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Opioid Mu and Delta Receptor Antagonists Reduce Wet Dog Shaking Elicited by Perforant Path Stimulation

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MITCHELL, C. L., M. I. BARNES, P. M. HUDSON AND J. S. HONG. *Opioid mu and delta receptor antagonists reduce wet dog shaking elicited by perforant path stimulation.* PHARMACOL BIOCHEM BEHAV **38**(4) 801–805, 1991.—Stimulation of the perforant path, a major input to the hippocampal formation, produced significant decreases in the hippocampal levels of methionine enkephalin, dynorphin A(1–8) and an increase in the hippocampal level of gamma-aminobutyric acid. In addition, it was also observed that both mu and delta opioid receptor antagonists reduce wet dog shakes elicited by perforant path stimulation. The antagonists did not affect the changes in hippocampal levels of methionine enkephalin, dynorphin A(1–8) or gamma-aminobutyric acid. The results demonstrate that endogenous opioids are involved in the wet dog shakes elicited by perforant path stimulation. Since electrographic seizure activity occurs in the hippocampus in conjunction with perforant path stimulation-induced wet dog shakes, these data provide further evidence that endogenous opioid peptides play an important role in regulation of limbic system epileptogenic phenomena.

Hippocampus Opioid peptides Opioid antagonists Wet dog shakes GABA Perforant path

THE major input to the hippocampal formation (dentate gyrus and Ammon's horn) from the neocortex is through the entorhinal cortex via the perforant path (6). Electrical stimulation of the entorhinal cortex (7) or the perforant path (4), under proper conditions, produces a stereotypic behavior exemplified by paroxysmal shaking of the head, neck and trunk in rats. This behavior has been referred to as "wet dog shakes" (WDS) because it resembles the behavior observed in a dog shaking itself while wet (16). Several lines of evidence suggest that dentate granule cells play an important role in WDS elicited by activation of the limbic system (4, 7, 9). Since these granule cells contain both enkephalin (8) and dynorphin (15), and since intrahippocampal injection of opioids induces WDS (14), there is considerable interest in the role hippocampal opioids may play in the generation of hippocampal epileptiform activity and accompanying WDS.

We have previously demonstrated that stimulation of the perforant path produces significant decreases in the hippocampal levels of methionine enkephalin (ME) and dynorphin A(1–8) (DYN) and an increase in the hippocampal level of gamma-aminobutyric acid (GABA) (17). The present study had three purposes. These were to determine (a) if the results of the first study could be repeated; (b) if either mu or delta opioid receptor antagonists, or both would reduce WDS elicited by perforant path stimulation; (c) whether or not mu or delta receptor antagonists would alter the changes in levels of ME, DYN, or GABA seen following perforant path stimulation in the absence of these antagonists.

METHOD

Animals

Male, Fischer-344 rats weighing 250–300 g were obtained from Charles River Breeding Company (Portage, MI). They were housed four per cage in a room maintained at 21 ± 2°C and humidity 50 ± 10% with a standard 12/12-h light/dark cycle. Food (NIH diet 31) and water were ad lib.

Surgery

Each animal received atropine sulfate, 2 mg/kg, SC followed approximately 10 min later with pentobarbital sodium, 50 mg/kg, IP. Methoxyflurane was used, as needed, to supplement anesthesia. In each animal, a bipolar electrode aimed at the left perforant path in the region of the angular bundle was chronically implanted under stereotaxic guidance. The electrodes were made from

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twisted 0.25 mm nichrome wire with a tip separation of approximately 0.5 mm. A stainless steel guide cannula (22 gauge, Plastic One Co., Roanoke, VA) was placed in the lateral ventricle. Coordinates were taken from the atlas of Paxinos and Watson (20) and were as follows: perforant path −7.8 mm posterior, 4.4 mm lateral, and 4.0 mm deep; lateral ventricle −0.8 mm posterior, 1.5 mm lateral, 2.5 mm deep (guide cannula) and 4.0 mm deep (injection cannula). Anterior-posterior measurements were made with reference to bregma, lateral measurements were made with reference to the sagittal suture, and depth was measured from the skull at the point of placement. All measurements were made with top of the skull horizontal. Upon completion of the implantation procedure, the electrode was inserted into an amphenol receptacle which was cemented, with the guide cannula, to the skull using dental acrylic. The guide cannula was sealed with a dummy injection cannula until the time of drug administration. Each animal was then given an injection of 75,000 units of penicillin-G, IM.

Treatments

Three separate experiments were conducted. These compared the effect of (a) β-funaltrexamine (β-FNA) (a mu receptor antagonist purchased from Research Biochemicals, Wayland, MA) vs. vehicle; (b) ICI 174864 (a delta receptor antagonist purchased from Cambridge Research Biochemists, Inc., Valley Stream, NY) vs. vehicle; and (c) combination of β-FNA and ICI 174864 vs. vehicle. There were at least 10 animals per group. Each group of animals was used for only one experiment.

Forty-eight hours after surgery all animals were stimulated to determine the threshold for eliciting WDS. Stimulus parameters consisted of an 8-s train of biphasic pulses, duration of 0.1 ms/phase and a train rate of 5 Hz. The initial stimulus intensity was 0.1 mA. Every 2 minutes the intensity was increased by 0.1 mA until WDS were observed. Any animal not exhibiting WDS was discarded.

Fourteen days following threshold determination the animals were divided into 4 groups of 10 each: (a) vehicle-injected, nonstimulated control; (b) antagonist-injected, nonstimulated control; (c) vehicle-injected animals stimulated to the point of exhibiting approximately 75 WDS; and (d) antagonist-injected and stimulated animals yoked to the preceding group. Thresholds for eliciting WDS were matched as closely as possible for groups (c) and (d). The stimulus parameters were the same as those used in the threshold determination except that the initial stimulus intensity was 0.2 mA below that previously determined to elicit WDS and secondly, once WDS were observed the animals were repeatedly stimulated at 5-minute intervals until they reached the criterion number of shakes. At each replication the initial current level was 0.2 mA below that which WDS were previously observed and increased, as stated above, until WDS again occurred. Each animal in group 4 was stimulated the same number of times as the animal to which it was yoked.

An injection cannula (28 gauge, stainless steel) was inserted through the guide cannula and each animal was injected with a volume of 5 μl. The vehicle was artificial cerebrospinal fluid (aCSF). β-FNA was injected in a dose of 10 μg, 24 h prior to testing. This procedure was selected since this dose and time postdose of β-FNA have been reported to antagonize the antinociceptive actions of morphine (as measured by tail-flick latencies) and also to antagonize the anticonvulsant actions of etorphine (26). ICI 174864 was injected in a dose of 2 μg, 10 minutes prior to testing. This procedure was selected since this dose and time postdose of ICI 174864 have been reported to inhibit epileptiform electrocorticogram patterns and myoclonic contractions induced by the delta opioid receptor agonist, [D-Ser^2, Leu^5]enkephalyl-Thr (DSLET) (10), as well as bladder contractions induced by the delta opioid receptor agonist, [D-Pen^2, D-Pen^5]enkephalin (DPDPE) (5). The antagonists were dissolved in aCSF. The aCSF was composed of NaCl, 130 mM; KCl 3.5 mM; NaH_2PO_4, 1.25 mM; $CaCl_2 \cdot 2H_2O$, 1.5 mM; $MgSO_4 \cdot 7H_2O$, 1.5 mM; and glucose, 10 mM.

Immediately following testing the animals were killed by decapitation and their brains removed. Those animals not stimulated were killed at the same time following drug injection as the stimulated animals. The hippocampus was dissected free bilaterally and stored at −70°C for subsequent biochemical analyses. ME and DYN were measured by radioimmunoassay (11). Tissue levels of GABA were determined by high-performance liquid chromatography (12).

Statistical Analysis

The thresholds for eliciting WDS and the number of WDS were compared for each antagonist or their combination vs. the corresponding aCSF control group using Mann-Whitney U-tests (23). The number of WDS occurring in each of the antagonist groups was compared by a one-way analysis of variance (ANOVA) (25). The levels of ME, DYN and GABA were analyzed separately for each experiment by ANOVA. Following a significant F ratio for treatment, the means were compared to the aCSF nonstimulated control group by Fisher's Least Significant Difference test (25).

RESULTS

The WDS generally occurred in clusters. The criterion of approximately 75 WDS was reached in 3 to 6 clusters. The total time of testing ranged from approximately 45 to 75 minutes. The opioid receptor antagonists had no effect on the threshold for eliciting WDS (data not shown). Both antagonists and their combination significantly reduced the number of WDS relative to their respective controls. At the doses studied, there was no significant difference in the efficacy of the 2 antagonists nor their combination in reducing WDS, $F(2,27) = 1.35$, $p = 0.028$. These data are shown in Fig. 1.

Stimulation of the perforant path caused a significant reduction in the hippocampal level of ME. This occurred in both hippocampi. The antagonists had little or no effect on this reduction in ME. The effects of the various treatments on the hippocampal levels of ME measured on the stimulated side are shown in Fig. 2. These data clearly show that the antagonists, given separately, had no effect on the reduction in ME induced by stimulation of the perforant path. However, the ME level in the stimulated group receiving both antagonists, although lowered, was not significantly different from the nonstimulated aCSF control group.

Perforant path stimulation markedly lowered hippocampal DYN levels and the antagonists did not alter this effect (Fig. 3).

Stimulation of the perforant path caused a significant elevation in the hippocampal level of GABA. The antagonists did not alter this effect. These data are shown in Fig. 4.

DISCUSSION

The present study confirmed that stimulation of the perforant path produces significant decreases in the hippocampal levels of ME and DYN and an increase in the hippocampal level of GABA (17). The fact that the opioid antagonists reduce WDS elicited by perforant path stimulation indicates that endogenous opioids are involved in this phenomenon. That both β-FNA and ICI 174864 reduce WDS suggests that (a) both mu and delta receptors are involved or (b) one of the antagonists (or both) is not selective un-

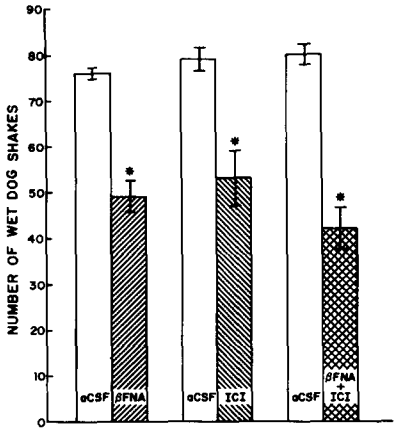

FIG. 1. Effect of β-FNA or ICI 174864 or their combination on the number of WDS elicited by perforant path stimulation. Values are expressed as mean ± S.E.M. There were 10 animals per group. All agents were injected into the lateral ventricle in a volume of 5 μl. β-FNA was administered in a dose of 10 μg, 24 h prior to testing. ICI 174864 was administered in a dose of 2 μg, 10 min prior to testing. The corresponding aCSF control groups received aCSF at the same interval prior to testing as that for the antagonists. *$p<0.002$ compared to the corresponding aCSF group (Mann-Whitney U-test, 2-tailed).

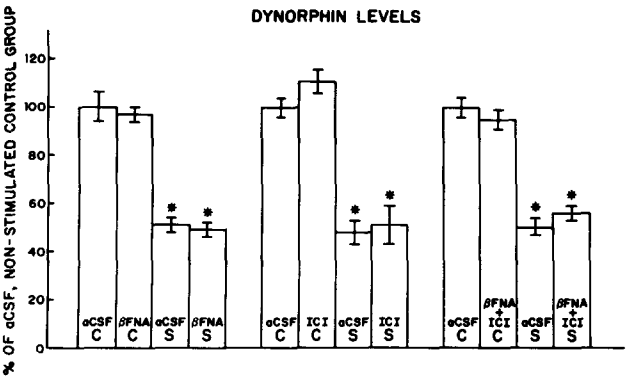

FIG. 3. Relative levels of dynorphin A(1–8) in the hippocampus following the various treatment. In this figure, C represents the nonstimulated groups and S represents the stimulated groups. Since the data were obtained from three separate experiments the levels are expressed as percent of the corresponding aCSF nonstimulated control group (± S.E.M.). There were 10 animals per group. All agents were injected into the lateral ventricle in a volume of 5 μl. β-FNA was administered in a dose of 10 μg, 24 h prior to testing. ICI 174864 was administered in a dose of 2 μg, 10 min prior to testing. The corresponding aCSF control groups received aCSF at the same interval prior to testing as that for the antagonists. *$p<0.05$ compared to the corresponding aCSF nonstimulated control group (Least Significant Difference Test following ANOVA).

der the conditions of this experiment. Indeed, Lee et al. have reported that when opioid agonists are injected directly into the ventral hippocampus, only activation of mu, not delta or kappa receptors, elicits WDS (14). Since we injected the antagonists by the ICV route, it is possible that blockade of delta receptors in a location other than the ventral hippocampus could have attenuated the WDS elicited by perforant path stimulation. In this regard, it should also be pointed out that intraperitoneal administration of the kappa agonist, U-50,488H, inhibits the appearance of WDS elicited by direct hippocampal stimulation (19) or kainic acid (13). It should be of interest, therefore, to know whether or not ICV or intrahippocampal injections of U-50,488H would inhibit perforant path stimulation-induced WDS. Obviously, these possibilities require further investigation.

The failure of the combination of β-FNA and ICI 174864 to exert an effect greater than either alone suggests that the maximal

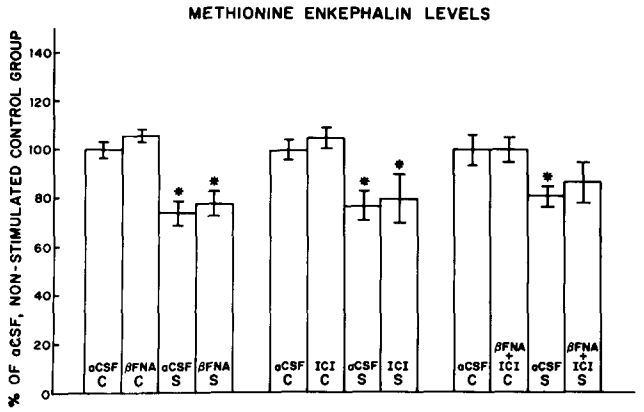

FIG. 2. Relative levels of methionine enkephalin in the hippocampus following the various treatments. In this figure, C represents the nonstimulated groups and S represents the stimulated groups. Since the data were obtained from three separate experiments the levels are expressed as percent of the corresponding aCSF-nonstimulated control group (± S.E.M.). There were 10 animals per group. All agents were injected into the lateral ventricle in a volume of 5 μl. β-FNA was administered in a dose of 10 μg, 24 h prior to testing. ICI 174864 was administered in a dose of 2 μg, 10 min prior to testing. The corresponding aCSF control groups received aCSF at the same interval prior to testing as that for the antagonists. *$p<0.05$ compared to the corresponding aCSF nonstimulated control group (Least Significant Difference Test following an ANOVA).

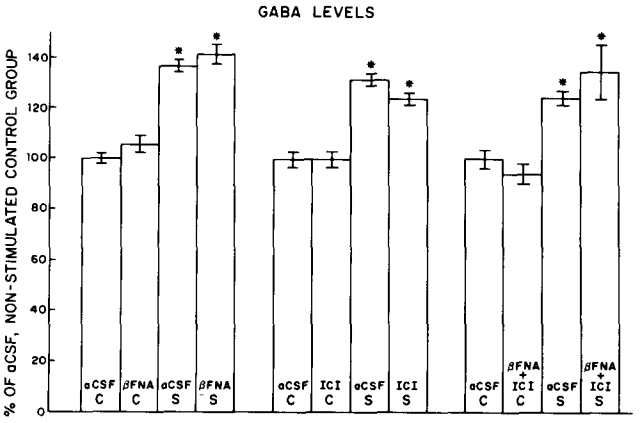

FIG. 4. Relative levels of GABA in the hippocampus following the various treatments. In this figure, C represents the nonstimulated groups and S represents the stimulated groups. Since the data were obtained from three separate experiments the levels are expressed as percent of the corresponding aCSF nonstimulated control group (± S.E.M.). There were 10 animals per group. All agents were injected into the lateral ventricle in a volume of 5 μl. β-FNA was administered in dose of 10 μg, 24 h prior to testing. ICI 174864 was administered in a dose of 2 μg, 10 min prior to testing. The corresponding aCSF control groups received aCSF at the same time interval prior to testing as that for the antagonists. *$p<0.05$ compared to the corresponding aCSF nonstimulated control group (Least Significant Difference Test following an ANOVA).

effect was obtained with the doses used for each of the compounds alone. Alternatively, prior occupancy of binding sites by β-FNA may have allosterically hindered the action of ICI 174864. Such an effect has been reported by D'Amato and Holaday (3). These possibilities also require further investigation.

The antagonists had little, or no, effect on the decrease in hippocampal levels of ME and DYN occurring coincident with WDS. There was clearly no effect of the antagonists, given separately or in combination, on the lowering of DYN levels induced by stimulation of the perforant path. However, their effect on ME levels was less clear-cut. Given separately, the antagonists had no effect on perforant path stimulation-induced reduction in ME. But, the ME level in the combined antagonist stimulated group, although lowered, was not significantly different from the corresponding aCSF nonstimulated control group. Yet, neither was it different from the corresponding aCSF-stimulated control group. Thus whether or not the combination of βFNA and ICI174864 effectively alter the reduction in ME induced by stimulation of the perforant path is a moot question.

The antagonists also had no effect on the increase in hippocampal levels of GABA occurring coincident with WDS. The increase in GABA levels could be due to an increase in synthesis, an increase in the reuptake or a reduction in release. Increases in hippocampal GABA have been observed under a variety of experimental conditions producing hippocampal epileptiform activity (1, 21, 22). With kainic acid, at least, the increase in GABA may reflect a decrease in release since intrahippocampal injection of kainic acid causes an increase in GABA in the contralateral hippocampus which is unaccompanied by changes in synthesis or turnover (22). It is tempting to speculate that the increase in GABA seen in our studies is due to a decrease in release since it has been postulated that the enhanced excitability of pyramidal neurons induced by application of opioid peptides to the hippocampus is due to inhibition of GABA containing interneurons (2, 19, 24). If this is the case, however, one might expect that the antagonists would have reduced the elevation in GABA levels induced by perforant path stimulation. This did not occur. Since we measured total hippocampal levels of GABA it is possible that local changes might have occurred which were undetected. Experiments are currently underway to examine this possibility by a microdialysis technique which measures the rate of release of GABA from different subregions of the hippocampus.

The fact that rather high doses of the antagonists only attenuated WDS suggests that the endogenous hippocampal opioid peptides may act to modulate the effect of a neurotransmitter (l-glutamate?). This notion is strengthened by the findings that systemic administration of MK-801, a noncompetitive NMDA receptor blocking agent, both elevates the threshold for and reduces the number of WDS induced by perforant path stimulation (Barnes, M. I. and Mitchell, C. L., unpublished) as does intrahippocampal injection of gamma-D-glutamyl-glycine (Xie, C. W., Lee, P. H. K., Mitchell, C. L. and Hong, J.-S., unpublished), a nonspecific glutamate receptor blocking agent. Further studies concerning this aspect are being conducted.

In summary, the present study confirmed our previous finding (17) that stimulation of the perforant path produces significant decreases in the hippocampal levels of ME and DYN and an increase in the hippocampal level of GABA. That endogenous opioids are involved in the WDS elicited by perforant path stimulation was demonstrated by the fact that the mu opioid antagonist, β-FNA and the delta opioid antagonist, ICI 174864 attenuated these WDS. Since electrographic seizure activity occurs in the hippocampus in conjunction with perforant path stimulation-induced WDS, these data provide further evidence that endogenous opioid peptides play an important role in the regulation of limbic system epileptogenic phenomena.

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