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Kappa Opioid Effects on Fetal Behavior: Central Administration of U50,488

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PETROV, E. S., E. I. VARLINSKAYA, S. R. ROBINSON, W. P. SMOTHERMAN, B. R. DE COSTA AND K. C. RICE. *Kappa opioid effects on fetal behavior: Central administration of U50,488*. *PHYSIOL BEHAV* 56(1), 175-182, 1994.—The kappa opioid agonist U50,488 was administered to E21 rat fetuses via intracisternal (IC), intrahemispheric (IH), or intrathecal (IT) injection. The IC administration of U50,488 promoted a threefold increase in motor activity, which was predominated by movements of caudal regions of the body (rearlimbs, body trunk, and tail). The agonist effect was reversed by IC administration of the selective kappa opioid antagonist nor-binaltorphimine. The IH injection of U50,488 had little effect on fetal behavior, but IT injection resulted in pronounced increases in fetal activity, including rearlimb, trunk, and tail movements. The IT administration of nor-binaltorphimine blocked U50,488 effects, whereas IH injection of the antagonist had little influence on fetal behavior. These findings suggest that kappa opioid receptors located in the spinal cord may play a role in the regulation of fetal motor behavior.

U50,488 Nor-binaltorphimine Kappa Opioid Rat fetus

CURRENT interest in the early development of endogenous opioid systems has been fostered by recognition of behavioral effects associated with prenatal or early postnatal exposure to opiate drugs (3,8). Moreover, experiments conducted with fetal and neonatal rodents indicate that opioids may play an important role in the regulation of motor and sensory behavior around the time of birth. In the rat, both the mu and kappa opioid systems can be activated through pharmacological manipulations to bring about changes in fetal behavior. Kappa opioid activity in particular appears to be important in promoting or facilitating changes in motor activity, sensory responsiveness, and the expression of organized action patterns (17). Peripheral administration of selective kappa agonist drugs, such as U50,488 or U69,593, has been found to cause pronounced increases in fetal motor activity and to suppress fetal responsiveness to cutaneous stimulation of the perioral area (13,19). More naturalistic manipulations of the rat fetus also have been shown to promote endogenous kappa opioid activity. Infusion of a small volume of milk into the oral cavity of the fetus, for instance, results in diminished perioral responsiveness (15), elevated levels of rearlimb activity, and expression of the fetal stretch response (12), an action pattern that resembles the behavior of neonatal rats upon milk letdown at the nipple (6). Blockade of opioid receptors with naloxone or selective kappa antagonists eliminates behavioral effects induced by

milk infusion or peripheral administration of kappa opioid drugs (15,16,19).

Although opioid effects on fetal behavior are well documented, little information is available regarding the neural mechanisms or localization of opioid effects within the central nervous system of the fetus. Kappa opioid receptors have been described within both the brain and spinal cord of the rat fetus (2,5), but also are widely distributed in peripheral organs, especially in the gut. It is possible that some of the behavioral effects promoted by U50,488 and similar kappa opioid compounds are the consequence of the drug binding at peripheral receptors, and are not the result of direct changes in neural activity within the fetal CNS. Some inferences about the localization of opioid effects in the fetus have been drawn from studies of opioid interactions with other neurochemical systems, notably the dopamine system. Manipulations of dopamine activity in the fetal rat appear to be mediated by changes in kappa opioid activity, suggesting that kappa receptors may be more closely associated with descending motor circuits or effector systems (13).

To better understand the influence of kappa opioids on behavioral regulation during early development, it will be important to determine the effects of opioid administration on central nervous system function in the fetus in vivo. The principal aim of this study was to administer the kappa opioid agonist drug

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U50,488 directly into the brain or spinal cord of individual rat fetuses and to measure ensuing changes in fetal motor behavior. A selective antagonist of kappa receptors also was administered to verify that the agonist U50,488 exerts its behavioral effects through central and not peripheral action. These experiments were conducted through the use of techniques that permit pharmacological manipulation, sensory stimulation, and behavioral observation of rat fetuses late in gestation (14,20).

GENERAL METHOD

Subjects

A total of 150 rat fetuses derived from 37 pregnancies served as subjects in this study. Fetuses were produced in timed matings of Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). Groups of three adult female rats were housed in plastic breeding cages (36 × 47 × 20 cm) with one male rat during a 4-day breeding period. Vaginal smears were collected daily to date conception, with the first day of visible sperm designated as embryonic day 0 (E0). Pregnant rats were maintained in a 22°C room and exposed to a 12-h light:12-h dark cycle (lights on at 0700 h) until the date of fetal testing (E21). Food and water were available ad lib. All animals were maintained and treated in accordance with guidelines for animal care established by the National Institutes of Health (PHS publication 86-23).

Prenatal Preparation

Fetal subjects were prepared for behavioral testing on day 21 of gestation (E21). Subjects were prepared in the morning (0900–1200 h) because the onset of parturition typically occurs in the afternoon or evening on E21. The pregnant rat was prepared by chemomyelotomy to permit access to fetal subjects. The rat was briefly anesthetized with ether and then received a 70- μ l injection of 100% ethanol into the spinal canal between the first and second lumbar vertebrae. Chemomyelotomy results in irreversible neural blockade in the spinal cord at the low thoracic level and eliminates sensation in the lower part of the body. The prepared rat was placed in a holding device, immersed to chest depth in a warm bath containing buffered isotonic saline maintained at body temperature (37.5°C), and was allowed to recover from the ether anesthetic. The pregnant rat was monitored visually during the period of fetal testing to ensure completeness of the spinal preparation. The uterus was exteriorized into the bath through a midline laparotomy. Individual fetuses were selected as experimental subjects and delivered from the uterus into the saline bath. No attempt was made to selectively assign male or female fetuses to a treatment group; male and female subjects were approximately equally represented within treatment groups. Whenever feasible, one subject from each pregnancy was assigned to each treatment group within an experiment to avoid confounding of treatment effects with litter effects (7). The surrounding embryonic membranes were removed to improve visibility of fetal movements and experimental access to the fetus. Throughout the period of testing, the umbilical cord of fetal subjects remained unobstructed and attached to the placenta, which remained inside the uterus. All fetal subjects exhibited pink coloration, indicating good oxygenation, for the duration of the experiment. After surgical preparation, a 20-min period elapsed before behavioral testing to provide time for the pregnant rat and subject fetuses to accommodate to the bath environment (14).

Central Administration of Kappa Opioid Agonist and Antagonist

Solutions containing the selective kappa opioid agonist U50,488 (U50), the selective kappa antagonist nor-binaltorphim-

ine diHCl (BNI), or the isotonic saline vehicle (SAL) were injected into the central nervous system of individual fetal subjects. The U50 was administered in a standard dosage of 5.5 μ g per fetus (1.0 mg/kg in an average E21 rat fetus); BNI was administered at dosages of 10 or 20 μ g per fetus. Dosages were selected to centrally administer the same amount of each drug that has been found to produce behavioral effects when administered by IP injection (19). Three different sites of injection were used: intracisternal (IC), intrahemispheric (IH), and intrathecal (IT) (20). In all central injections, a 30-ga hypodermic needle attached to a length of transparent polyethylene tubing (PE-10) was inserted under visual guidance into the target site. For IC injection the needle was inserted into the foramen magnum between the occipital bone and the first cervical vertebra, with the tip placed in the cisterna magna. The IH injections targeted the lateral ventricle of the cerebral hemisphere and involved insertion of the needle to a depth of 1.5 mm at a point located 1.0 mm anterior and 1.0 mm lateral from bregma. These coordinates correspond to the location of the lateral ventricle in the brain of the term rat fetus (E21–P0) (9). In each experiment, half of the subjects received the IH injection into the left hemisphere, half into the right. For IT injection, the needle was inserted into the upper thoracic spinal canal at the T3–T5 level. Successful placement of the needle into the target site in IC and IT injections was immediately confirmed by the appearance of a small volume of cerebrospinal fluid in the tubing. Drug and vehicle solutions were injected slowly (8–10-s pulse) in a volume of 1.0 μ l with a 0.2-ml micrometer syringe. Solutions were prepared daily from frozen aliquots.

Behavioral Observation

In all experiments, the observer was unaware of the specific drug treatment administered to any given subject. Effects of drug treatments were assessed by observing changes in fetal motor behavior. The observer noted each instance of fetal movement and called the appropriate behavioral category to an assistant, who recorded the data with a microcomputer-based event recorder. Six categories of fetal movement were distinguished by reference to the region of the fetal body that was involved in the movement: forelimb (movement involving one or both forelimbs), rearlimb (movement involving one or both rearlimbs), trunk (flexion or extension of the body trunk), tail (extension of the tail away from the ventrum), head (change in head position relative to the body trunk), and mouth (opening and closing of the mouth). These movement categories differ slightly from previous studies of fetal behavior in our laboratory; in particular, pilot experiments indicated the need for finer distinctions among movements of the caudal half of the body (trunk, rearlimb, and tail movements). The sum of movement events in these six categories was used as a measure of overall fetal activity. This method of recording and quantifying fetal motor behavior preserves information about the frequency and timing of fetal movements and is highly consistent between observation sessions (reliability > 0.90).

A seventh category of fetal behavior, facial wiping, was distinguished to assess changes in fetal responsiveness to a perioral cutaneous stimulus. Facial wiping is a coordinated action pattern involving placement of one or both forepaws against the side of the head and movement of the forepaw(s) in a rostral direction, with contact sliding across the face. Facial wiping is uncommonly expressed by fetal rats in the absence of explicit sensory stimulation (11), but is reliably evoked by sensory stimulation of the perioral area (15). To measure fetal responsiveness, a perioral probe was administered at the end of the observation session by

applying a stiff bristle (3.7 g force) near the corner of the mouth twice in rapid succession. The presence or absence of a wiping response following application of the probe was scored for each fetal subject.

Data Analysis

Each category of fetal behavior was summarized by 1-min interval over the observation session. An overall analysis comprising all treatment groups involved a multifactor analysis of variance (ANOVA), with movement counts during successive minutes of observation treated as a repeated measure. Where significant main effects of drug treatment were evident, pairwise comparisons of group means were conducted by the method of Scheffé. The number of fetal subjects that exhibited a facial wiping response to the perioral probe was compared among treatment groups by nonparametric chi-square test.

EXPERIMENT 1: CENTRAL ADMINISTRATION OF U50

Peripheral administration of U50 has been shown to influence motor behavior and sensory responsiveness in the E21 rat fetus (1,13,19). Intraperitoneal injection of U50 results in elevated motor activity, changes in body posture, and reduced responsiveness to a perioral cutaneous stimulus. The objective of Experiment 1 was to determine the behavioral effects of U50 when administered into the brain or spinal cord of the E21 rat fetus.

A total of 80 fetuses from 16 pregnancies served as subjects in Experiment 1. Fetuses received a drug injection of the kappa agonist (U50; 5.5 μ g per fetus), or a control injection of the isotonic saline vehicle (SAL). The U50 or SAL was injected at one of three sites (IC, IH, IT). The combination of solution and injection site yielded six treatment groups in Experiment 1: U50-IC ($n = 16$ fetuses), U50-IH ($n = 17$), U50-IT ($n = 17$), SAL-IC ($n = 10$), SAL-IH ($n = 10$), and SAL-IT ($n = 10$). Immediately following injection, the behavior of fetal subjects was recorded in an 8-min observation session.

Results and Discussion

Overall fetal activity was compared in a two-factor repeated-measures ANOVA (six drug treatments \times 8 min), which indicated the significant main effect of drug treatment, $F(5, 74) = 24.3, p < 0.001$ (Fig. 1). Post hoc comparison of average motor activity in the six groups indicated that fetal movements were significantly elevated in the U50-IC and U50-IT groups relative to the U50-IH group, and that all fetuses injected with U50 were more active than fetuses in the three SAL control groups (Scheffé, p -values < 0.05). The increase in fetal activity in the U50-IC and U50-IT groups amounted to 250% of levels exhibited by SAL-treated subjects. The absence of main or interaction effects involving time suggested that elevated levels of fetal movement remained stable over the 8-min observation session.

Similar ANOVAs were calculated to examine effects of the kappa opioid agonist on specific categories of fetal movement (Fig. 2). The main effect of drug treatment was found for movements of forelimbs, $F(5, 74) = 3.8, p < 0.005$. Post hoc tests revealed that forelimb movements were modestly elevated in the U50-IC group relative to SAL-injected subjects ($p < 0.05$). The main effect of treatment also was found for rearlimbs, $F(5, 74) = 38.8, p < 0.001$, with a pronounced increase in rearlimb movements in the IC and IT groups compared to all SAL groups (all $p < 0.05$), but no change in rearlimb movements when U50 was administered IH. However, IH injection of U50 resulted in a significant increase in head movements, as indicated by the main effect of treatment, $F(5, 74) = 5.2, p < 0.001$; head activity was

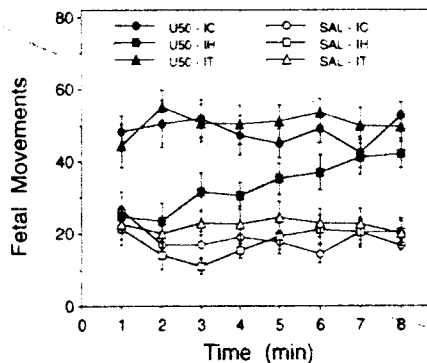


FIG. 1. Overall motor activity of E21 rat fetuses following intracisternal (IC), intrahemispheric (IH), or intrathecal (IT) injection of U50 or the isotonic saline vehicle (SAL) in Experiment 1. Points depict the mean number of fetal movements per minute; vertical lines show SEM.

greater in the U50-IH group than the SAL-IH group ($p < 0.05$). The main effect of treatment indicated a modest effect of U50 on mouth movements, $F(5, 74) = 3.4, p < 0.01$, with more mouthing expressed by fetuses in the U50-IT group than SAL-injected controls ($p < 0.05$).

Central administration of U50 also influenced movements of the body trunk and tail. The main effect of treatment was found for trunk movements, $F(5, 74) = 11.2, p < 0.001$. Trunk activity was greater in the U50-IT group compared to the U50-IH and three SAL groups (all $p < 0.05$), but did not differ from the U50-IC group. Qualitatively, U50 appeared to promote dorsiflexion of the trunk, resulting in an overall lengthening of body posture. Such body extension was observed in all 17 subjects in the U50-IT group, 15 of 16 subjects (94%) in the U50-IC group, 11 of 17 subjects (65%) in the U50-IH group, and 13 of 30 subjects (43%) in the three SAL groups. The presence or absence of body extension was found to vary significantly among these groups, $\chi^2(3) = 22.0, p < 0.001$ ($n = 80$). Pairwise comparisons of each U50 group with SAL controls indicated a significantly elevated incidence of body extension following IT injection, $\chi^2(1) = 12.7, p < 0.001$ ($n = 47$), and IC injection, $\chi^2(1) = 9.1, p < 0.01$ ($n = 46$). Body extension in the U50-IH group did not differ from the three SAL groups ($p > 0.25$).

The significant interaction of drug treatment and time was evident for tail movements, $F(35, 518) = 1.7, p < 0.01$. To simplify this interaction, one-way ANOVAs were conducted to assess simple main effects of treatment for each minute of observation. These comparisons indicated significant differences at each minute (all $p < 0.001$). An increase in tail movements was evident in the U50-IT group relative to all other groups during minutes 1–3, and relative to all groups except U50-IC during minutes 4–8. Tail movements also were elevated in the U50-IC group compared to the U50-IH and three SAL groups during minutes 2–8. The IH injection of U50 did not increase tail movements at any time.

The results of Experiment 1 demonstrate that central administration of U50 significantly alters the expression of fetal motor behavior. Effects of U50 are apparent in nearly all categories of fetal movement, but are most evident when U50 is administered into the cisterna magna or spinal cord. The IC or IT injection of U50 causes modest changes in movements involving rostral parts of the body (mouth, forelimbs) and pronounced changes in movements involving caudal parts (rearlimbs, trunk, and tail). The sites of injection that are most effective, considered with the categories of fetal movement most affected, suggest that U50 influences

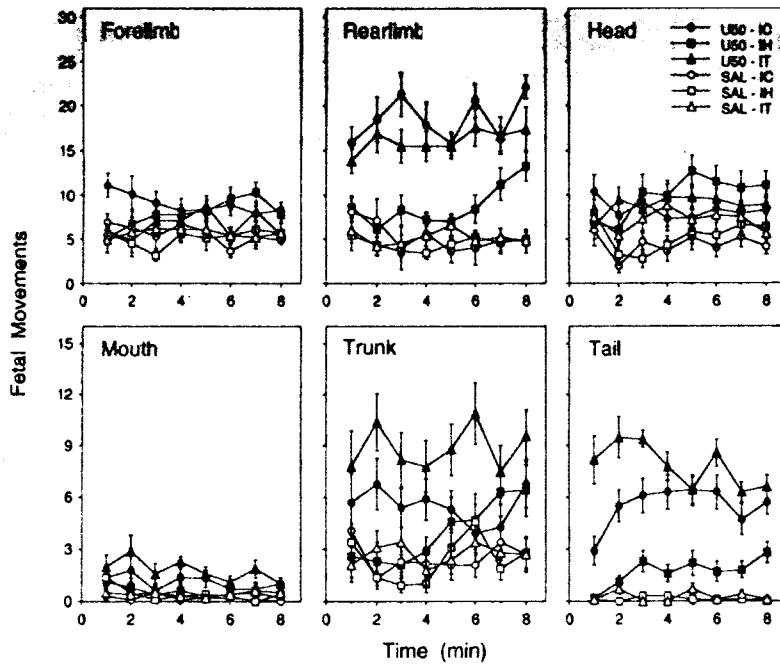


FIG. 2. Changes in six categories of fetal behavior following IC, IH, or IT injection of U50 or SAL in Experiment 1. Data for forelimb, rearlimb, head, mouth, trunk, and tail movements are shown.

fetal behavior through its interaction with opioid receptors in caudal elements of the central nervous system, such as the fetal spinal cord and/or brain stem. Close inspection of the behavioral effects of U50 administered into the cerebral hemisphere supports this interpretation. Within the IH group, there was little evidence for behavioral effects during the first 4 min of observation, but movements of rearlimbs, trunk, and tail increased during the second half of the observation session. Time-dependent changes in overall fetal activity were indicated by a one-way ANOVA, $F(7, 112) = 3.6, p < 0.01$. This finding is consistent with the interpretation that IH injection of U50 produces a delayed effect on fetal activity, which may be attributed to spread of the drug from the site of injection.

EXPERIMENT 2: REVERSAL OF U50 EFFECTS WITH BNI

Experiment 1 confirmed that central administration of a kappa opioid agonist results in a significant increase in fetal motor activity, principally including rearlimb, trunk, and tail movements. Previous experiments have shown that blockade of kappa receptors by peripheral administration of the antagonist BNI is effective in blocking the behavioral effects promoted by endogenous kappa opioid activity (15,18) and exogenous treatment with opioid agonists (19). The objective of Experiment 2 was to confirm that the behavioral effects produced by central administration of U50 are mediated by the kappa opioid receptor. Specifically, U50 was administered by IC injection to all fetal subjects. After a period of time sufficient for behavioral effects to be expressed, a second IC injection involving BNI was administered in an attempt to reverse changes in fetal motor behavior induced by the kappa opioid agonist.

A total of 30 rat fetuses from 10 pregnancies served as subjects in Experiment 2. All subject fetuses received an initial IC injection consisting of $5.5 \mu\text{g}$ of U50 and fetal behavior was observed for 5 min. A second IC injection then was delivered

consisting of the saline vehicle (SAL; $n = 10$), $10.0 \mu\text{g}$ ($n = 10$), or $20.0 \mu\text{g}$ ($n = 10$) of BNI. After a delay of 2 min to allow time for the second injection, fetal behavior was recorded for an additional 5 min. At the conclusion of this observation session, each fetal subject was presented with the perioral probe to assess changes in cutaneous responsiveness.

Results and Discussion

An initial analysis examined overall fetal activity during the first 5 min of the observation session to confirm the effect of U50 on fetal behavior. This two-factor repeated-measures ANOVA indicated the significant main effect of time, $F(4, 108) = 8.7, p < 0.001$. After administration of U50, subjects in all groups exhibited a high level of motor activity (Fig. 3), which was most pronounced during minutes 2–5. Fetal activity in these groups

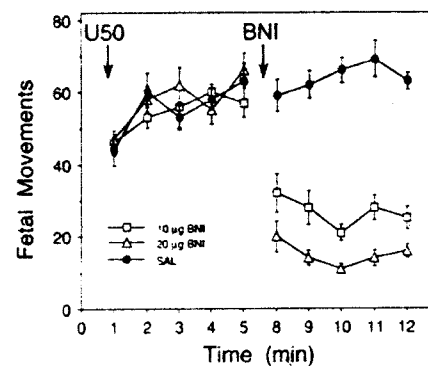


FIG. 3. Overall motor activity in Experiment 2. Fetuses received an IC injection of U50 at the beginning of the session. Five minutes later, subjects received an IC injection of one of two doses of the kappa opioid antagonist BNI (10.0 or $20.0 \mu\text{g}$) or SAL.

was comparable to that observed in the U50-IC group in Experiment 1, and was significantly elevated relative to the 10–20 movements per minute that are consistently expressed by unmanipulated fetal subjects (e.g., SAL-injected subjects in Experiment 1). The absence of main or interaction effects involving the drug treatment factor confirmed that no differences were evident among the three groups during the first 5 min of the session.

To assess effects of IC administration of the kappa opioid antagonist, overall fetal activity during the last 5 min of the session was compared in a two-factor repeated-measures ANOVA (three drug treatment groups \times 5 min), which indicated the significant main effect of drug treatment, $F(2, 27) = 142.0$, $p < 0.001$. Subjects in the SAL group continued to exhibit high levels of motor activity through the last 5 min of the session. However, decreases in overall activity were evident in both the 10.0 and 20.0 μg BNI groups (Fig. 3). Post hoc comparison of group means by the method of Scheffé revealed that overall activity was reduced in the 10.0 μg BNI group relative to SAL controls, and was further reduced in the 20.0 μg BNI group (all $p < 0.05$). Qualitative comparison of the level of fetal activity in these two BNI groups with SAL-injected subjects from Experiment 1 suggested that fetal movements remained modestly elevated in the 10 μg BNI group, but were completely reversed by IC injection of 20 μg of BNI.

Additional two-factor repeated-measures ANOVAs were conducted to assess the effects of BNI on individual movement categories during the last 5 min of the observation session (Fig. 4). Main effects of drug treatment were found for movements of forelimbs, $F(2, 27) = 10.1$, $p < 0.001$, rearlimbs, $F(2, 27) = 413.5$, $p < 0.001$, head, $F(2, 27) = 19.5$, $p < 0.001$, mouth, $F(2, 27) = 5.8$, $p < 0.01$, tail, $F(2, 27) = 73.5$, $p < 0.001$, and trunk, $F(2, 27) = 17.6$, $p < 0.001$. Post hoc comparison of group means indicated that forelimb movements were significantly greater in the 10 μg BNI group than in the 20 μg BNI group ($p < 0.05$). The patterns of group differences in the remaining five categories

were identical: the 10 μg and 20 μg dosages of BNI were equally effective in reducing fetal movements relative to SAL-injected controls (all $p < 0.05$).

Fetuses in the three groups also differed in their tendency to express a facial wiping response to the perioral probe at the end of the session. Only one of 10 subjects (10%) that received IC SAL after U50 injection exhibited a wiping response to the probe, compared to five of 10 subjects (50%) in the 10 μg BNI group and nine of 10 subjects (90%) in the 20 μg BNI group. The number of subjects exhibiting a wiping response differed significantly among the three groups, $\chi^2(2) = 12.8$, $p < 0.005$ ($n = 30$), suggesting that the low dose of BNI partially reversed and the higher dose completely reversed the effect of U50 on perioral cutaneous responsiveness.

The findings of Experiment 2 are consistent with the interpretation that IC injection of BNI was effective in reversing the behavioral effects produced by IC injection of U50 in the fetal rat. In particular, the most distinctive effects of U50, namely the pronounced increases in rearlimb, tail, and trunk movements, were completely reversed by IC administration of BNI. There is no evidence that peripheral or central administration of BNI suppresses fetal activity. In the absence of other sensory or pharmacological treatments, BNI has no effect on behavior on E20 and promotes a modest increase in motor activity on E21 (15,18). These data further support the interpretation that BNI reverses the effects of U50 through its action at the kappa opioid receptor. This experiment also confirmed that central administration of U50 reduces fetal responsiveness to a cutaneous stimulus applied to the perioral region: only one subject in the SAL group exhibited a facial wiping response to the perioral probe. The IC injection of BNI reversed the effect of the kappa agonist on perioral responsiveness. Because previous studies have consistently reported that 80–90% of fetal subjects will exhibit a wiping response to the probe in the absence of pharmacological manipulation (15), it would seem that only the 20 μg dose of BNI was

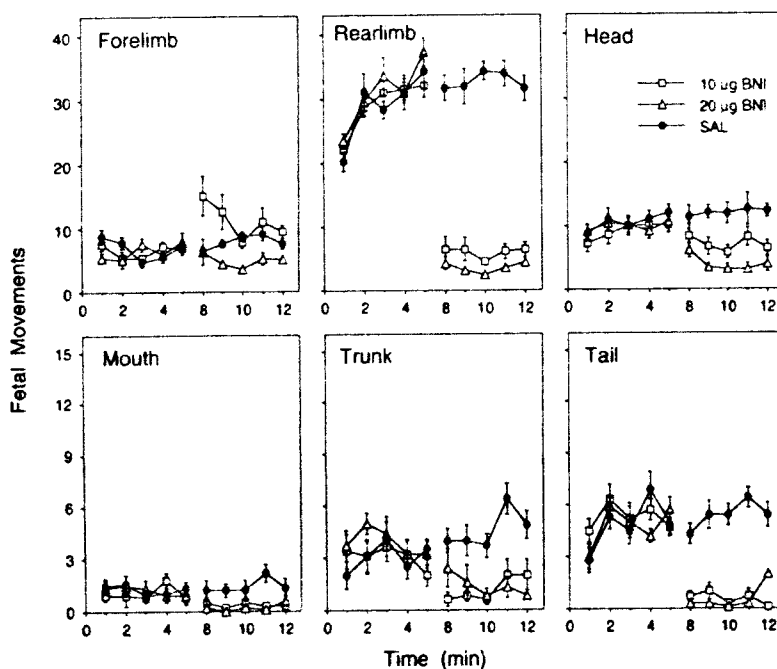


FIG. 4. Changes in six categories of fetal behavior after IC injection of U50 followed by IC injection of BNI or SAL in Experiment 2. Data for forelimb, rearlimb, head, mouth, trunk, and tail movements are shown.

completely effective in reversing the agonist-induced reduction in fetal responsiveness.

EXPERIMENT 3: BLOCKADE OF U50 EFFECTS WITH BNI ADMINISTERED IH OR IT

The results of Experiment 1 confirm that distinctive behavioral effects are produced by administration of U50 into the spinal cord of the E21 rat fetus, but comparable effects are not observed following IH injection of U50. Moreover, the categories of behavior most influenced by U50 encompass caudal regions of the body, including rearlimbs, tail, and trunk. A parsimonious interpretation of these results is that kappa opioid receptors associated with motor control pathways in the brain stem or spinal cord are responsible for mediating the behavioral effects of central U50 administration. If U50 acts in caudal elements of the fetal central nervous system, then it should be possible to reverse the effects of IC injection of U50 by IT administration of BNI. However, because IH injection of U50 is without behavioral effect, IH administration of the kappa antagonist should not influence changes in fetal behavior induced by U50 injected IC. These predictions were tested in Experiment 3, in which E21 fetuses received an IC injection of U50 followed by IT or IH injection of BNI or the saline vehicle.

A total of 40 E21 fetuses from 11 pregnancies were used as subjects in Experiment 3. Fetuses received an initial IC injection consisting of 5.5 μg of U50. Immediately after this initial injection, subjects were observed for 5 min, then received a second injection of either saline or 20.0 μg BNI administered either IH or IT. After a 2-min delay to accommodate the second injection, the behavior of all fetal subjects was recorded for an additional 5 min. At the conclusion of the session, all subjects were tested with the perioral probe to assess changes in cutaneous responsiveness. The combination of solution and injection site of the kappa antagonist yielded four drug treatment groups: BNI-IH, BNI-IT, SAL-IH, and SAL-IT ($n = 10$ per group). It was expected that the kappa antagonist would reverse the effects of the IC injection of U50 on fetal behavior when administered IT, but not when administered IH.

Results and Discussion

As in Experiment 2, an initial two-factor repeated-measures ANOVA comparing overall fetal activity during the first 5 min of the observation session was used to measure the effectiveness of the IC injection of U50. This analysis indicated the significant main effect of time, $F(4, 144) = 35.2, p < 0.001$. Fetal activity increased during the first 2 min of the observation session, reaching a stable plateau during minutes 3–5. No main or interaction effects involving the drug treatment factor were evident, confirming that U50 promoted increases in fetal motor activity in all four groups.

To assess effects of IH or IT administration of the kappa opioid antagonist, overall fetal activity during the last 5 min of the session was compared in a two-factor repeated-measures ANOVA (four drug treatment groups \times 5 min). This analysis indicated the significant main effect of drug treatment, $F(3, 36) = 77.8, p < 0.001$ (Fig. 5). Subjects in both SAL groups continued to exhibit high levels of motor activity through the end of the session, as did subjects receiving an IH injection of BNI. However, fetuses that received an IT injection of BNI showed an immediate and marked reduction in overall activity. Post hoc comparison of group means confirmed that motor activity was significantly reduced in the BNI-IT group relative to all three other groups (all $p < 0.05$). The level of activity expressed by fetuses in the BNI-IT group were comparable to the SAL groups

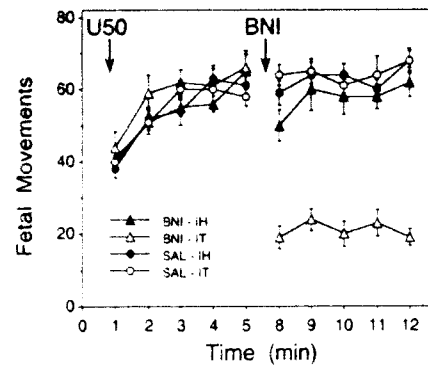


FIG. 5. Overall motor activity in Experiment 3. Fetuses received an IC injection of U50 at the beginning of the session, followed 5 min later by IH or IT injection of BNI (20 μg) or SAL.

in Experiment 1, and the 20 μg BNI group in Experiment 2. Fetuses in the BNI-IH group did not differ in overall motor activity from the two SAL injection groups.

Additional two-factor repeated-measures ANOVAs were conducted to assess the effects of drug treatment on individual movement categories during the last 5 min of the observation session (Fig. 6). No main or interaction effects were found for forelimb movements ($p > 0.10$). But main effects of drug treatment were found for movements of rearlimbs, $F(3, 36) = 109.0, p < 0.001$, head, $F(3, 36) = 12.5, p < 0.001$, mouth, $F(3, 36) = 5.3, p < 0.005$, tail, $F(3, 36) = 55.5, p < 0.001$, and trunk, $F(3, 36) = 6.5, p < 0.005$. Rear limb and head movements were significantly reduced in the BNI-IT group relative to all other groups (all $p < 0.05$), whereas the BNI-IH group did not differ from SAL controls. Tail movements were significantly reduced in the BNI-IH group compared to both SAL groups, and was further reduced in the BNI-IT group (all $p < 0.05$). Mouthing and trunk movements were expressed less frequently by BNI-IT subjects than by SAL-IT subjects (all $p < 0.05$), but the BNI-IT and BNI-IH groups did not differ. Qualitative differences in the effect of the two BNI injections on trunk movements were especially evident: although body extension was observed in nine of 10 subjects (90%) in the BNI-IH group, seven of 10 subjects (70%) in the SAL-IH group, and eight of 10 subjects (80%) in the SAL-IT group, body extension was not expressed by fetuses after IT injection of BNI (zero of 10 subjects). The presence or absence of body extension varied significantly among these groups, $\chi^2(3) = 20.8, p < 0.001$ ($n = 40$). Pairwise comparisons indicated no difference in the incidence of body extension in the two IH groups ($p > 0.50$), but body extension was significantly reduced in the BNI-IT group relative to the SAL-IT group, $\chi^2(1) = 10.2, p < 0.01$ ($n = 20$).

Fetuses also differed in their tendency to express a facial wiping response to the perioral probe at the end of the session. None of the 10 subjects (0%) that received BNI-IH after U50 injection exhibited a wiping response to the probe. Reductions in responsiveness were evident in fetuses injected with SAL-IT (one of nine subjects; 10%) or SAL-IH (one of nine subjects; 10%). In contrast, all 10 subjects (100%) in the BNI-IT group exhibited a wiping response to the probe. The number of subjects exhibiting a wiping response differed significantly among the four groups, $\chi^2(3) = 31.4, p < 0.001$ ($n = 40$). This finding suggested that BNI injected into the spinal cord was effective in reversing the effect of U50 on perioral cutaneous responsiveness.

CONCLUSIONS

The three experiments of this study demonstrate that a kappa opioid agonist and antagonist can exert pronounced effects on

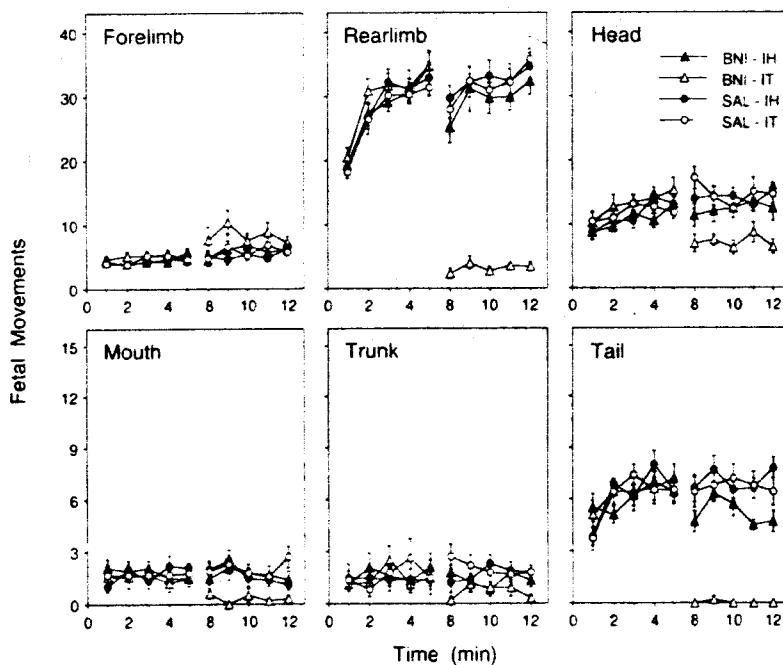


FIG. 6. Changes in six categories of fetal behavior after IC injection of U50 followed by IH or IT injection of BNI or SAL in Experiment 3. Data for forelimb, rearlimb, head, mouth, trunk, and tail movements are shown.

motor behavior and sensory responsiveness when administered into the central nervous system of the E21 rat fetus. In Experiment 1, IC or IT injection of U50,488, a selective kappa agonist, resulted in a threefold increase in fetal activity, and IH injection promoted a more modest increase in motor behavior. Although most categories of fetal movement were affected, the kappa agonist appeared to preferentially promote movements in caudal regions of the body, including the rearlimbs, body trunk, and tail. These findings accord well with earlier studies of fetal behavior, in which kappa opioid agonists (U50,488 or U69,593) were administered to fetuses by IP injection. Peripherally administered kappa agonists promote rearlimb activity (13,19), postural extension of the body trunk (1), and expression of the fetal stretch response upon intraoral infusion of milk or other fluids (1,16). The stretch response in particular appears to depend upon changes in caudal motor patterns: stretching is contingent on an antecedent period of elevated rearlimb movements and involves the coordination of trunk dorsiflexion, pelvic tilting, and caudal extension of the rearlimbs (12). Similar behavioral elements—rearlimb activity, body extension, and tail movements—are facilitated by central administration of U50,488, especially by IT injection.

The results of Experiments 2 and 3 confirm that the behavioral effects produced by U50,488 are mediated by the kappa class of opioid receptors. The IC injection of nor-binaltorphimine, a selective kappa antagonist, reversed changes in motor activity induced by U50,488. The IT injection of the kappa antagonist also was very effective in reversing agonist-induced changes in motor behavior, but IH injection of the antagonist was not. The clear implication of the efficacy of IC and IT injections and the expression of caudal motor patterns is that U50,488 and nor-binaltorphimine interact with kappa opioid receptors associated with neurons in the spinal cord or brain stem of the fetus. This is consistent with other studies that kappa opioid drugs are most effective when administered into the spinal cord of developing

rats (4). Kappa receptors in the spinal cord appear to exhibit a bimodal distribution in relative concentration during development, with peaks during the late prenatal period and end of the first postnatal week (2). The distribution and early abundance of kappa receptors are consistent with the hypothesis that U50,488 engages spinal opioid receptors to bring about distinctive changes in fetal motor behavior (13).

Central manipulations of kappa opioid activity also were found to influence fetal responsiveness to perioral stimulation. Unmanipulated fetal subjects, or fetuses that receive control injections of saline, typically exhibit a facial wiping response to a cutaneous stimulus applied to the perioral area. The wiping response can be suppressed by sensory-induced changes in kappa opioid activity (15), or by pharmacological activation of the kappa opioid system through peripheral (19) or central administration of U50,488 (Experiments 2 and 3, this study). The IH injection of nor-binaltorphimine had no apparent influence on fetal responsiveness to the perioral stimulus, but both IC and IT injections of the antagonist completely reversed the effects of U50,488 on facial wiping. Although less intuitive than the effects of kappa opioid activity on fetal motor activity, kappa-induced changes in fetal responsiveness to perioral stimulation also may be due to opioid receptors located in caudal portions of the fetal central nervous system.

Central administration of the kappa agonist U50,488 promotes changes in fetal behavior that closely resemble the behavioral effects evoked by infusion of milk. Presentation of milk into the mouth of the fetus results in elevated mouthing and rearlimb activity (12), an increase in body extension (1), and reduced responsiveness to a perioral cutaneous stimulus (15). Milk-induced changes in fetal behavior can be blocked by pretreatment with nor-binaltorphimine, indicating kappa involvement. The effectiveness of IT administration of U50,488 or nor-binaltorphimine to engage these patterns of behavior, as documented in the present study, suggests that milk modifies fetal behavior

by promoting activity at kappa opioid receptors located in caudal portions of the fetal CNS. The pattern of interaction between the kappa opioid system and the dopamine system provides corroborative evidence for this hypothesis. Drug-induced changes in dopamine activity appear to exert behavioral effects in the term rat fetus through subsequent activity at kappa receptors (10,13). The absence of dopamine activity in caudal elements of the fetal CNS implies the involvement of an intermediate system or sys-

tems that complete the chain between orosensory processing of milk and kappa opioid-mediated changes in fetal behavior.

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