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EEG Spectral Analysis of the Neuroprotective *Kappa* Opioids Enadoline and PD117302[1]

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ABSTRACT

The present study characterized the electroencephalographic (EEG) effects of the neuroprotective *kappa* opioids enadoline and PD117302 in conscious, freely moving rats with the use of computer-assisted spectral analysis (CASA). Enadoline (25–100 μg/kg) or PD117302 (1.25–5.0 mg/kg) was administered intravenously to rats implanted with cortical EEG electrodes. Although both drugs produced an immediate, mild sedation, there were no signs of head-weaving or ataxia, and there was little visual evidence of opioid-like EEG slow-wave bursts or seizures. Both drugs produced only modest increases in total EEG power that were not dose dependent. In contrast, CASA revealed significant dose-dependent frequency shifts in relative power distributions, thereby identifying distinct *kappa* opioid alterations in awake EEG activity; EEG power decreased in the 0- to 4-Hz frequency band with concomitant increases in power measured in the 4- to 8-Hz frequency range. The *kappa* opioids produced a dose-dependent consolidation of the EEG waveform centered about a peak frequency of 5.0 Hz (for enadoline) or 4.8 Hz (for PD117302) and a significant shift in the mean EEG frequency from 6.6 Hz (predrug) to 6.2 Hz (postdrug). Further CASA revealed significant postdrug decreases in the edge frequency, mobility and complexity of the EEG. Both drugs produced moderate increases in the latency to slow-wave sleep (SWS). Overall, enadoline (ED_{50} = 18 μg/kg) was ~94 times more potent than PD117302 (ED_{50} = 1690 μg/kg) in producing the *kappa* EEG profile. Because the *kappa*-induced EEG changes were stereospecific for the (−)-enantiomers and inhibited by norbinaltorphimine (nor-BNI), the EEG "fingerprint" described in this study could be attributed to specific activation of brain *kappa* opioid receptors.

Since the development of the *kappa* opioid benzomorphan analgesics, several putative *kappa* ligands expressing varying affinities and selectivities for *kappa vs. mu* opioid binding sites have been developed (Rees, 1992). The benzomorphan *kappa* opioids such as bremazocine and ethylketocyclazocine are recognized to interact with comparable potency at *mu* opioid sites as well as to exhibit activity at nonopioid binding sites (Hunter *et al.*, 1990; Millan, 1990). In addition, the relatively selective nonbenzomorphan *kappa* opioids such as the prototype arylacetamide ligands U50,488 and spiradoline have been determined to possess nonopioid actions and exhibit different pharmacological profiles for their enantiomers (Hayes *et al.*, 1988; Meecham *et al.*, 1989; Tortella *et al.*, 1990, Young, 1989).

In general, *kappa* opioids share a pharmacological profile in rodents exhibiting similar analgesic, behavioral, diuretic and EEG properties (Campi and Clarke, 1995; Millan, 1990; Tortella *et al.*, 1980) Unfortunately, of those *kappa* opioids evaluated in humans, including the benzomorphinans MR2034, ketocyclazocine and cyclazocine (Haertzen, 1970; Pfeiffer *et al.*, 1986; Kumor *et al.*, 1986), and the arylacetamide spiradoline (Dionne *et al.*, 1991; Peters and Gaylor, 1989), all have displayed a predisposition for eliciting CNS disturbances, including altered perception, dysphoria and psychotomimetic reactions.

Enadoline and PD117302 represent a series of highly selective arylacetamide *kappa* opioids originally developed as analgesics (Halfpenny *et al.*, 1989; Hunter *et al.*, 1990; Leighton *et al.*, 1987) and possessing ~900- and ~9000-fold selectively, respectively, for *kappa vs. mu* or *delta* opioid binding sites, with negligible affinity for nonopioid sites, including PCP or *sigma* sites (Clark *et al.*, 1988; Hunter *et al.*, 1990). Furthermore, as the resolved (−)-enantiomer, the pharmacological profile exhibited by enadoline is consistent with *kappa* opioid activity (Hunter *et al.*, 1990). More importantly, clinical evaluations with enadoline have yet to reveal dysphoria

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ABBREVIATIONS: EEG, electroencephalogram; CASA, computer-assisted spectral analysis; SWB, slow-wave burst; SWS, slow-wave sleep; nor-BNI, norbinaltorphimine; FAP, frequency analysis program; CNS, central nervous system.

or psychotomimetic activity. Specifically, although CNS effects, including lethargy, dystaxia, emotional lability, abnormal thinking and other perceptual effects, have been reported at high doses (Dawkins *et al*., 1991; Reece *et al*., 1994), the results of tests to assess drug-induced sedation, euphoria and psychotomimetic activity revealed effects of minimal intensity that, when corrected for placebo, were not considered clinically significant (Reece *et al*., 1994).

The results of several recent studies have confirmed that enadoline and PD117302 are neuroprotective in experimental models of CNS injury (Tortella and DeCoster, 1994). Although these data have been obtained almost exclusively in rodent models of ischemic injury, evidence derived from a gyrencephalic injury model (*i.e.*, focal cerebral ischemia in cats) confirms a neuroprotective property of enadoline in higher species (Mackay *et al*., 1993). Its neuroprotective mechanism of actions remains to be elucidated, but results from *in vitro* and *in vivo* studies indicate that enadoline acts presynaptically to inhibit the release of glutamate (Hill and Brotchie, 1995; Lambert *et al*., 1991; Millan *et al*., 1995; Pinnock, 1992), thereby attenuating one of the primary excitotoxic signals responsible for neuronal injury in the mammalian CNS. On the basis of these findings, enadoline is currently entering clinical trials in stroke patients.

The purpose of the present study was to conduct a comprehensive pharmacological evaluation of the effects of enadoline and PD117302 on spontaneous cortical brain function in the rat. Using CASA, an EEG "fingerprint" has been identified representing *kappa* opioid activity that is distinct from that described previously for other psychotomimetic *kappa* opioid and nonopioid drugs.

Methods

Animals. Fifty male Sprague-Dawley rats (200–250 g; Zivic-Miller) were anesthetized with ketamine HCl and xylazine (70 mg/kg and 6 mg/kg i.m., respectively) and surgically prepared with a chronic indwelling jugular vein catheter and epidural stainless-steel screw (0–80 × ⅛ in.) electrodes chronically implanted and fixed to the skull using dental acrylate cement. The four recording electrodes were implanted bilaterally over the right and left frontal (3 mm anterior, 2 mm lateral to bregma) and parietal (3 mm posterior, 2 mm lateral to bregma) cortices. A fifth reference electrode was implanted over the occipital cortex (Tortella *et al*., 1987). Ten rats were also prepared with an 27-gauge indwelling cannulae aimed at the right lateral ventricle for intracerebroventricular injections. After surgery, all animals were allowed 3 to 5 days for recovery before testing. During this time, they were acclimated to the test environment by being individually housed in clear Plexiglas recording chambers (30 × 30 × 49 cm). A 12 hr light/dark cycle (lights on at 6:00 a.m.) was maintained, and food and water were provided *ad libitum*.

EEG analysis. The recording chambers described above were equipped with custom designed multichannel mercury swivel commutators (Dragonfly Inc., Silver Spring, MD). The rats were connected to the swivel system by flexible Microdot cables. This recording set-up provided a noise-free connection from the unrestrained rat to a Grass model 7D polygraph, permitting freedom of movement by the animals during all phases of the experiment. In addition to the on-line EEG record, the experiments were simultaneously recorded on a Hewlett-Packard FM tape recorder and time-coded (see Tortella *et al*., 1987, for details). The direct bipolar EEG was filtered at the polygraph at 35 Hz (high pass) and analyzed on-line and off-line by spectral analysis on a Nicolet Pathfinder II signal analysis system (Nicolet Instrument Co., Madison, WI) using the FAP (frequency analysis program) developed by Nicolet and the Pathlab II software package developed by R. P. Gussio (see Marquis *et al*., 1989). On-line, predrug and postdrug EEG epochs of 1-min duration were analyzed continuously during the entire experiment by digitizing six consecutive 10-sec samples of analog EEG activity at a rate of 126 Hz and subjected to fast Fourier transformation to provide real-time power density spectra. Using these comprehensive power density spectra, selected 1-min EEG epochs chosen for subsequent off-line analysis were averaged to yield single plots of EEG spectral power shifts in the frequency domain. EEG spectral estimates, including peak frequency, mean frequency, total power, 97% edge frequency and the Hjorth parameters mobility and complexity (Hjorth, 1973), were calculated for the 1- to 30-Hz global band (band 5) frequency range. The EEG power (μV^2) residing in specific frequency bands was expressed as a percentage of the total power (*i.e.*, relative power) in the global band. This estimate of relative power was determined for the following designated frequency bands: 1 to 4.0 Hz (band 1), 4.0 to 8.0 Hz (band 2), 8.0 to 13.0 Hz (band 3) and 13 to 30 Hz (band 4). Importantly, the EEG data from all animals were included in the calculations of each of the CASA parameters quantified throughout the study, with the exception of the peak frequency data seen in figure 5. In this case, only rats eliciting a *kappa* EEG response (see fig. 6) could be included because changes in EEG power would otherwise be distributed evenly across the defined frequency bands.

Experimental protocol. Throughout these experiments, all rats were drug naive and used only once. Beginning the morning of each experiment, control EEG recordings were collected such that testing was initiated only after each subject exhibited normal EEG and behavioral SWS patterns. All experiments routinely began between 9:00 and 11:00 a.m. and were considered complete on the reemergence of SWS. With this protocol, each animal served as its own control.

After the onset of normal SWS rhythms and behavior, the experiment was initiated by giving an intravenous injection of the control vehicle (1 ml/kg, distilled deionized water). For the nor-BNI experiments, rats also received an intracerebroventricular control vehicle injection (7 μl). With the reemergence of EEG SWS after the control injections (usually within 10–25 min), the animals (n = 4–6/dose) were injected intravenously with either enadoline (25, 50 or 100 μg/kg), PD117302 (1.25, 2.5 or 5.0 mg/kg) or the respective (+)-enantiomers CAM569 (500 μg/kg) and PD123497 (5.0 mg/kg). *Kappa* opioid receptor specificity was determined using the selective kappa antagonist nor-BNI. For these experiments, nor-BNI (25 nmol) was administered 30 min before either *kappa* agonist, a pretreatment time demonstrated previously to provide *in vivo kappa* receptor antagonism (Tortella *et al*., 1989).

For the duration of the EEG experiments, each animal was observed for behavioral signs of head-weaving, sedation, locomotion, ataxia and myoclonic or clonic behaviors. The behavioral state of each animal was defined as follows: (1) head-weaving, side to side, lateral head movements most evident while the animal is otherwise motionless; (2) sedation, the absence of overt behaviors in an awake state and distinguished from normal quiet awake behavior by the appearance of drug-induced, synchronized EEG slowing; (3) ataxia, unsteady or impaired gate during walking/locomotion; and (4) sleep, normal sleep posture, eyes closed and EEG SWS patterns. The behavioral responses for each animal were noted and recorded on the EEG polygraph records as a correlate to their respective changes in EEG activity.

Compounds. Enadoline [(5*R*)-5α, 7α, 8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxzspiro [4,5] dec-8-yl]-4-benzofuranacetamide monohydrochloride], PD117302 [(±)-*trans*-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide monohydrochloride] and their respective (+)-isomers (CAM569 and PD123497) were synthesized by the Parke-Davis Research Unit (Cambridge, UK). nor-BNI was purchased from Research Biochemicals (Natick, MA). All compounds were dissolved in distilled deionized water immediately before testing and administered as a 1 ml/kg volume. Intracerebroventricular injections of nor-BNI were delivered as a 5-μl volume

followed by a 2-μl flush. Importantly, all injections (i.v. and i.c.v.) were administered as a 1-min infusion without handling or disturbance of normal animal behavior.

Results

On the morning of testing, EEG and behavioral SWS activity was routinely observed by 9:00 a.m. for each rat. Control intravenous or intracerebroventricular vehicle injections were followed by quiet-awake behavior and normal desynchronized cortical EEG activity consisting of low-amplitude waveforms, which on CASA of the EEG, failed to show any predominate EEG spectral peak (fig. 1, top). On-line visual monitoring of the analog EEG activity and real-time, compressed frequency analysis of the EEG using the FAP program revealed little change in the amplitude of the waveform after either enadoline or PD117302 injections. Furthermore, although the EEG waveforms clearly appeared more synchronized after enadoline and PD117302 injections, inspection of the cortical EEG activity showed little evidence of the classic opioid-like EEG SWBs and no signs of EEG (or behavioral) seizures (fig. 1). As demonstrated by the representative power spectra generated for enadoline (fig. 1, left) or PD117302 (fig. 1, right)-induced EEG activity, with increasing doses both *kappa* opioids produced a marked consolidation of EEG power centered about the 5.0-Hz frequency band.

As shown in figure 2, changes in total EEG power (band 5) measured for enadoline and PD117302 were not dose dependent and revealed only moderate increases measured at the 50 μg/kg and 5 mg/kg doses, respectively. In contrast, figure 3 shows that analysis of relative power shifts across the defined frequency bands demonstrated a dose-dependent decrease in EEG power measured in band 1, which was associated with concomitant increases in EEG power measured in band 2. The dose-dependent EEG power shifts between bands 1 and 2 are further distinguished in figure 4, in which with increasing doses, a maximal 25% to 30% shift in power from band 1 to band 2 was measured (fig. 4). Quantitatively, there were no changes in the EEG power distributions measured for the higher (*i.e.*, α or β) frequency bands (fig. 3, bands 3 and 4).

Additional CASA of the enadoline and PD117302 EEG response identified the peak frequency of their respective EEG waveforms as 5.0 $\pm$ 0.2 and 4.8 $\pm$ 0.2 Hz (fig. 5, top). In addition, compared with predrug activity, enadoline and PD117302 significantly decreased the mean frequency of the EEG by ~0.4 Hz (fig. 5, bottom). Finally, significant postdrug decreases in the edge frequency, mobility and complexity of the EEG were measured for both drugs (table 1).

The percentage of rats per treatment group responding to doses of enadoline or PD117302 by producing the *kappa* EEG profile is shown in figure 6. Quantal analysis of these data (Tallarida and Murray, 1987) demonstrated that the respective ED_{50} values (and confidence limits) for enadoline and PD117302 are 18 (6.8–49.7) μg/kg and 1,690 (1200–2466)

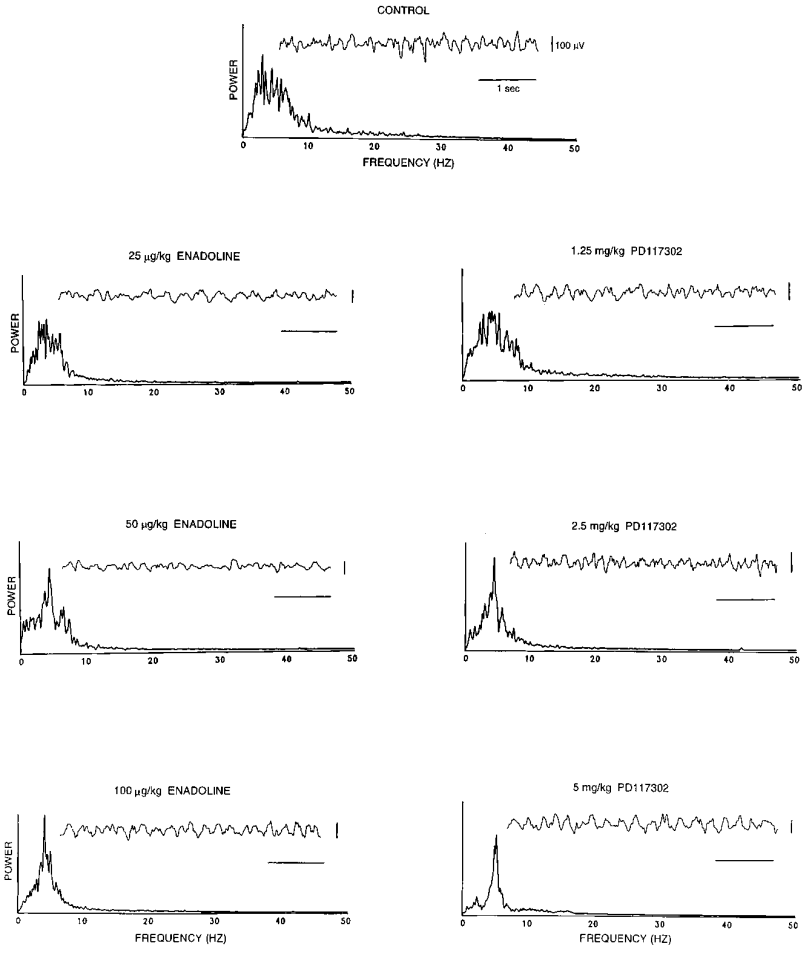

Fig. 1. Representative analog cortical EEG (insets) and digitized EEG power spectra profiles produced by increasing intravenous doses of enadoline (left) or PD117302 (right) in naive rats. Each panel shows the averaged power spectra analyzed from 1 min of EEG activity typified in the respective inset. Each case represents the EEG activity predominant during the initial 2 to 10 min after injection and associated with behavioral sedation.

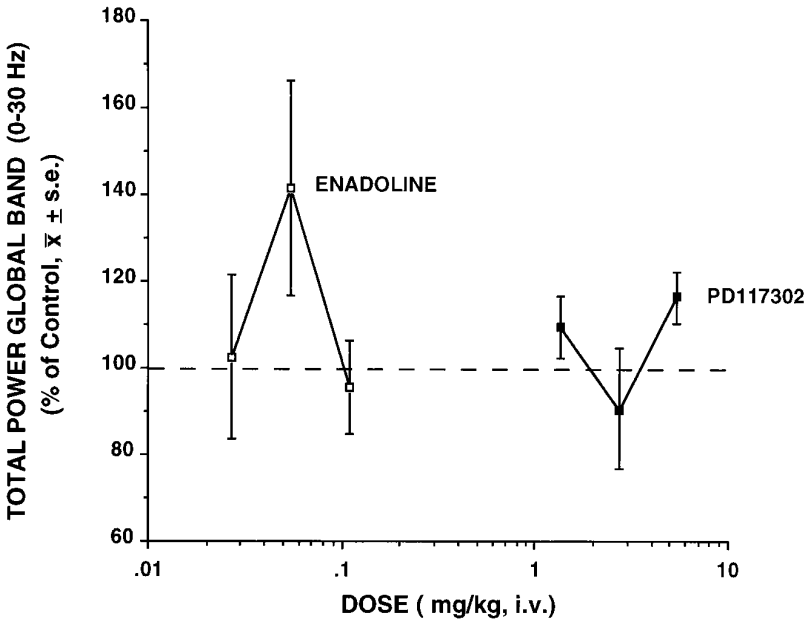

Fig. 2. Dose-response effect of enadoline or PD117302 on total EEG power in the global (0–30 Hz) frequency band. Each point represents the mean ± S.E.M. (n = 5–6 dose group) of the EEG spectral power calculated as a percentage of the predrug control awake EEG power.

μg/kg, indicating that enadoline is 94-fold more potent than PD117302.

The immediate behavioral response to acute injections of enadoline or PD117302 was sedation. However, the animals were awake, upright and responsive to external stimuli. Also, in most animals, an immediate brief Straub tail reaction lasting 15 to 30 sec was observed. After the period of sedation, both *kappa* opioids produced intermittent, brief episodes of sniffing and locomotion. At the highest dose tested, the duration of enadoline- and PD117302-induced behavior and associated *kappa* EEG changes was 26 ± 3 and 25 ± 4 min, respectively. Regardless of dose, neither drug produced behavioral signs of ataxia or head-weaving.

Figure 7 describes the dose-dependent effect of enadoline or PD117302 to delay the onset to SWS. Control SWS latencies for the rats tested across all treatment groups ranged from 3.5 ± 1.1 to 15.2 ± 5.3 min. At the highest doses tested, enadoline and PD117302 increased the latency to SWS a maximum of 62 ± 2 and 46 ± 5 min, respectively (fig. 7).

Control intracerebroventricular injections of nor-BNI failed to produce any significant changes in the EEG or behavior of the rat (not shown). However, pretreatment with the *kappa* antagonist significantly attenuated the EEG and behavioral effects of enadoline or PD117302 (figs. 3, 4, 6 and 7). Furthermore, the (+)-enantiomers of enadoline (CAM569) or PD117302 (PD123497) had no effect on the EEG or behavior of the rat (figs. 6 and 7).

Discussion

Kappa opioids represents a chemically diverse, pharmacologically complex class of drugs possessing a spectrum of opioid and nonopioid receptor affinities and an array of pharmacological properties. Originally developed as analgesics, *kappa* opioids historically have been limited by side effects ranging from diuresis and acute dysphoric reactions to psychotomimesis and dependence. However, with the successful development of highly selective ligands for the *kappa* opioid binding site, there is increasing evidence to suggest that a clinically useful *kappa* drug can be developed without severe limiting side effects.

As research in the field has advanced from the benzomorphinan ligands to the arylacetamide series of *kappa* opioids, several drugs prototyped after the selective *kappa* opioid U-50488 have been developed as *kappa* agonists possessing analgesic activity in a number of preclinical models of nociception. Most notable have been the Upjohn compound spiradoline (U62066) and the Parke-Davis drug PD117302, both representing racemic mixtures of two enantiomers (Rees, 1992). As racemates, each drug displayed preclinical efficacy as analgesics. However, their (+)-isomers were determined to exhibit affinity at *mu* opioid binding sites (Meecham *et al.*, 1989) and, at least in the case of spiradoline [140-fold *mu* selectivity for its (+)-isomer], subsequently determined to possess dysphoric properties in humans (Dione *et al.*, 1991; Peters and Gaylor, 1989). In contrast, the furanyl derivative of PD117302, enadoline, was developed by Parke Davis as the resolved (−)-enantiomer and demonstrated to be highly selective for the *kappa* opioid receptor (K_i = 0.11 nM; *μ/kappa* selectivity ratio of 905) and exhibit a pharmacological profile in rodents typical of *kappa* ligands (Hunter *et al.*, 1990). Thus, unlike its predecessors, enadoline is a potent analgesic (Davis *et al.*, 1992; Hunter *et al.*, 1990; McLaughlin *et al.*, 1995) and neuroprotective (Tortella and DeCoster, 1994) drug that on early clinical evaluation appears to be devoid of psychotomimetic activity (Dawkins *et al.*, 1991; Reece *et al.*, 1994).

The results of the present study identified and characterized EEG properties of enadoline and PD117302 in conscious rats that were stereoselective and antagonized by nor-BNI. The emergence of the *kappa* opioid EEG spectral changes was dose dependent such that with increasing doses of either enadoline or PD117302, a synchronization of cortical activity emerged despite minimal, nonsignificant changes measured in total EEG power. For either drug, CASA of the power spectral shifts analyzed in the frequency domain revealed a consolidation of EEG power centered around the 4.8- to 5.0-Hz frequency band and an overall mean frequency of 6.2

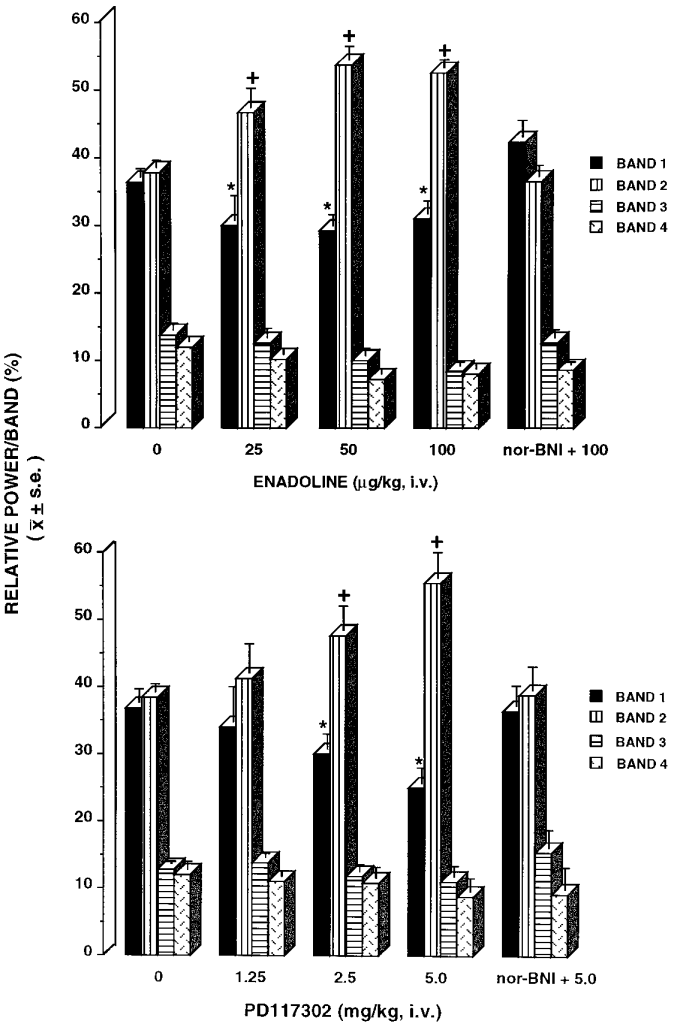

Fig. 3. Changes in the relative EEG power distribution (calculated as the percent of total power residing in each of the defined frequency bands) associated with increasing doses of enadoline or PD117302 (n = 5–6 per dose group) and antagonism by nor-BNI * Significantly different from predrug control (band 1), P < .05. * Significantly different from predrug control (band 2), P < .05 (analysis of variance/Newman-Keuls test).

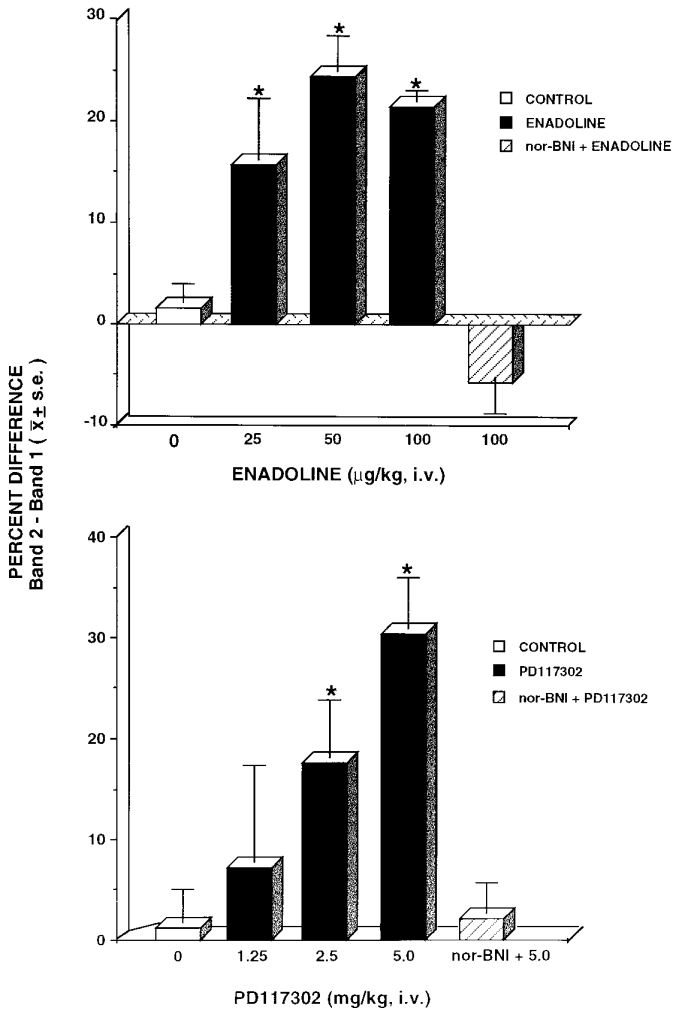

Fig. 4. Percent difference in the respective power spectral shifts between bands 1 and 2 for each dose of enadoline or PD117302 depicted in figure 3 (n = 5–6 per group) and antagonism by nor-BNI (n = 5 per group). * Significantly different from control, $P < .05$ (Mann-Whitney U test).

Hz. Therefore, using this highly quantitative approach, a distinct "EEG fingerprint" can be attributed to *kappa* opioid receptor activation, at least in rats.

Although a similar *kappa* opioid-induced EEG peak frequency has been described for other *kappa* ligands, certain qualitative and quantitative differences can be seen that are likely related to differences in *kappa* binding site affinity and selectivity profiles. For example, previous EEG studies in rats examining less-selective *kappa* ligands, such as cyclazocine, ethylketocyclazocine and ketocyclazocine, U-50,488, or spiradoline and BRL52656, have demonstrated either significant increases or decreases in total EEG power or amplitude (Campi and Clarke 1995; Carruyo *et al.*, 1968; Colasanti and Khazan, 1973; Tortella *et al.*, 1980; Young *et al.*, 1993; Young and Khazan, 1984); the induction of high-amplitude SWB in awake EEG (Campi and Clarke, 1995; Colasanti and Khazan, 1973; Tortella *et al.*, 1980; Young and Khazan, 1984) or, in the case of spiradoline, a "dual peak" power spectra profile (Campi and Clarke, 1995; Tortella *et al.*, 1990). These putative *kappa*-induced EEG effects are, for the most part, inconsistent with those described in the present study for either enadoline or PD117302 and likely attributed to the differences reported in the opioid binding affinities and selectivities for these drugs. Therefore, it is plausible that the different EEG profiles reported for the less-selective *kappa* ligands involve differential interactions at *mu, kappa*, and possibly even *sigma* or dopaminergic binding sites (Tortella *et al.*, 1980; Young *et al.*, 1989, 1993).

In addition to receptor heterogeneity, differences in the EEG analysis procedures may account in part for the differences in EEG activity described in this study for enadoline and PD117302 *vs.*, for example, that reported elsewhere for enadoline or spiradoline (Campi and Clarke, 1995) and other *kappa* opioids such as U50,488 and ketocyclazocine (Young *et al.*, 1993; Young and Khazan, 1984). In these latter studies, EEG CASA was usually preformed *posthoc* (*i.e.*, off-line) and/or only at designated postdrug injection intervals (usually 15–30 min). Therefore, in the final analysis, this CASA protocol may not capture the dynamic changes in the EEG occurring immediately and possibly intermittently. This was not the case in our experiments because EEG CASA was

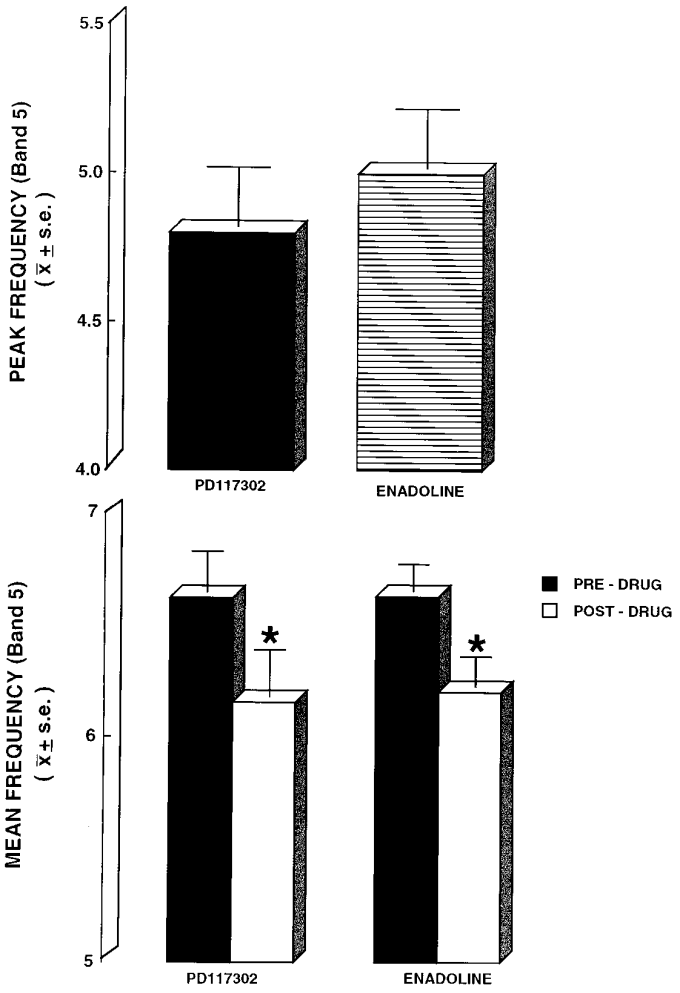

Fig. 5. Effects of enadoline (n = 17, all doses) and PD117302 (n = 14, all doses) on the EEG peak frequency and mean frequency of the global band (0–30 Hz). * Significantly different from predrug control, $P < .05$ (paired t test).

performed continuously on-line (*i.e.*, using the FAP) from predrug injection until the reemergence of postdrug SWS. As such, the computer-derived EEG fingerprints described here for enadoline and PD117302 represents the predominant EEG effect caused by these drugs as measured in real time rather than the measured effect of the drug response at a designated postinjection time interval, which may be different. Importantly, using our approach, the "dual peak" power spectra profile for spiradoline and its resolved isomers has been identified to be qualitatively and quantitatively distinct from that for enadoline or its (+)-isomer CAM569 (Tortella *et al.*, 1990).

To date, all *kappa* opioids (with the exception of enadoline) entering into clinical trials have produced unwanted psychotomimetic/hallucinogenic episodes in patients (*vidae infra*). The identification and prediction of drug-induced psychotomimetic activity based on preclinical experimental studies in a subhuman species have been difficult (if not impossible), with most efforts having focused on animal behavior studies that, by their nature, represent the highest level of integrative CNS activity. How the EEG findings described in the present study relate to this issue of drug-induced psychotomimesis in experimental animals remains to be determined. In contrast to a study of solely animal behavior, the present study measured brain function directly by recording the electrical field potentials in the rat cortical EEG. Although this end point offers a lower level of CNS integration, it has been shown to provide some insight toward identifying and understanding drug-induced CNS disturbances in rodents. For example, using rat EEG and CASA, Dimpfel *et al.* (1989) reported an ability to distinguish hallucinogenic from nonhallucinogenic amphetamine derivatives. Moreover, their results demonstrated that hallucinogenic compounds selectively increased EEG power in the α_1 frequency range (7.0–9.5 Hz). Earlier studies by this same group (Dimpfel *et al.*, 1988) showed a similar pattern of changes for LSD. More recently, a number of putative psychotomimetic drugs have been studied using a rat model of spontaneous cortical EEG activity and CASA similar to that described in the present study. Injections of phencyclidine have been shown to induce a dual peak spectra (Mattia and Moreton, 1986), not unlike that reported for the psychotomimetic NMDA antagonist MK-801 (Marquis *et al.*, 1989; Tortella and Hill, 1996), the benzomorphan phencyclidine/*sigma* ligands (+)-SKF-10047 and (+)-cyclazocine (Marquis *et al.*, 1989; Tortella and Robles, 1991) or the psychotomimetic *kappa* opioid spiradoline (Campi and Clarke, 1995; Tortella *et al.*, 1990). For example, as mentioned earlier, studies evaluating the EEG effects of spiradoline in rats (Tortella *et al.*, 1990) determined that this somewhat selective *kappa* opioid induces an EEG spectral profile similar to other putative psychotomimetic drugs (*i.e.*, a dual peak spectra profile with frequency peaks centered around 1.9 and 5.0 Hz). Furthermore, it was determined that the initial spectral peak produced by spiradoline was selective for the (+)-enantiomer U63639 and antagonized by a low dose of naloxone (probably *mu*), whereas the second *kappa*-like peak was selective for the (−)-enantiomer U63640. Campi and Clarke (1995) also described a double peak EEG power spectra for analgesic doses of spiradoline in rats that was not seen at equivalent antinociceptive doses of enadoline. Critically, in the present study, CASA of the rat cortical EEG activity after injections of enadoline or PD117302 failed to reveal either changes in α_1 power or the dual peak EEG spectral profile, apparently associated with certain psychoactive drugs.

The novel EEG fingerprint produced by enadoline and described in the present study was stereoselective for the (−)-isomer and, like PD117302, was antagonized by pretreatment with the selective *kappa* antagonist nor-BNI. This suggests that these unique EEG changes represent a true functional activation of the *kappa* opioid receptor. These results are consistent with previous reports describing different EEG effects of non-*kappa* opioids in rats or *kappa* opioids possessing mixed selectivity for *mu, delta* and *kappa* opioid receptors (*vidae infra*). For example, using similar CASA EEG techniques, Khazan and coworkers (Khazan, 1975; Young *et al.*, 1981) and Tortella *et al.*, (1987) have shown that *mu* opioids such as morphine and [D-Ala^2-N-methly-Phe^4-Gly^5-ol]enkephalin produce predominantly an EEG slowing characterized by high amplitude bursts at low doses characterized by an increase in power in the 0- to 10-Hz frequency band and continuous synchronization and seizures at high doses. The *mu*-mediated EEG slowing is quantitatively measured as a distinct broad, single peak frequency spectra compared with the more-consolidated power spectra of enadoline of PD117302. These *mu*-mediated EEG effects have been shown to be highly sensitive to antagonisms by naloxone or [D-Pen^2-D-Pen^5]enkephalin β-funaltrexamine (Khazan, 1975;

TABLE 1
Effect of enadoline and PD117302 on the EEG spectral estimates of mobility, complexity and 97% edge frequency

Drug (*n*)[a]	Mobility		Complexity		Edge frequency (Hz)	
	Predrug	Postdrug	Predrug	Postdrug	Predrug	Postdrug
Enadoline (17)	8.54 ± 0.15	7.85 ± 0.16[c]	15.49 ± 0.19	14.45 ± 0.38[b]	21.98 ± 0.39	19.71 ± 0.55[c]
PD117302 (14)	8.60 ± 0.19	8.04 ± 0.32[b]	15.54 ± 0.20	14.44 ± 0.46[b]	22.07 ± 0.61	19.73 ± 0.92[c]

[a] Total number of rats tested per drug (all doses).
[b] $P < .05$, [c] $P < .01$, paired *t* test.

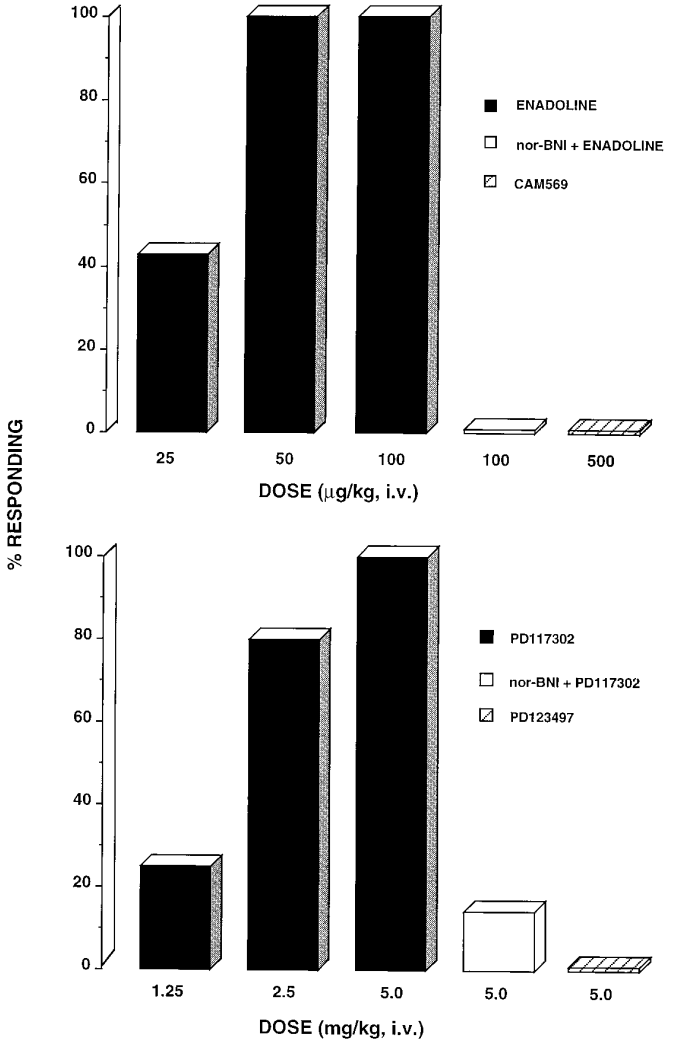

Fig. 6. Quantal dose-response effect of enadoline (top) or PD117302 (bottom) to produce the *kappa* EEG profile in rats and antagonism by nor-BNI and the lack of effect of the respective (+)-isomers of enadoline (CAM569) or PD117302 (PD123497). Each histogram represents the percentage of rats responding per group (n = 5–6).

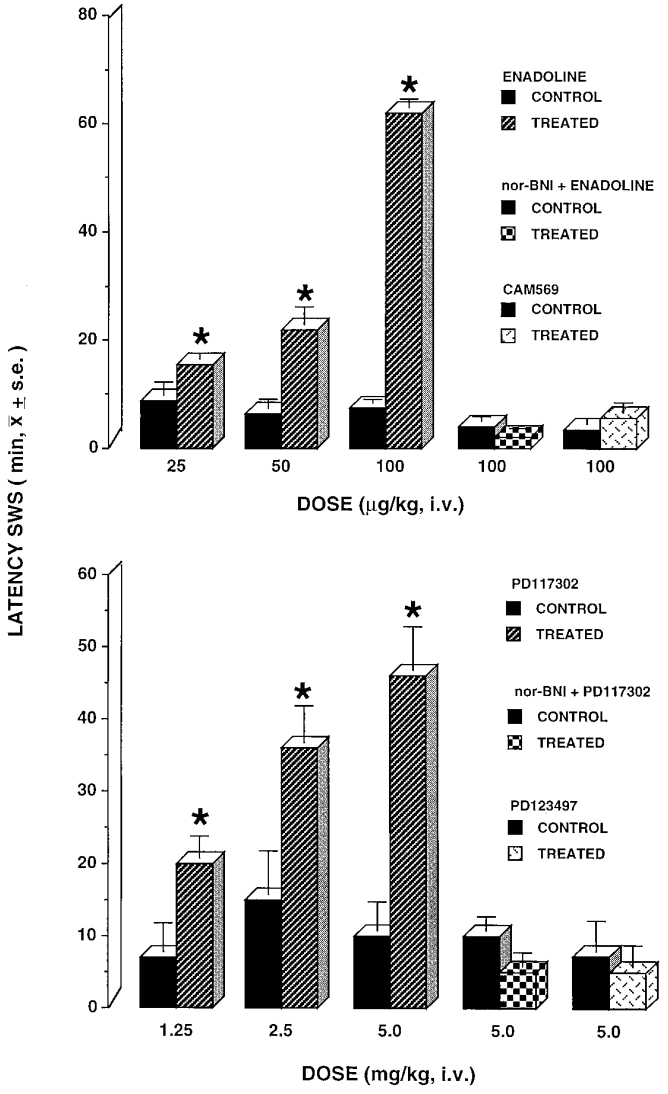

Fig. 7. Effect of enadoline or CAM569 (top) and PD117302 or PD123497 (bottom) on the latency to behavioral and EEG SWS in the rat and antagonism by nor-BNI. * Significantly different from control, $P < .05$ (paired *t* test). Each histogram represents the mean response of 5 or 6 rats per group.

Tortella *et al.*, 1987). In contrast, *delta* opioid ligands such as [D-Pen^2-L-Pen^5]enkephalin and DPLPE, deltorphin or SNC-80 also produce a consolidated single peak power spectra in the rat EEG but of a higher peak frequency (at 5.5–6.5 Hz) compared with enadoline and are antagonized by the *delta* opioid antagonists ICI 174,864, naltrindole or naltriben (Tortella *et al.*, 1987). Depending on their route of administration, *mu* and *delta* opioids can also induce EEG seizures in rats (Tortella, 1993), another effect apparently not shared by enadoline or PD117302 (present study) or other highly selective *kappa* opioids (Campi and Clarke, 1995; Tortella *et al.*, 1990; Young *et al.*, 1989). Finally, although enadoline and PD117302 induced a brief Straub tail reaction, a behavioral response considered synonymous with a *mu* receptor activation, we observed similar Straub tail behaviors in rats after the intravenous injection of several nonopioid drugs, including sodium channel blockers, glycine antagonists, metrazol, some *sigma* compounds and even the excitatory amino acid N-methyl-D-aspartate. Importantly, in the present study, the Straub tail induced by enadoline or PD117302 was antagonized by nor-BNI.

In conclusion, the neuroprotective *kappa* opioids enadoline and PD117302 were determined to induce changes in spontaneous cortical EEG activity in unanesthetized rats that were (1) *kappa* opioid receptor specific, (2) distinct from EEG effects described for non-*kappa* opioids or *kappa* opioids possessing mixed receptor binding affinities and (3) unlike those described in rats for known hallucinogenic or psychotomimetic drugs. Although high doses of enadoline may produce clinical signs of lethargy, dystaxia, emotional lability, abnormal thinking and other perceptual effects, drug-induced sedation, euphoria and psychotomimetic activity do not appear to be of clinical significance (*vide infra*). Therefore, it is possible that this *kappa* drug, which is entering into clinical trials for the treatment of stroke and ischemic brain attacks, may not be burdened with the unwanted CNS side effects associated with earlier *kappa* opioid drugs. Finally, the results of this study suggest that the method of EEG CASA in rats provides a sensitive and highly quantifiable *in vivo* approach for the characterization and study of *kappa* opioid activity and may be a predictor of preclinical drug-induced psychotomimesis and/or related CNS disturbances.

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