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Effects of the δ-Opioid Agonist, [D-Pen^2,D-Pen^5]-Enkephalin, on Fetal Lamb EEG

HAZEL H. SZETO,[1] PETER Y. CHENG, DUN-LI WU AND YI SOONG

Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021

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SZETO, H. H., P. Y. CHENG, D.-L. WU AND Y. SOONG. *Effects of the δ-opioid agonist, [D-Pen^2,D-Pen^5]-enkephalin, on fetal lamb EEG.* PHARMACOL BIOCHEM BEHAV **49**(4) 795–800, 1994.—Opiates are known to exert biphasic effects on level of arousal, with excitation at low doses and depression at higher doses. It has been suggested that this dual excitatory and depressant actions of opiates may be mediated by different receptor subtypes. We have previously shown that activation of μ_1-opioid receptors evoked EEG activation in the fetal lamb. The purpose of the present study was to quantitate the effects of DPDPE, a highly selective δ-opioid agonist, on fetal EEG. When infused ICV (4.6–154 nmol/h), DPDPE elicited dose-dependent activation of fetal EEG, with a reduction in power distribution in the delta (1–4 Hz) band, and an increase in the beta (15–32 Hz) band. This activation was reflected by an increase in the spectral edge frequency. This EEG activation was greatly attenuated at DPDPE doses greater than 154 nmol/h, resulting in a U-shaped dose–response curve. The EEG activation was completely blocked by naloxone or naltrindole (δ antagonist), but not by naloxonazine (μ_1 antagonist). These results indicate that the activation of δ-opioid receptors will evoke EEG activation in the fetal lamb.

Opioids δ-Opioid receptor DPDPE Naltrindole EEG Behavior Fetus Pregnancy

IN ADULT rats, morphine and methadone have been reported to exert biphasic effect on level of arousal, with relatively high doses in naive animals leading to stuporous behavior and synchronization of the electroencephalogram (EEG) (3,13). This initial period of behavioral depression is followed by a secondary phase of behavioral excitation in which an alert EEG and increased motor activity occur. Treatment with low doses of morphine can produce increased locomotor activity (2) and desynchronization of the EEG (8). A similar biphasic action of opiates on EEG and motor activity has also been observed when the fetus is exposed to opioids (20,22,25,28). Both excitatory and depressant actions appear to be mediated by specific opioid receptors as they were antagonized by naloxone (NLX).

The discovery of multiple subtypes of the opioid receptor has led to the suggestion that this dual excitatory and depressant actions of opiates on EEG and locomotor activity may be mediated by different receptor subtypes. Opioid agonists with selectivity for the μ-opioid receptor increased locomotor activity in rats at low doses, whereas higher doses produced an initial period of immobility that was followed by an increase in locomotor activity (11,12,15,19). The locomotor stimulation was found to be mediated by μ_1-opioid receptors, whereas the catalepsy was thought to be mediated by μ_2-opioid receptors (19). On the other hand, δ-opioid agonists were found to be associated with monophasic stimulation of locomotor activity (6,7,12,15,18,27). Thus, μ- and δ-opioid receptors appear to mediate different behavioral profiles. However, their effects on EEG has not been studied as extensively using highly selective agonists.

In the fetal lamb, we have shown that morphine-induced activation of EEG can be blocked by pretreatment with naloxonazine (NALZ), suggesting that it is mediated by the μ_1-opioid receptor (4). The effects of selective δ-opioid receptor agonists on fetal EEG are not known. A large number of studies have reported that both acute and chronic use of opiates are known to result in a variety of adverse effects on pregnancy outcome. In addition, prenatal opiate exposure is associated with a variety of behavioral disturbances, many of which persist into early childhood. An understanding of the role of opioid receptor subtypes on fetal EEG may be important in the design of receptor-selective opioid agonists for use

[1] To whom requests for reprints should be addressed.

in pregnant women. The purpose of the present study was to quantitate the effects of [D-Pen^2,D-Pen^5]-enkephalin (DPDPE), a highly selective δ-opioid agonist, on EEG in the fetal lamb. The intracerebroventricular (ICV) administration of DPDPE resulted in a dose-dependent activation of fetal EEG, which was effectively blocked by the selective δ-opioid antagonist, naltrindole (NTI).

METHOD

Animal Preparation

A total of 56 studies were carried out in 34 fetal lambs between 120 and 140 days of gestation (term being 145 ± 5 days). Fetal lambs were surgically instrumented for chronic intrauterine recordings of EEG, in accordance with guidelines approved by the Institution for the Care and Use of Animals. Details of the surgical procedure have been described previously (20,24). Briefly, two stainless steel screws (size 0–80) were implanted over the parietal cortex for recording EEG activity. A polyvinyl catheter was placed in the lateral cerebroventricle for infusion of drugs. In addition, polyvinyl catheters were placed in the distal aorta to permit arterial blood gas determinations (BMS3MK2, Radiometer, Copenhagen, Denmark) and in the inferior vena cava for drug infusions. The EEG leads and catheters were tunneled SC to the maternal flank and stored in a pouch. This preparation permitted continuous intrauterine recording of fetal EEG in an unrestrained, unanesthetized animal. Intraoperatively, 2 g of ampicillin was placed in the amniotic cavity and 1 g in the peritoneal cavity of the ewe.

Study Design

To allow ample time for recovery from the surgical procedure, all drug studies were performed at least 5 days after surgery. Studies were carried out with the ewe standing or lying quietly in a cart with free access to food and water throughout the study. All studies were performed at the same time of the day (0900–1700 h). An individual animal only received one study per day, with at least 2 days allowed between studies for recovery. In each animal, a 3-h control period was obtained for analysis before drug administration. Only fetuses with arterial pH > 7.3, PCO_2 < 50 mmHg, and PO_2 > 16 mmHg were included in the study (10).

EEG Analysis

Details of the EEG acquisition method have been published previously (21). Briefly, the EEG signal was recorded on a Gould 2800S analog recorder after amplification to about 1 V and with a bandpass filter of 1–100 Hz. The amplified, filtered signal was also recorded concurrently onto FM tape (TEAC XR-310) for storage and off-line analysis. Analog-to-digital conversion at 256 Hz was accomplished by a Data Translation 2801A board.

The EEG is an extremely complex signal that can only be fully characterized by both amplitude and frequency. Although visual analysis will reveal amplitude changes, it does not permit assessment of changes in frequency components. Power spectral analysis with fast Fourier transform (FFT) was therefore used to provide a quantitative assessment of changes in frequency components. Fast Fourier transform (FFT) was performed on consecutive series of 1024 data points (4 s) following trend removal and the application of a Hamming (raised cosine) window. Details of the method can be found in earlier publications (21,22). The resolution of the FFT was 0.25 Hz. Five power spectra were averaged to produce a single mean power spectrum every 20 s. Averaging of spectra is necessary, as the error associated with each point estimate in a single power spectrum is 100%. Comparison of a large number of power spectra can be rather difficult and generally involves dividing the spectral data into several different wavebands (delta, 1–4 Hz; theta, 5–7 Hz; alpha, 8–13 Hz; beta, 15–32 Hz). Relative power in each of these wavebands was calculated by dividing the power in each waveband by total power. The 90% spectral edge frequency (SEF) was also calculated as the frequency below which 90% of the power resides (21). The SEF essentially provides a measure of the overall frequency of the signal, and has been used very effectively in demonstrating age-related changes in the fetal EEG (21) and dose-related changes after morphine exposure (22).

DPDPE Administration

DPDPE (Cambridge Research Biochemicals, UK) was dissolved in saline and infused ICV to fetal lambs at five different doses ranging from 4.6 to 463 nmol/h. This dose range was chosen based on the effective doses reported for stimulation of fetal breathing movements (5). A total of 5–11 animals were studied at each dose level. For each dose, the infusion was maintained for 1 h at a constant flow rate of 0.3 ml/h. The effects of DPDPE on fetal EEG activity were quantitated during the hour of DPDPE infusion and for 2 additional hours after infusion. Each animal received only one dose of DPDPE in each study. As a vehicle control, the effects of saline infusion were compared to control, presaline values, with each animal serving as its own control (n = 9).

DPDPE Administration With Antagonists

To determine if the response to DPDPE was mediated by specific opioid receptors, the effects of 46 nmol/h DPDPE ICV were determined in the presence of naloxone 6 mg/h (IV, n = 5). The infusion of naloxone, dissolved in saline, was started 1 h before the DPDPE infusion and was maintained for 2 additional hours after the end of DPDPE infusion. Possible involvement of the δ- and the μ_1-opioid receptors in the EEG response to 46 nmol/h DPDPE was examined by administering the selective δ-opioid antagonist, NTI (RBI, Natick, MA), and the selective μ_1-opioid antagonist, NALZ (a generous gift from Dr. Gavril Pasternak, Memorial Sloan-Kettering Cancer Center, New York, NY). The infusion of NTI (665 nmol/h, ICV, n = 4) was started 1 h before DPDPE and was maintained throughout DPDPE infusion and for 1 additional hour after DPDPE infusion had stopped. Although NTI is known to readily cross the blood-brain barrier (31), it was administered ICV due to our limited supply of the drug and possible significant clearance across the placenta to the mother. The dose of NTI was calculated based on the effective dose reported to block DPDPE-induced stimulation of breathing movements in fetal lambs (5). NALZ was administered to the fetus at a dose of 34 mg (52 μmol, IV, n = 5) infused over a period of 50 min. The effect of NALZ on the fetal EEG response to DPDPE was tested 24 h after administration of NALZ. This dose of NALZ has previously been shown to be effective in antagonizing EEG activation and respiratory stimulation caused by morphine in the fetal lamb (4,23).

Statistical Analysis

All data are presented as mean ± SEM. Due to the relatively large interindividual variation in spectral characteristics

as a function of gestational age of the fetus, the effects of drug administration were compared to control predrug values with each animal serving as its own control. The data were normalized as a percent change from control values. Statistical significance in the dose-response study was determined using one-way analysis of variance (ANOVA; factor = dose), and the Dunnett's test was used for comparing each dose to saline. ANOVA and the Dunnett's test were also used to compare the effects of DPDPE, with and without various antagonists, to that of saline.

RESULTS

Effects of ICV DPDPE on Fetal EEG

Time action. The time to onset of the response varied greatly between animals, but generally occurred by 1 h after onset of DPDPE infusion. Peak response was observed between 1 and 3 h. The time course of drug action was dose dependent for doses of DPDPE ranging from 4.6–154 nmol/h. At the highest dose, there appeared to be a delay in the response (see Figs. 1 and 2).

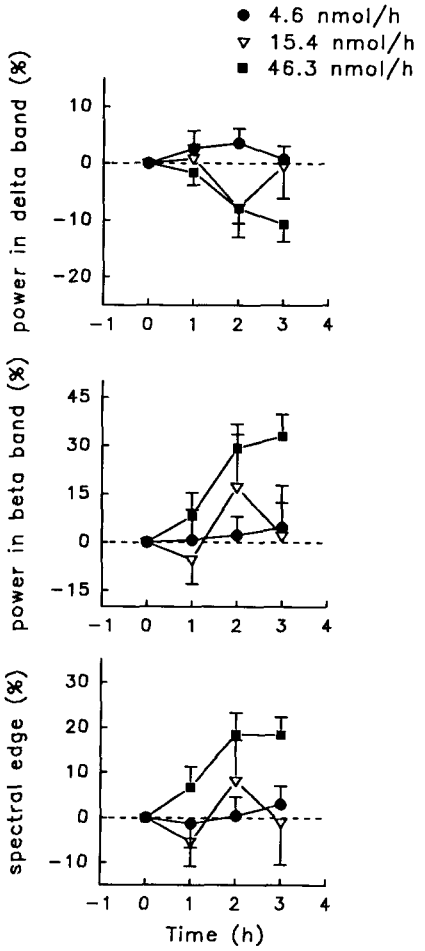

FIG. 1. Time course of DPDPE actions (4.6–46.5 nmol/h, ICV) on fetal EEG spectral parameters. Top panel: percent change in power in the delta band; middle panel: percent change in power in the beta band; bottom panel: percent change in the spectral edge frequency. DPDPE was infused between 0–1 h (4.6 nmol/h, n = 6; 15.4 nmol/h, n = 5; 46.5 nmol/h, n = 11).

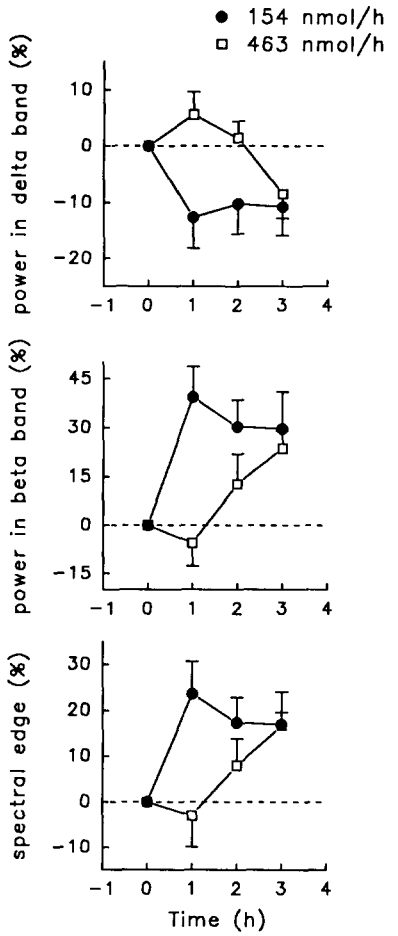

FIG. 2. Time course of DPDPE actions (154–463 nmol/h, ICV) on fetal EEG spectral parameters. Top panel: percent change in power in the delta band; middle panel: percent change in power in the beta band; bottom panel: percent change in the spectral edge frequency. DPDPE was infused between 0–1 h (154 nmol/h, n = 5; 463 nmol/h, n = 5).

Dose-response. Saline infusion did not result in any significant changes in the fetal EEG. Doses of DPDPE between 4.6 and 154 nmol/h reduced power in the delta band in a dose-related manner, and increased power in the beta band (Fig. 3). DPDPE administration did not significantly affect power in the alpha band. The activation of fetal EEG by DPDPE was also reflected by a dose-related increase in the 90% spectral edge frequency (Fig. 3). This EEG activation was greatly attenuated with further increase in DPDPE dose, resulting in a U-shaped dose-response curve. The changes in EEG pattern were not accompanied by any significant changes in fetal arterial blood pH, PCO_2 or PO_2.

Selective Antagonism of DPDPE Responses

Concurrent IV administration of NAL (6 mg/h) or ICV administration of NTI (665 nmol/h) completely abolished the effects of 46 nmol/h oof DPDPE on the spectral properties of the fetal EEG (Fig. 4). In contrast, the effects of DPDPE on the EEG were not affected by pretreatment with NALZ (Fig. 4).

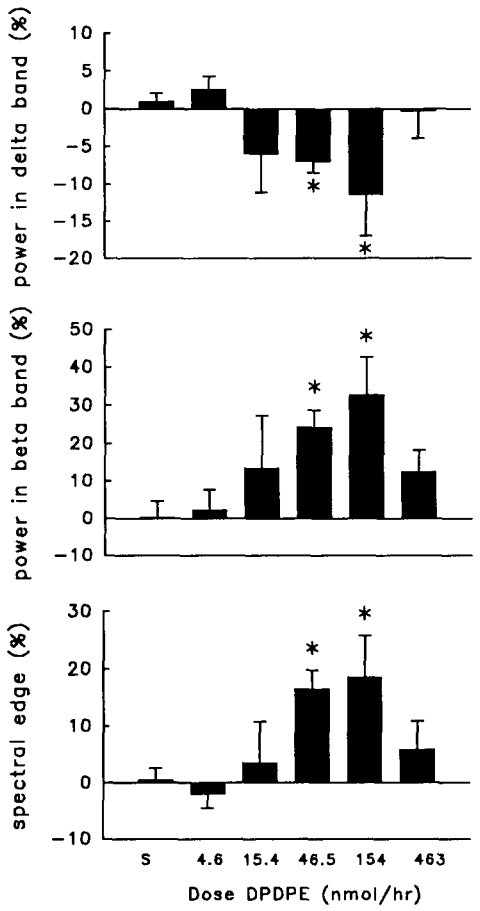

FIG. 3. Dose-related effects of DPDPE on fetal EEG spectral parameters. Data are expressed as percent change after DPDPE administration (1 h infusion + 2 h postinfusion) from control (S = saline, n = 9; 4.6 nmol/h, n = 6; 15.4 nmol/h, n = 6; 46.5, n = 11; 154 nmol/h, n = 5; 463 nmol/h, n = 5). *$p < 0.05$.

DISCUSSION

The present studies provide data to show dose-dependent effects of the δ-selective opioid receptor agonist, DPDPE, on fetal EEG activity. Activation of the EEG is supported by a reduction of power in the delta (1–4 Hz) band and an increase in the beta band (15–32 Hz). The increase in the spectral edge frequency reflects the overall increase in frequency of the EEG. These effects were attenuated at higher doses of DPDPE, resulting in a U-shaped dose-response curve.

Although the effects of DPDPE on adult EEG are not known, there are reports that DPDPE can stimulate locomotor activity in adult rats (6,15,16,18,27). In one study, the increase in locomotor activity by DPDPE was shown to be mediated by δ-opioid receptors because it was blocked by ICI 154,129 (16). In a recent study, [D-Ala^2]-deltorphin, a highly selective δ-opioid agonist, was also reported to increase locomotor activity in adult rats, and that effect was completely blocked by naltrindole (12). In our study, the excitatory action of DPDPE on fetal EEG was clearly mediated via the δ-opioid receptor, as the effects were completely blocked by concurrent administration of naltrindole. Because we had previously found that activation of the μ_1-opioid receptor also resulted in an activation of the fetal EEG (4), it was important to demonstrate that this effect of DPDPE was not mediated by the μ_1-opioid receptor. Our results showed that the EEG activation by DPDPE could not be blocked with a dose of NALZ that had previously been shown to be adequate in blocking the EEG activation caused by morphine (4).

The attenuation of the response at higher doses of DPDPE may be explained by the loss of δ-opioid receptor selectivity at those doses. A similar U-shaped dose-response curve was found for the effect of DPDPE on fetal breathing (5). At doses that resulted in activation of fetal EEG, DPDPE significantly increased fetal breathing activity. This respiratory stimulant action of DPDPE was also attenuated at higher doses, but with no evidence of respiratory depression. U-shaped dose-response curves have also been reported for both the impairment and enhancement of avoidance performance produced by DPDPE in adult rats (14). The authors interpreted this U-shaped dose-response relationship as indicating the involvement of opposing processes, each of which mediates a different response and is maximally stimulated by a different concentration of drug. However, it is also possible that at the higher doses, DPDPE is no longer selective for the δ receptor. In binding studies, DPDPE has been shown to have an affinity

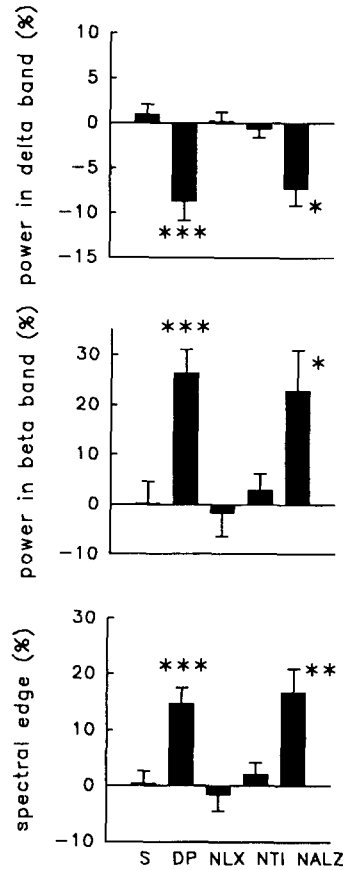

FIG. 4. Peak effect of saline (n = 9), DPDPE alone (46.5 nmol/h, ICV; n = 12), and DPDPE (46.5 nmol/h, ICV) in the presence of NTI (665 nmol/h, ICV; n = 4), NLX (6 mg/h, IV; n = 5), or NALZ (34 mg, IV, 24 h earlier; n = 5) on fetal EEG spectral parameters. Data are expressed as percent change from control. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$ compared to the saline group.

for δ receptors that is only ~100-fold higher than for μ receptors (17). Thus, at low doses, DPDPE may preferentially bind to the δ receptor. At higher doses, DPDPE may bind to both δ and μ receptors.

The effects of DPDPE on the fetal EEG were distinct from the effects of morphine reported in previous studies (22). Although both drugs resulted in a reduction of power in the delta band (1-4 Hz) and an increase of power in the beta band (15-32 Hz), the maximal effects caused by morphine were approximately twofold greater than DPDPE. In addition, fetal EEG activation by morphine was accompanied by a significant increase of power in the theta band (5-7 Hz), in contrast to DPDPE, which had no effect in that frequency band. Theta activity is generally thought to be hippocampal in origin, and administration of opiates to adult animals has been reported to elicit a theta rhythm (3-10 Hz) in hippocampal recordings (9). Furthermore, opioid agonists have long been known to produce excitation of hippocampal pyramidal neurons (29). More recent studies showed that both μ- and δ-opioid agonists enhance the amplitude of the primary population spike in CA_1 (1). However, it appears that only μ-opioid agonists release the secondary population spike (30). This may account for the difference between morphine and DPDPE on EEG theta activity in our studies.

These results provide data to show dose-dependent effects of the δ-opioid receptor-selective agonist, DPDPE, on fetal EEG pattern, and a confirmation of the involvement of the δ-opioid receptor with antagonist studies. These studies did not examine the neurochemical mechanisms by which δ-selective agonists produce EEG activation. Recent studies in adult rats have suggested a role for the limbic dopamine system in the stimulant actions of [D-Ala^2]-deltorphin (12). Both ICV and intra-accumbens infusion of [D-Ala^2]-deltorphin evoked dose-dependent motor stimulation characterized by locomotor activity, grooming, and oral stereotypies. These effects were abolished after blockade of dopamine D_1 receptors with SCH 23390. In contrast, D_1 dopamine transmission did not appear to play a role in the motor activity of μ-opioid agonists (12,26). We have previously reported that the EEG activation elicited by low doses of morphine in the fetal lamb was mediated by μ_1-opioid receptors, and appeared to involve muscarinic pathways (4,22). Thus, μ- and δ-opioid receptors may modulate behavioral activity via different neurochemical systems. Studies are currently in progress to ascertain the role of dopamine pathways in the excitatory actions of δ-opioid agonists in the fetus.

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