Administered and Endogenously Released Kappa Opioids Decrease Pilocarpine-Induced Seizures and Seizure-Induced Histopathology

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Accepted for publication November 13, 1997 This paper is available online at http://www.jpet.org

ABSTRACT

The effects of kappa opioids on seizures and seizure-induced histopathology were investigated with the pilocarpine model of temporal lobe epilepsy. Rats treated with the kappa opioid receptor agonist U50488h before pilocarpine showed: 1) increased seizure latency; 2) decreased seizure duration; 3) decreased mossy fiber sprouting; and 4) increased hilar neuron survival when compared with rats pretreated with saline. Behavioral effects of U50488h were blocked by the kappa opioid receptor antagonist norbinaltorphimine (nBNI), whereas the changes caused by U50488h in the histological response to pilocarpine were not blocked by nBNI. Rats treated with nBNI before pilocarpine exhibited: 1) increased incidence of seizures; 2) increased mossy fiber sprouting; and 3) increased hilar neuron loss when compared with rats treated with pilocarpine alone. These changes suggest a protective role of endogenously released kappa opioids in this seizure model. The location of functional kappa opioid receptors in the rat dentate gyrus was documented electrophysiologically to enable correlation with kappa opioid effects on histopathology. The kappa selective agonist, U69593, reversibly decreased the amplitude of excitatory postsynaptic potentials in the middle molecular layer of the dentate gyrus from the ventral but not the more dorsal portion of the hippocampal formation. Thus, kappa opioids decreased the severity and incidence of behavioral seizures and secondarily decreased seizure-induced histopathology via the decreased incidence of seizures.

Complex partial epilepsy of temporal lobe origin is one of the most common forms of epilepsy (Hauser and Kurland, 1975) and is often associated with histopathology termed Ammon's horn sclerosis (Margesson and Corsellis, 1966). Ammon's horn sclerosis is characterized by neuronal loss in the hilar, CA3 and CA1, but not CA2 regions of the hippocampal formation (Babb and Brown, 1987) as well as sprouting of the granule cell axons (mossy fibers) into the inner molecular layer of the dentate gyrus (Nadler et al., 1980; Sutula et al., 1989; Houser et al., 1990). Pharmacotherapy for TLE is generally unsatisfactory because approximately 40% of the patients with this disease are not responsive to currently available anticonvulsant medications (Elwes et al., 1984; Mattsen et al., 1985). Kappa opioids may represent one alternative treatment.

Kappa opioids decrease excitatory neurotransmission in numerous brain regions (McFadzean et al., 1987; Moore et al., 1988; Wagner et al., 1992) and, in the guinea pig hippocampus, decrease excitatory transmission by modulating glutamate release from presynaptic terminals (Gannon and Terrian, 1991; Simmons et al., 1994). Kappa opioids have been reported to act as anticonvulsants in a variety of epilepsy models (see Tortella, 1988; Simmons and Chavkin, 1996) and specifically the kappa opioid receptor agonists PD117302 and U69593, inhibit acute pilocarpine-induced seizures and neurotoxicity in mice (Przewlocka et al., 1994). Kappa opioids also are neuroprotective both in vivo (Hall and Pazara, 1988; Hayward et al., 1992; Mackay et al., 1993; Widmayer et al., 1994) and in vitro (DeCoster et al., 1994).

In the present study, the pilocarpine model of TLE was used to investigate the anticonvulsant and neuroprotective effects of both administered and endogenously released kappa opioids. The pilocarpine model is a well established model of TLE in which a single high dose (300–400 mg/kg; Turski et al., 1989) of the cholinergic agonist, pilocarpine, produces behavioral and electroencephalographic seizures (Turski et al., 1983). This acute phase of seizure activity is followed by a chronic phase in which animals exhibit recurrent spontaneous seizures. Systemic administration of many anticonvulsant drugs used to treat human forms of epilepsy prevent acute pilocarpine-induced seizures in rats. The effectiveness of these drugs against the chronic recurrent spon-

ABBREVIATIONS: ANOVA, analysis of variance; fEPSPs, field excitatory postsynaptic potentials; LSD, least significant difference; nBNI, norbinaltorphimine; SE, status epilepticus; TLE, temporal lobe epilepsy.
taneous seizures has not been reported (Turski et al., 1989). Pilocarpine-induced seizures also lead to hippocampal pathology which mirrors human Ammon's horn sclerosis, including cell loss and mossy fiber sprouting (see Mello et al., 1992).

The aims of this study were 1) to replicate previous results in mice using an alternative kappa opioid receptor agonist, U50488h (Lahti et al., 1982; VonVoigtlander et al., 1983) and 2) to investigate the hypothesis that endogenous kappa opioids act as anticonvulsants and neuroprotectants against pilocarpine-induced seizures. Blockade of kappa opioid receptors with the selective antagonist, nBNI (Takemori et al., 1988a) was used to investigate the effects of endogenous kappa opioids on acute pilocarpine-induced seizures and histopathology. Furthermore, the location of functional kappa opioid receptors and the physiological effects of kappa opioid receptor activation in the rat dentate gyrus were documented electrophysiologically by the kappa selective agonist U69593 (Lahti et al., 1985). The location of functional kappa opioid receptors within the dentate gyrus was then correlated with histopathological changes.

Materials and Methods

Pilocarpine and opioid injections. Male Sprague-Dawley rats (110–150 g; Bantin and Kingman, Bellevue, WA) were injected with pilocarpine to induce chronic epilepsy (Turski et al., 1983) as described previously (Bausch and Chavkin, 1997). Rats were injected with methyl-scopolamine nitrate (1 mg/kg in saline i.p.; Sigma, St. Louis, MO) 30 min before pilocarpine hydrochloride injection (275–375 mg/kg in saline i.p.; Sigma) to minimize the peripheral effects of pilocarpine (Baez et al., 1976, Turski et al., 1983). Control animals also received methyl-scopolamine, but were injected with saline instead of pilocarpine. Animals were observed for 2.5 hr and monitored for 6 to 8 hr after injection with pilocarpine. To reduce the mortality rate, all rats were administered diazepam (4 mg/kg i.p.) 1 hr before SE, and every 2 hr as necessary thereafter to control seizures (Mello et al., 1993). All animals that received pilocarpine were given rat chow soaked in Gatorade and sucrose for 2 days after injection.

In experiments investigating the effects of kappa opioids, U50488h (Research Biochemicals International, Natick, MA) was injected (10–20 mg/kg in saline s.c.) 45 min before pilocarpine injection. Although U69593 generally is used for selective activation of the kappa-1 type opioid receptor, the related benzeneacetamide analog, U50488h, was used for systemic treatment because it penetrates the blood brain barrier more readily (Bianchi, 1989). nBNI (Research Biochemicals International, Natick, MA) was injected (10 mg/kg in saline s.c.) 0.5, 2 or 18 hr before the pilocarpine injection. In some experiments in which nBNI was given 18 hr before pilocarpine, a second smaller dose of nBNI (0.4 mg/kg in saline s.c.) was administered 2 hr before pilocarpine injection. Kappa opioid injection protocols were derived from previous studies investigating the effects of U50488h and nBNI on physiological measures (Takemori et al., 1988b, Milanes et al., 1991; Leyton and Stewart, 1992; Veeranna and Bhargava, 1993). Pilocarpine was given at a dose of 325–375 mg/kg (Turski et al., 1983) in U50488h experiments, and it was given at a lower dose (275 mg/kg) in studies of endogenous kappa opioid action to avoid a ceiling effect. Rats in the ‘no pilocarpine’ control group were pretreated with saline (n = 6), U50488h (n = 6) or nBNI (n = 6) and were then injected with methyl-scopolamine nitrate. Finally, all rats in this control group were injected with saline instead of pilocarpine. Data from all animals receiving ‘no pilocarpine’ were averaged since there were no significant differences in histological or behavioral responses.

Histology. Rats were sacrificed by CO2 narcosis 4 weeks after treatment. The brains were removed, blocked and immersion fixed for 1 hr in 0.1% sodium sulfide followed by 2 to 3 days in 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) containing 30% sucrose. Brains were cut into 40-μm transverse sections using a freezing sliding microtome, sections placed into 0.1 M phosphate buffer and mounted on subbed glass slides. Mounted sections were then stained with cresyl violet or with neo-Timm stain (Holm and Geneser, 1991). For the neo-Timm stain, sections were postfixed through 95%, 70% and 50% ethanol, dehydrated in distilled water for 20 to 30 min, then dipped in 0.5% gelatin and allowed to dry overnight. Slides were developed in a solution of 0.11% silver lactate, 0.85% hydroquinone, 30% gum arabic collod (all w/v) in 0.2 M citrate buffer for 1 to 1.5 hr and then rinsed, counterstained with Neutral Red, dehydrated, cleared and coverslipped. Images were collected with a Leitz Dialux 20 microscope for figures 2 and 3 or with a Nikon Diaphot microscope with Image 1 software for analysis of cresyl violet-stained sections.

Histological data analysis. Sections for analysis were taken from horizontal stereotaxic coordinates B = −5.60, IA 4.40 to B = −5.32, IA 4.68 and B = −7.34, IA 2.66 to B = −7.10, IA 2.90 according to the atlas of Paxinos and Watson (1986). These regions correspond to the middle and ventral portions of the hippocampus, respectively. One section from each of the appropriate coordinates was chosen randomly from each animal for detailed analysis. Images for quantitative analysis were collected with a Nikon Diaphot microscope with Image 1 software for the cresyl violet-stained sections and imported into MetaMorph Image analysis program. Sections were assigned coded numbers to permit a blind analysis. The hilus was defined as the region between the two blades of the granule cell layer and was delimited at the open end by a perpendicular line drawn between the two blades of the granule cell layer, excluding the CA3c pyramidal cell layer. Hilus area was determined by the image analysis programs (calibrated with a square stage micrometer). Large blood vessels were excluded from area measurements. Cells were counted manually if the stained somata was greater than 10 × 10 μm.

Changes in mossy fiber sprouting were scored subjectively by viewing mounted neo-Timm stained sections with a Leitz Dialux 20 microscope. Slides were assigned coded numbers to permit a blind analysis. Timm staining was scored as: 0, occasional or no supragranular staining; 1, scattered staining in all supragranular regions or continuous light band of staining in the supragranular region of the infrapyramidal blade of the dentate gyrus; 2, continuous light band of staining in all supragranular regions; 3, dense continuous band of staining in all supragranular regions (Tauck and Nadler, 1985).

Electrophysiology. Electrophysiological experiments were performed with the in vitro hippocampal slice preparation (Dingledine et al., 1980). Rats were decapitated, brains immediately removed and placed in ice-cold buffer. The brain was blocked, attached to a warming block with cyanoacrylate glue and 500-μm transverse slices cut by a Campden vibratome. Starting from the ventral surface of the brain, the first three hippocampal slices from the temporal pole were collected as “ventral” slices, slices from the next 1 mm were discarded; and the next three slices were collected as “middle” slices. These slices correspond to the approximate horizontal stereotaxic coordinates B = −5.60, IA 4.40 to B = −5.26, IA 4.74 (middle) and B = −7.60, IA 2.40 to B = −6.38, IA 3.62 (ventral) according to the atlas of Paxinos and Watson (1986). Slices were submerged in a recording chamber, warmed to 34°C and superfused continuously with oxygenated Krebs-bicarbonate buffer (mM): NaCl, 120; KCl, 3.5; NaH2PO4, 1.25; MgCl2, 1.3; CaCl2, 2.5; glucose, 10; NaHCO3, 25.6; equilibrated with 95% O2, 5% CO2. Slices were allowed to equilibrate for at least 1 hr in the recording chamber before beginning experiments. A concentric bipolar electrode (SNE 100, Rhodes Medical Supply, Woodland Hills, CA) was placed in the molecular layer of the dentate gyrus and perforant path fibers were stimulated (0.3 ms square pulse, 0.015 Hz) at current intensities sufficient to evoke a population spike or fEPSP of half-maximal amplitude. Glass recording microelectrodes (3–5 MΩ) were filled with 3 M NaCl and placed in the granule cell...
layer or the middle molecular layer of the exposed blade to extracellularly record population spikes and fEPSPs, respectively. Data were collected with an Axopatch 200 amplifier (Axon Instruments, Foster City, CA) (2-kHz analog filter). Population spike amplitudes were measured from the first peak to the nadir of the population spike waveform; fEPSP amplitudes were measured from base line to nadir. U69593 (Research Biochemicals International) and nBNI were diluted in recording buffer immediately before use and applied by bath superfusion. Base-line amplitude values were recorded for 5 min. Amplitude values were recorded again 15 min after application of U69593 and 15 min after application of nBNI or after 60 min of U69593 washout.

**Statistical analysis.** Data fitting a nonparametric distribution (e.g., Timm scores) were tested for significance by use of Kruskal-Wallis ANOVA by ranks. Data fitting a normal parametric distribution (e.g., behavioral and electrophysiological data) were tested for significance by a one-way ANOVA with least significant difference post hoc comparison. ANOVA tests were performed with Statistica software (StatSoft, Inc., Tulsa, OK). Data were tested for correlation with Spearman Rank Order Correlation by SigmaStat software (Jandel Scientific, Inc., San Rafael, CA).

**Results**

**Behavioral observations.** Administration of anticonvulsant drugs prevent acute pilocarpine-induced seizures in rats, whereas the effectiveness of these drugs against chronic recurrent spontaneous seizures is unknown (Turski et al., 1989). Therefore, this study focused on acute pilocarpine-induced seizures and the effects of kappa opioids on these acute seizures. After injection with a high dose of pilocarpine (325–375 mg/kg), rats exhibited gustatory automatisms, salivation and head scratching within 12 ± 2 min (range, 3–37 min; n = 23). These actions are not correlated with electroencephalographic seizures (Turski et al., 1989) and are considered to be a type of preconvulsive behavior (Przewlocka et al., 1994). Most (72%) of the animals injected with a high dose of pilocarpine displayed behavioral motor seizures. These seizures occurred with a mean latency of 26 ± 4 min (range, 10–78 min; n = 18) with the duration of the longest motor seizure averaging 51 ± 5 min (range, 2–60 min; n = 18) (table 1). The upper limit for duration measures was 60 min because rats were administered diazepam after 1 hr to decrease mortality (Mello et al., 1993) and to standardize the treatment. Many rats (60%) exhibited SE for the full 60 min before diazepam administration (table 1) and displayed a median of three (range 2–8; n = 18) motor seizures before progressing to SE.

Treatment of rats with the kappa opioid receptor agonist U50488h before a high dose of pilocarpine (325–375 mg/kg) did not significantly affect preconvulsant behavior (data not shown). However, as reported previously for other kappa agonists (Przewlocka et al., 1994), rats pretreated with U50488h did exhibit a significant dose-dependent increase in seizure latency, decrease in seizure duration and decrease in the number of animals that exhibited SE for the full 60 min; these effects were blocked by the kappa opioid receptor antagonist nBNI (table 1). nBNI had no significant effect on any of the behavioral observations listed in table 1 when given before a high dose (325–375 mg/kg) of pilocarpine, although nBNI tripled the mortality rate caused by pilocarpine. Rats treated with U50488h alone (no pilocarpine; n = 6) displayed mild sedation.

The observed anticonvulsant effects of U50488h led us to investigate whether endogenous kappa opioids may also limit seizure activity and whether blockade of kappa receptors would exacerbate the effects of pilocarpine. High doses of pilocarpine (300–400 mg/kg; Turski et al., 1989) may produce maximal levels of hyperexcitability and neuropathology. Thus, norBNI-induced increases in these measures would be difficult to detect. Furthermore, excessive hyperexcitability could overwhelm the capacity of the endogenous kappa opioid system. Rats were treated with the kappa opioid receptor antagonist, nBNI, before a lower “threshold” dose of pilocarpine (275 mg/kg) to avoid this possible ceiling effect. Doses of pilocarpine less than 275 mg/kg (100 and 200 mg/kg) were previously shown to cause preconvulsive behavior but not fully developed electroencephalographic seizures and only mild neuropathological alterations in nonhippocampal regions (Turski et al., 1983). After injection with the lower dose of pilocarpine (275 mg/kg), approximately 25% fewer rats exhibited motor seizures and SE for at least 60 min when compared with the high dose of pilocarpine (table 1). As predicted, treatment of rats with nBNI before the lower dose of pilocarpine significantly increased the number of rats exhibiting seizures and nearly doubled the number of animals exhibiting the full 60 min of SE (table 1). Additionally, pretreatment with nBNI almost tripled the mortality rate (table 1). Whereas no correlation was found between the dose of nBNI and mortality rate (rs = 0.249, P > .05), a positive

**TABLE 1**

<table>
<thead>
<tr>
<th>Behavioral observations</th>
<th>Dose of Drug</th>
<th>Latency to First Seizure</th>
<th>Longest Seizure</th>
<th>Animals Exhibiting SE &gt;60 min</th>
<th>Animals Exhibiting No Seizures</th>
<th>Mortality Rate</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>min</td>
<td>min</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>325–375 mg/kg pilocarpine</td>
<td>0 mg/kg U50488h</td>
<td>26 ± 4 (18)</td>
<td>51 ± 5 (18)</td>
<td>60</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg U50488h</td>
<td>42 ± 6 (9)</td>
<td>24 ± 9 (9)</td>
<td>40</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg U50488h</td>
<td>50 ± 14 (9)</td>
<td>24 ± 9 (9)</td>
<td>14</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg U50 + nBNI</td>
<td>21 ± 3 (5)</td>
<td>42 ± 12 (5)</td>
<td>67</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>275 mg/kg pilocarpine</td>
<td>0 mg/kg nBNI</td>
<td>25 ± 4 (9)</td>
<td>46 ± 8 (9)</td>
<td>33</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg nBNI</td>
<td>39 ± 10 (16)</td>
<td>54 ± 4 (16)</td>
<td>65</td>
<td>16</td>
<td>32</td>
</tr>
</tbody>
</table>

* Data are means ± S.E.M. with the number of animals shown in parentheses.

 Significant difference from 0 mg/kg U50488h (one-way ANOVA, LSD post hoc comparison, P < .01).

 Significant difference from 0 mg/kg U50488h (one-way ANOVA, LSD post hoc comparison, P < .05).

 Significant difference from 0 mg/kg U50488h (Kruskal-Wallis ANOVA by ranks, P < .05).

 Significant difference from 0 mg/kg U50488h (Kruskal-Wallis ANOVA by ranks, P < .05).
correlation was found between seizures and mortality rate \((rs = 0.540, P < .01)\). As expected for an antagonist, injection of nBNI alone (no pilocarpine; \(n = 6\)) elicited no measurable behavioral effects and no mortality. The results show that nBNI did exacerbate the effects of the lower dose of pilocarpine and suggest that the increased incidence of seizures caused by nBNI was responsible for the increased mortality rate. Because nBNI is a \(kappa\) opioid receptor antagonist, the most plausible explanation for its action is through blocking the physiological effects of an endogenously released \(kappa\) opioid. Therefore, these data support the hypothesis that endogenous \(kappa\) opioids have an anticonvulsant role.

**Electrophysiological effects of \(kappa\) opioids in the dentate gyrus.** We then compared seizure-induced histopathology with the location of functional \(kappa\) opioid receptors. The dentate gyrus was chosen for study for the following reasons. First, the hilar region of the dentate gyrus shows the most consistent significant cell loss after pilocarpine-induced seizures (Mello et al., 1993). Second, the zinc-rich mossy fiber axons of the granule cells “sprout” into the inner molecular layer after pilocarpine-induced seizures (see Mello et al., 1992). This sprouting is readily detectable by the neo-Timm stain for heavy metals (Nadler et al., 1980; Sutula et al., 1989; Houser et al., 1990; Holm and Geneser, 1991; Mello et al., 1992). Last, a recent report showed that \(kappa\) opioid receptor immunoreactivity was present in the middle molecular layer of the ventral but not the more dorsal regions of the rat dentate gyrus (McGinty et al., 1994), thus providing a convenient differential distribution of receptors within one brain region. Electrophysiological experiments were done to confirm anatomical data and to determine whether these receptors were functional. Consistent with anatomical findings, the \(kappa\) opioid receptor agonist, U69593, reversibly decreased the fEPSP measured in the middle molecular layer of the dentate gyrus in the ventral but not more dorsal regions of the hippocampal formation (fig. 1).

**Histological findings.** Sections from rats injected with the lower dose of pilocarpine showed no significant hilar cell loss (table 2; fig. 2) and no significant increase in the score for mossy fiber sprouting (table 3; fig. 3) when compared with sections from rats treated with saline. However, sections from rats treated with nBNI before the lower dose of pilocarpine showed significant neuronal loss in the hilus of the middle but not ventral hippocampal formation (table 2; fig. 2). Alternate sections from these same animals showed an increase in the median score for mossy fiber sprouting in both the ventral and middle hippocampal formation (table 3; fig. 3). Again, these results show that blockade of \(kappa\) opioid receptors does exacerbate the effects of the lower dose of pilocarpine. No correlation between histological changes and the differential distribution of functional \(kappa\) opioid receptors in the dentate gyrus was evident. There were, however, positive correlations between seizures and cell loss (ventral \(rs = 0.520, P < .01\); middle \(rs = 0.456; P < .01\)), seizures and scores for mossy fiber sprouting (ventral \(rs = 0.927, P < .01\); middle \(rs = 0.922, P < .01\)) and seizures and dose of nBNI (\(rs = 0.433, P < .05\)). These correlations suggest that the neuroprotective effects of \(kappa\) opioid receptor activation are mediated secondarily via a decreased incidence of seizures.

Histological analysis of the dentate gyrus was then performed in both the ventral and middle portions of the hippocampal formation to investigate the difference in seizure-induced histopathology in regions with and without functional \(kappa\) opioid receptors. No supragranular Timm staining was observed in sections from the middle portion of the hippocampal formation taken from control rats (0 mg/kg pilocarpine; table 3; fig. 3). However, a continuous light band of supragranular staining was seen in the infrapyramidal blade of the dentate gyrus in sections from the ventral portion of the hippocampal formation (table 3). Supragranular Timm staining of mossy fibers has been documented previously in the dentate gyrus of normal rats (Laurberg and Zimmer, 1981; Ribak and Peterson, 1991; Seress, 1992). An increase in the score for mossy fiber sprouting was noted in both the ventral and middle portions of the hippocampal formation.
Fig. 2. Hilar cell loss. Transverse rat brain sections were stained with cresyl violet stain as described under “Materials and Methods.” Sections from rats injected with a high (325–375 mg/kg) dose of pilocarpine (B) exhibited gliosis (suggested by the increased number of small cell bodies) and a loss of hilar neurons when compared with U50488h (no pilocarpine)-injected controls (A). However, sections from animals that were treated with U50488h before a high dose of pilocarpine (C) showed no significant loss of hilar neurons. Sections from rats injected with the lower dose (275 mg/kg) of pilocarpine (E) exhibited no significant loss of hilar neurons when compared with nBNI (no pilocarpine)-injected controls (D). However, sections from animals that were treated with nBNI before the lower dose of pilocarpine (F) did exhibit gliosis and a significant loss of hilar neurons. Sections shown are from the middle portion of the hippocampal formation and were taken from the same animals as the respective treatment groups in figure 3. Abbreviations: g, granule cell layer; h, hilus; m, molecular layer. Scale bar in A applies to A through F; 250 μm.
formation from rats that were injected with a high dose of pilocarpine (table 3; fig. 3). Alternate sections from these same rats that were stained with cresyl violet showed a 24% and 48% loss of hilar neurons in the ventral and middle hippocampal formation, respectively (table 2; fig. 2). Treatment with 20 mg/kg U50488h before the high dose of pilocarpine markedly decreased the hilar cell loss (table 2; fig. 2) and significantly reduced the median score for mossy fiber sprouting (table 3; fig. 3) in both the ventral and middle portions of the hippocampal formation. However, the kappa opioid receptor antagonist, nBNI, did not prevent the histological changes caused by U50488h (tables 2 and 3). These results suggest that U50488h may have exerted its neuroprotective effects through a mechanism not mediated by the kappa opioid receptor. Given these data, it was not surprising to find a lack of correlation between histological changes and the differential distribution of functional kappa opioid receptors in the dentate gyrus.

**Discussion**

**Effects of endogenous kappa opioids on pilocarpine-induced seizures and histopathology.** This is the first report describing the effects of endogenous kappa opioids on pilocarpine-induced seizures and histopathology. The data showing that kappa opioid receptor blockade exacerbated the behavioral effects of a low dose of pilocarpine support the hypothesis that endogenous kappa opioids have an anticonvulsant role. This interpretation is consistent with the effects of administered kappa opioids (present study; Przewlocka et al., 1994). Furthermore, the data showing increased cell death after kappa opioid receptor blockade support the hypothesis that endogenous kappa opioids are neuroprotective. The mechanism for the neuroprotective effects is likely to be indirect because there was no correlation between the location of functional kappa receptors in the dentate gyrus and seizure-induced histopathology in this same region. The positive correlations between seizures and cell loss, mossy fiber sprouting and kappa receptor blockade suggest that the anticonvulsant properties of endogenous kappa opioids also may be responsible for the neuroprotective effects.

The data showing no effect of nBNI on either behavioral seizures or seizure-induced histopathology when given before a high dose of pilocarpine are consistent with the results of Przewlocka et al. (1994) and are not surprising, because many of the animals treated with this pilocarpine dose demonstrated near-maximal durations of seizures in our testing paradigm.

The specific site of anticonvulsant action of endogenous and/or administered kappa opioids within the brain is unclear. The hippocampus, amygdala, cortex and nucleus accumbens are the primary regions of early seizure activity after pilocarpine treatment. Our recent work showing kappa-mediated inhibition of excitatory neurotransmission and excitatory synaptic plasticity in the hippocampal slice makes the hippocampus a candidate for kappa opioid anticonvulsant actions; however, similar control of excitatory transmission by kappa opioids at other sites in the neural circuit is also likely. Indeed, the substantia nigra, entopeduncular nucleus, caudate putamen and deep prepiriform cortex have been implicated in altering the threshold and/or propagation of pilocarpine-induced motor and limbic seizures (see Turski et al., 1989). Both kappa opioid receptors and the opioid peptide precursors, prodynorphin and proenkephalin, have been detected in or near these brain regions (see Mansour et al., 1988). Clearly, further studies with use of microinjection into individual brain regions will be necessary to determine the region responsible for the anticonvulsant actions of endogenous kappa opioids.

We have reported that high-frequency stimulation releases the endogenous opioids, the enkephalins and dynorphins (Wagner et al., 1990, 1991, 1993; Bramham et al., 1988, 1991; Caudle et al., 1991; Xie and Lewis, 1991, 1995; Simmons et al., 1992; Weisskopf et al., 1993). Furthermore, hippocampal enkephalins and dynorphins are decreased immediately after seizures, which implies release of the endogenous opioids (Hong et al., 1980; Kanamatsu et al., 1986a, b). Dynorphin has its greatest affinity for kappa receptors and is thought to be the endogenous kappa opioid receptor ligand (Chavkin et al., 1982; Corbett et al., 1982). Antisera against the dynorphin peptides blocked the activation of kappa opioid receptors effectively after the stimulated release of endogenous transmitters, whereas antisera against enkephalins did not (Wagner et al., 1991, 1993). Thus, the most likely candidates for the endogenous anticonvulsant opioid peptide are the dynorphin opioids.

**Effects of administered kappa opioids on pilocarpine-induced seizures and histopathology.** The effects of U50488h on behavioral motor seizures agree with the previous study in mice (Przewlocka et al., 1994) which reported that the kappa opioid receptor agonists PD117302 and U69593 significantly increased the latency and decreased the severity score of motor seizures after a high (400 mg/kg) dose of pilocarpine. Furthermore, the kappa opioid receptor antagonist, nBNI, reversed the effects of U50488h (present study), PD117302 and U69593 (Przewlocka et al., 1994) on pilocarpine-induced seizures, which suggests that the anticonvulsant actions of these kappa agonists are mediated via kappa opioid receptors.

Results from the present study extend previous histological analyses (Przewlocka et al., 1994) by showing quantification of cell loss in the hilus, the region of the hippocampal formation shown to be affected most consistently by pilocarpine-induced seizures (Mello et al., 1993). Przewlocka et al. (1994) found previously that kappa agonists reduced the

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Mossy fiber sprouting</th>
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<tr>
<td><strong>Dose of Drug</strong></td>
<td><strong>Ventral</strong></td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>325–375 mg/kg pilocarpine</td>
<td>1.5 (22)*</td>
</tr>
<tr>
<td>10 mg/kg U50488h</td>
<td>2 (10)</td>
</tr>
<tr>
<td>20 mg/kg U50488h</td>
<td>1 (12)*</td>
</tr>
<tr>
<td>275 mg/kg pilocarpine</td>
<td>1 (6)</td>
</tr>
<tr>
<td>0 mg/kg nBNI</td>
<td>1 (15)</td>
</tr>
<tr>
<td>10 mg/kg nBNI</td>
<td>2 (12)*</td>
</tr>
<tr>
<td>0 mg/kg pilocarpine</td>
<td>1 (15)</td>
</tr>
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</table>

* Significant difference from 0 mg/kg pilocarpine (Kruskal-Wallis ANOVA by ranks, P < .01).
* Significant difference from 0 mg/kg U50488h (Kruskal-Wallis ANOVA by ranks, P < .05).
* Significant difference from 0 mg/kg U50488h (Kruskal-Wallis ANOVA by ranks, P < .01).
* Significant difference from 0 mg/kg nBNI (Kruskal-Wallis ANOVA by ranks, P < .05).
Fig. 3. Mossy fiber sprouting. Transverse rat brain sections were stained with neo-Timm stain as described under “Materials and Methods.” No mossy fiber sprouting was observed in sections from animals treated with U50488h (A) or nBNI (D) before saline (0 mg/kg pilocarpine controls). Sections from rats injected with a high (325–375 mg/kg) dose of pilocarpine (B) did exhibit mossy fiber sprouting, whereas sections from animals that were treated with U50488h before a high dose of pilocarpine (C) showed no mossy fiber sprouting. Sections from rats injected with the lower dose (275 mg/kg) of pilocarpine (E) exhibited no significant mossy fiber sprouting. However, sections from animals that were treated with nBNI before the lower dose of pilocarpine (F) did exhibit significant mossy fiber sprouting. Sections shown are from the middle portion of the hippocampal formation and were taken from the same animals as the respective treatment groups in figure 2. Abbreviations: g, granule cell layer; h, hilus; m, molecular layer. Scale bar in A applies to A through F; 250 μm.
mild to moderate cellular destruction in CA1, CA3 and pyriform cortex caused by pilocarpine-induced seizures by subjective analysis. However, contrary to the results of Przewlocka et al. (1994), who showed that the neuroprotective effects of PD117302 and U69593 were reversed by the kappa antagonist nBNI, the histological changes caused by U50488h were not blocked by nBNI in our study. These data suggest that the neuroprotectant effects of U50488h are either mediated via a non-kappa opioid receptor-mediated mechanism or that the treatment paradigm used for nBNI in the present study was not optimized to prevent the histological changes caused by U50488h. The latter explanation seems unlikely because nBNI did prevent the anticonvulsant actions of U50488h with the same paradigm. Two non-kappa opioid receptor-mediated mechanisms are possible. First, that U50488h may be acting through a mu or delta opioid receptor, or second, that U50488h may be acting through a non-opioid receptor to decrease cell loss and inhibit mossy fiber sprouting. Indeed, several reports have suggested that U50488h has effects both in vivo (Hayes et al., 1988; Spencer et al., 1988; Nencini and Graziani, 1990) and in vitro (Hayes et al., 1988; Uts et al., 1995) that are not mediated through opioid receptors. Theoretically, the nonselective opioid receptor antagonist, naloxone, could be used to distinguish between the two non-kappa receptor-mediated mechanisms. Naloxone is also an antagonist at mu and delta opioid receptors, however, and thus would block the binding of endogenously released opioids to these receptors. Because mu and delta receptor agonists promote epileptogenesis (see Tortella, 1988; Simmons and Chavkin, 1996) and the mu opioid receptor agonist, morphine, exacerbates pilocarpine-induced seizures and cell loss (Turski et al., 1985), data from these experiments would be difficult to interpret. In fact, similar to U50488h, naloxone given before high doses of pilocarpine also moderately suppresses pilocarpine-induced seizures and cell loss (Turski et al., 1985).

**Physiological effects of kappa opioids in the rat dentate gyrus.** Radioligand autoradiography has shown that kappa opioid receptors are present in the granule cell layer and adjacent layers in the rat dentate gyrus (McLean et al., 1987; Zukin et al., 1988). Previous studies looking at kappa opioid receptor-mediated effects in this same region reported no effects of tiifuodam or U50488h on granule cell population spike amplitudes (Neumaier et al., 1988) and no effects of U69593 or dynorphin on the fEPSPs measured in the outer two thirds of the molecular layer (Salin et al., 1995). The recent anatomical report showing kappa opioid receptor immunoreactivity in the middle molecular layer of the ventral but not the middle or dorsal regions of the rat dentate gyrus (McGinty et al., 1994) prompted us to reexamine the effects of kappa agonists in the rat dentate gyrus. The anatomical observations were extended by showing that these receptors are functional and that activation by a selective kappa receptor agonist leads to a decrease in fEPSP amplitude in regions showing kappa receptor immunoreactivity. The effects of U69593 on perforant path-evoked EPSPs in the present study are similar to those previously reported in the guinea pig (Wagner et al., 1992), which suggests that kappa opioid agonists also may presynaptically inhibit glutamate release from perforant path terminals in the rat.

**Possible role of endogenous kappa opioids in epilepsy.** In the hippocampal formation, the dynorphin-contain-


