-piperidinecarboxylic acid (2-PC; pipecolic acid) and 3-piperidinecarboxylic acid (3-PC; nipecotic acid) to block maximal electroshock (MES)-induced seizures, elevate the threshold for electroshock-induced seizures and be neurotoxic in mice was investigated. Protective index (PI) values, based on the MES test and rotorod performance, ranged from 1.3 to 4.5 for 2-PC benzylamides and from <1 to >7.2 for 3-PC derivatives. PI values based on elevation of threshold for electroshock-induced seizures and rotorod performance ranged from >1.6 to >20 for both types of derivatives. Since preliminary data indicated that benzylamide derivatives of 2-PC displace [^{3}H]1-[1-(2-thienyl)-cyclohexyl]piperidine (TCP) binding to the phencyclidine (PCP) site of the *N*-methyl-D-aspartate (NMDA) receptor in the micromolar range and such low affinity uncompetitive antagonists of the NMDA receptor-associated ionophore have been shown to be effective anticonvulsants with low neurological toxicity, the 2-PC derivatives were evaluated in rat brain homogenates for binding affinity to the PCP site. Although all compounds inhibited [^{3}H]TCP binding, a clear correlation between pharmacological activity and binding affinity was not apparent. Select compounds demonstrated minimal ability to protect against pentylenetetrazol-, 4-aminopyridine- and NMDA-induced seizures in mice. Corneal and amygdala kindled rats exhibited different sensitivities to both valproic acid and the nonsubstituted 2-PC benzylamide, suggesting a difference in these two models. Enantiomers of the α-methyl substituted benzylamide of 2-PC showed some ability to reduce seizure severity in amygdala kindled rats.

Keywords—2-piperidinecarboxylic acid, 3-piperidinecarboxylic acid, corneal kindling, amygdala kindling, uncompetitive NMDA antagonists, anticonvulsant.

Prior studies have shown that derivatives of 3-piperidinecarboxylic acid (3-PC or nipecotic acid) provide protection against chemically induced seizures (Crider *et al.*, 1982, 1984; Hinko *et al.*, 1984, 1988, 1992). Nipecotic acid is one of the most potent inhibitors of gamma-aminobutyric acid (GABA) uptake into rat cerebral cortex (Krogsgaard-Larsen and Johnston, 1975) and mouse whole brain mini-slices (Wood *et al.*, 1979). Both alkyl and phenyl esters of nipecotic acid have varying degrees of ability to inhibit GABA uptake into whole mouse brain mini-slices (Hinko *et al.*, 1988). Presumably the anticonvulsant activity of these nipecotic acid prodrugs results, at least in part, from a GABA-mimetic action.

Previous reports have also demonstrated that 2-piperidinecarboxylic acid (2-PC or pipecolic acid), a metabolite of the amino acid lysine, reduces synaptosomal and glial uptake of GABA in rat brain (Nomura *et al.*, 1981). L-Pipecolic acid, when administered by intraperitoneal (i.p.) injection, significantly increases the onset of clonic and tonic seizures induced by pentylenetetrazol in mice (Chang *et al.*, 1988). Kohn *et al.* (1990, 1991, 1993) have reported that benzylamide derivatives of amino acids provide excellent protection against maximal electroshock (MES) seizures in mice, although a mechanism for these compounds has not been proposed. In preliminary testing done in this laboratory, benzylamides of pipecolic acid demonstrate anticonvulsant

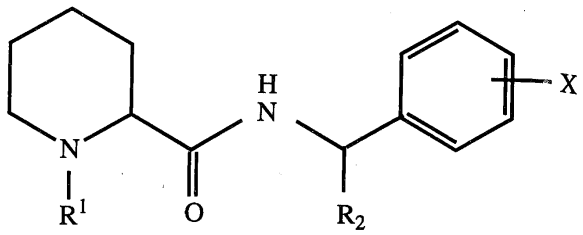
*To whom correspondence should be addressed.

activity against MES seizures. Receptor binding studies indicated that the benzylamides displace [^{3}H]1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) binding to the phencyclidine (PCP) site of the *N*-methyl-D-aspartate (NMDA) receptor in the micromolar range. The latter finding suggests that these derivatives may be functioning as low-affinity, uncompetitive NMDA antagonists (Hinko *et al.*, 1994).

High-affinity uncompetitive antagonists of the NMDA receptor-associated ionophore, such as PCP and dizocilpine (MK-801), are highly effective as anticonvulsant agents (Leander *et al.*, 1988; Chapman and Meldrum, 1989). Unfortunately, they produce significant neurotoxicity, with i.p. protective index (PI) values with respect to motor impairment in mice of less than one. It is unlikely, therefore, that they will be useful clinically in the treatment of epilepsy. Recently, it has been suggested that low-affinity uncompetitive antagonists, such as phencyclohexylamine (PCA) and 5-aminocarbonyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (ADCI), are effective anticonvulsants with low neurological toxicity and i.p. PI values in the range of 3–4. The affinity of these analogs for the PCP site of the NMDA receptor is in the low-micromolar range, whereas the so called high-affinity uncompetitive antagonists have affinities in the low nanomolar range (Rogawski, 1993).

The objective of the present study was to evaluate the anticonvulsant activity of alkyl and phenyl derivatives of nipecotic acid and benzylamide derivatives of pipecolic acid, by testing their ability (1) to protect mice against MES seizures; (2) to elevate the threshold to electroshock-induced seizures and; (3) to be neurotoxic. It has been suggested that the MES test, with its strong seizure stimulus may result in false negatives and rejection of potential anticonvulsants in early phases of testing. This may be particularly true with GABA-mimetic compounds (Löscher and Schmidt, 1988). The use of the MES test and elevation of threshold test in parallel allows for a comparison of activity. In addition, the calculation of PI values using seizure threshold models may avoid underestimation of anticonvulsant selectivity of test agents (Löscher and Nolting, 1991).

Additional objectives of this study included the assessment of the ability of 2-PC benzylamides to displace [^{3}H]TCP binding to the PCP site of the NMDA receptor in rat brain, to determine if binding activity correlates with pharmacological activity. Select 2-PC benzylamides were tested for their ability to protect against pentylenetetrazol (PTZ)-, 4-aminopyridine (4-AP)- and NMDA-induced seizures. The low-affinity uncompetitive NMDA antagonist, ADCI, has been shown to provide protection in the MES test and against PTZ-, 4-AP- and NMDA-induced seizures. Finally, select compounds were evaluated in corneal and amygdala kindling for their abilities to reduce seizure severity and alter seizure parameters. Both kindling models have been suggested to be models of human complex partial seizures, in that the pharmacological effectiveness of antiepileptic drugs in corneal kindled rats is very similar to that observed in amygdala kindled rats (Albright and Burnham, 1980; Kupferberg, 1989), although results from this laboratory with novel compounds suggested differences between these two models (Edafiogho *et al.*, 1992; Scott *et al.*, 1993).



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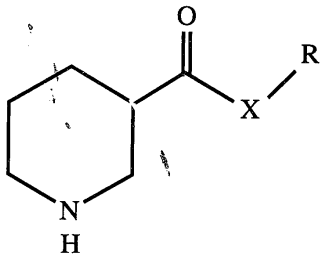
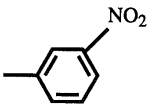


2

a: X=O; R=CH_2CH_3

b: X=O; R=$(CH_2)_8CH_3$

c: X=O; R= 

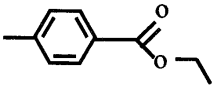
d: X=NH; R= 

Fig. 1. Parent structures for derivatives of (1) 2-piperidinecarboxylic acid and (2) 3-piperidinecarboxylic acid. Structural modifications of (1) are listed in Table 1.

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