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Opioid Receptor Blockade Throughout Prenatal Life Confers Long-Term Insensitivity to Morphine and Alters μ Opioid Receptors

IAN S. ZAGON,* STEVEN W. TOBIAS,† STACI D. HYTREK† AND PATRICIA J. MCLAUGHLIN*

*Departments of *Neuroscience and Anatomy and †Comparative Medicine, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033*

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ZAGON, I. S., S. W. TOBIAS, S. D. HYTREK AND P. J. MCLAUGHLIN. *Opioid receptor blockade throughout prenatal life confers long-term insensitivity to morphine and alters μ opioid receptors.* PHARMACOL BIOCHEM BEHAV **59**(1) 201–207, 1998.—The influence of maternal opioid receptor blockade (50 mg/kg naltrexone, NTX) or saline (controls) throughout pregnancy on nociception and brain opioid receptor characteristics of rat offspring were examined; all animals were crossfostered to untreated mothers at birth. At 21 and 30 days, NTX-exposed pups weighed 8.2–24.3% more than controls, but both NTX and control groups were of similar body weights at 48, 60, and 80 days. Rats in the NTX and control groups displayed comparable baseline reactions to the hotplate. Morphine challenge tests and nociceptive measures revealed that NTX-subjected offspring examined at 21, 30, 48, and 60 days did not react to dosages that invoked 42–132% decreases from baseline levels in controls. Animals exposed prenatally to NTX were analgesic when injected with the opioid butorphanol or the nonopioid xylazine. The binding affinity (K_d) and capacity (B_{max}) of δ and κ opioid receptors were similar in NTX and control groups at 21 and 80 days. However, the B_{max}, but not the K_d, of μ opioid receptors was subnormal in NTX offspring by about 20% in contrast to control rats at 21 and 80 days. The results imply that the interactions of some endogenous opioids with opioid receptors during development are determinants of certain aspects of pain sensitivity as well as the density of particular opioid receptors in the postnatal period. © 1998 Elsevier Science Inc.

Opioids Naltrexone Development Prenatal Postnatal Maternal Analgesia Nociception Opioid receptors Pain

ENDOGENOUS opioids interact with opioid receptors to form the endogenous opioid systems. These native opioid peptides serve in a variety of capacities, including the modulation of neurotransmission (1) and growth (2). Opioids and opioid receptors are present, and function, in the early life of humans and animals [e.g., (2,5,6,16,19,25)].

If endogenous opioid systems are involved with such physiological processes as neurotransmission, and both opioids and opioid receptors enjoy a developmental period that extends from prenatal to postnatal life, then the hypothesis may be constructed that opioid function in the postnatal period is dependent on the proper ontogeny of endogenous opioid systems. As a corollary, disruption of the embryology of endogenous opioids and opioid receptors, particularly the requirement for their interaction during development, may lead to irregularities in opioid function.

Recent investigations (12,13) have shown that opioid receptor blockade throughout the entire course of prenatal life has a profound influence on postnatal events. McLaughlin and colleagues (12) have found that continuous maternal opioid receptor blockade throughout gestation does not interfere with the course of pregnancy or influence maternal well-being. However, body weights, crown–rump lengths, and wet and dry weights of the brain, heart, kidney, liver, and skeletal muscle in neonates delivered by opioid antagonist-treated mothers were substantially elevated compared to control pups. Moreover, at weaning (day 21), the body weights of these opioid antagonist-exposed rats (crossfostered at birth to untreated lactating females) were 36% greater than controls, and organ weights increased from 18 to 246% in this group. In a subsequent report (13), these rats displayed an accelera-

Requests for reprints should be addressed to Dr. Ian S. Zagon, Department of Neuroscience and Anatomy, The M. S. Hershey Medical Center, 500 University Drive, Hershey, PA 17033–0850.

tion in the ontogeny of physical characteristics, spontaneous motor behavior, and reflexive abilities compared to control offspring.

The present study was conducted to explore the ramifications of completely blocking the interaction of endogenous opioids and opioid receptors during prenatal life on modalities related to opioid receptors. In particular, because opioids are involved with pain, we examined the response of animals born to mothers exposed to an opioid antagonist throughout pregnancy with regard to nociceptive behavior in postnatal life. These data were correlated with information about the binding affinity (K_d) and binding capacity (B_{max}) of μ, δ, and κ opioid receptors in the brain.

METHOD

Animals

Nulliparous female (195–230 g) and male (250–300 g) Sprague–Dawley rats (Charles River Labs, Wilmington, MA) were used in this study. Animals were housed in an environment of 21 ± 0.5°C with a relative humidity of 50 ± 10%. The room had a complete exchange of air 15–18 times per hour and a 12 L:12 D cycle with no twilight; water and Harlan Teklab Purina 8604 Rodent Chow were continuously available.

Animals were mated (one male to two females) and the presence of sperm indicated pregnancy (= day 1 of gestation); all animals were maintained in stainless steel, wire-bottom cages except where noted. Three days prior to parturition, the pregnant females were separated and placed individually into solid-bottom cages to deliver their pups.

Drug Injections

On day 1 of pregnancy, animals were randomly assigned to two groups, and received daily intraperitoneal (IP) injections of either 50 mg/kg NTX (Sigma, St. Louis, MO) or an equivalent volume (0.2 ml) of saline (control). Pregnant rats were weighed daily and the dose of NTX adjusted. All injections were given between 0900 and 0930 h. NTX was prepared weekly and stored at 4°C.

Body Weights

At birth, litters were culled to 10 pups per mother, with an equal number of males and females. All offspring (both NTX and controls) were crossfostered to nontreated lactating females. Pups were weighed at birth and left untreated with foster mothers until weaning (postnatal day 21). Rats were weighed on postnatal days 21, 30, 48, 60, and 80, and weights recorded for separate sexes. At day 30, male and female rats were placed into separate cages. At least 10 animals/sex/treatment group, randomly selected from three or more litters/treatment, were examined.

Nociceptive Tests

On postnatal days 21, 30, 48, and 60, NTX and control pups were tested for latency of response (i.e., licking of paws or rapid removal of hindpaws) on a hotplate apparatus (55°C; Analgesia Meter, Technilabs Instruments Inc., Pequannock, NJ). A baseline latency was recorded for each animal; 10 rats (representative of both sexes and randomly selected from three or more litters) were tested at each age. Within 15 min, morphine sulfate was injected intraperitoneally and 30 min later, rats were retested on the hotplate. The dosage of morphine was 5 mg/kg at 21 days of age, 10 mg/kg on days 30 and 48, and 12.5 mg/kg on postnatal day 60. These dosages were selected based on preliminary dose–response studies in controls (Tobias et al., unpublished observations) showing that they invoked a fairly comparable antinociceptive response across age groups in control subjects.

A second group of rats prenatally exposed to NTX or saline was allowed to grow undisturbed until postnatal day 57. At that time, animals were divided randomly into three groups and each tested on the hot plate. Within 15 min of establishing their baseline latencies, rats were injected with either 12.5 mg/kg morphine (intraperitoneally), 23 mg/kg of butorphanol (intramuscularly), or 10 mg/kg of xylazine (intraperitoneally); these drug dosages were selected based on preliminary tests and values obtained from the literature (23). Rats were retested on the hotplate 30 min following drug injection. Groups of rats were rotated such that every 3 days (postnatal days 60 and 63) each animal received a different analgesic drug. Twenty-one animals exposed prenatally to NTX and 24 rats exposed prenatally to saline, with representation of males and females and randomly selected from 10 litters treatment, were tested in this series of experiments.

Receptor Binding

Receptor binding studies were conducted at 10, 21, and 80 days of age. At 10 and 21 days, each of two or more assays consisted of six rats (an equal number of randomly selected males and females representative of three litters) from each group of animals exposed prenatally to either NTX or saline were anesthetized with pentobarbitol (30 mg/kg) and killed by rapid decapitation; samples of males and females of each group were combined. At 80 days of age, 15 male and 15 female rats were randomly selected from 10 litters/group of animals exposed prenatally to either NTX or saline, killed, and brains collected for receptor binding analyses. Each brain was analyzed independently and represented one assay, permitting evaluation of males and females.

Brains were removed, weighed, and quickly frozen in liquid nitrogen. Whole brains were homogenized (polytron, setting 6, 2 × 10 s) in a 1:20 (wt/vol) solution of cold 50 mM Tris-HCl buffer with 0.1 mg/ml bacitracin, 1 μg/ml leupeptin, 60 nM thiorphan, 1 mM EGTA, and 0.6 μg/ml PMSF, pH 7.4, at 4°C; this buffer is Tris/all. Homogenates were centrifuged (39,000 × g) for 20 min to obtain a P0 pellet.

Protein homogenates (400–800 μg/ml) were incubated at 22°C for 20 min to remove endogenous opioid peptides. Duplicate tubes of protein at a final volume of 1 ml were incubated for 60 min at 22°C with 50 μl of [^{3}H]-DAMGO, [^{3}H]-DPDPE, or [^{3}H]-U69,593. Saturation assays were conducted using a variety of concentrations of radiolabeled ligand ranging from 0.2 to 10 nM. Incubation was terminated by rapid filtration through Whatman GF/B filters under vacuum pressure using a Brandel Cell Harvester. Filters were rinsed three times with 5 ml volumes of ice-cold 50 mM Tris buffer, dried at 60°C for 1 h, and counted by liquid scintillation spectrometry (Beckman LS-2800). Nonspecific binding was determined in the presence of 100 nM of DAMGO, DPDPE, or U69,593.

Protein concentrations were determined by the BioRad method with gamma globulin as a standard.

Receptor binding data were analyzed with the Lundon I (Saturation Isotherm Binding Analysis) computer program (Lundon Software, Cleveland, OH) and GraphPad Prism (Prism Software, San Diego, CA). Both programs utilized nonlinear least-squares regression. Binding isotherms and Scatchard plots were computed directly by the software.

Chemicals

All radioligands were purchased from DuPont-New England Nuclear Research Products (Boston, MA). [^{3}H]-[D-Ala^2, MePhe^4, Gly ol^5] (DAMGO), [^{3}H]-[D-Pen^{2,5}]-enkephalin (DPDPE), and [^{3}H]-U69,593 had specific activities of 55.3 Ci/mmol, 31.7 Ci/mmol, and 47.5 Ci/mmol, respectively. Other compounds were acquired from the indicated sources: bacitracin, DPDPE, leupeptin, ethylene-bis (β-aminoethylether)-tetraacetic acid (EGTA), phenylmethylsulfonylfluoride (PMSF), thiorphan, and morphine sulfate, Sigma (St. Louis, MO); U69,593, Peninsula Laboratories (Belmont, CA), xylazine (Rompun©; Miles Inc. Agriculture Division, Shawnee Mission, KS); butorphanol (Torbugesic©; Fort Dodge Laboratories Inc., Fort Dodge, IA).

Statistical Analysis

Body weights were evaluated using a two-way analysis. Comparisons of latencies on the hotplate tests were made between baseline and postdrug (i.e., morphine, butorphanol, xylazine) values by a one-way analysis of variance. Mean binding affinities and capacities were assessed for each receptor type using a one-way analysis of variance. All subsequent comparisons were made with the Newman–Keuls tests; $p < 0.05$ was considered to be statistically significant.

RESULTS

Body Weights

The body weights of male and female offspring exposed maternally to NTX were increased 8.2 and 19.7%, respectively, from controls at weaning (i.e., postnatal day 21) (Table 1). At 30 days, male and female rats born to mothers treated during gestation with NTX weighed 16.3 and 24.3%, respectively, more than their control counterparts (Table 1). Male rats and females in the NTX and control groups did not differ in body weight at 48, 60, and 80 days (Table 1).

TABLE 1

BODY WEIGHTS OF RATS PRENATALLY EXPOSED TO NALTREXONE

Age (days)	Sex	Control	Naltrexone
21	M	43.8 ± 0.8	47.4 ± 1.0**
	F	44.7 ± 0.9	53.5 ± 1.1**
30	M	86.0 ± 1.8	100 ± 3**
	F	86.9 ± 1.7	108 ± 3**
48	M	234.4 ± 4.2	249 ± 7
	F	181.5 ± 3.5	174 ± 9
60	M	385.3 ± 27.1	357 ± 8
	F	230.4 ± 1.6	236 ± 7
80	M	439.9 ± 7.0	448 ± 9
	F	255.2 ± 5.2	268 ± 5

All animals were maternally exposed to 50 mg/kg naltrexone or an equivalent volume of saline daily throughout gestation; offspring were crossfostered to untreated females at birth. Data represent means (g) ± SEM; n = 10 to 14 rats/sex/treatment group. Significantly different from controls at $p < 0.01$ (**).

Nociception

In the developmental studies of nociception using morphine challenge, baseline latencies were comparable between NTX-exposed and control offspring at any age examined (Fig. 1). Control animals given morphine at 21, 30, 48, and 60 days of age exhibited increases (i.e., longer) in latencies of 63, 61, 42, and 132%, respectively, when tested 30 min following opioid agonist injection; in all cases the longer latencies on the hot plate differed significantly from baseline values. In contrast, latencies for rats born to mothers exposed to NTX throughout gestation and given morphine did not differ from baseline values. Evaluation of males and females separately at each age showed similar patterns of insensitivity to morphine.

To evaluate whether the offspring of mothers given NTX during pregnancy responded to analgesic drugs other than morphine, groups of rats that were 57–63 days in age were given injections of another opioid analgesic, butorphanol, or a nonopioid analgesic, xylazine; one group of animals were subjected to morphine for comparative purposes (Fig. 2). Control rats injected with either morphine, butorphanol, or xylazine exhibited increases in latencies of 88, 75, and 95%, respectively, from baseline values. Analgesics injected into the NTX-exposed offspring revealed that both butorphanol and xylazine elevated the latencies on the hotplate to 74 and 96%, respectively, of baseline levels. These changes in latency in response to butorphanol or xylazine shown by the NTX group were consistent with the changes in hot plate scores observed when these drugs were injected into control rats. In concert with earlier observations (see above), morphine injection into offspring exposed to NTX in early life had no effect on hot plate latencies relative to baseline scores. Analysis of males and females separately showed no differences in the pattern of response between NTX and control offspring.

Receptor Binding

Opioid receptor binding assays were conducted on animals born to mothers receiving daily injections of either NTX or saline throughout gestation (Tables 2 and 3 and Fig. 3). The binding affinity (K_d) and binding capacity (B_{max}) for δ and κ opioid receptors for the NTX-exposed and control offspring were similar at 21 and 80 days (Tables 2 and 3). Evaluation of males and females separately at 80 days revealed no differences in either δ or κ opioid receptor characteristics between NTX and control offspring. The K_d values for DAMGO, a ligand selective for μ receptors, were comparable between NTX and saline groups at 21 days, and at 80 days (Tables 2 and 3). However, the B_{max} for DAMGO in 21- and 80-day old animals of the NTX group were reduced by approximately 20% at both time points in comparison to their control counterparts (Tables 2 and 3 and Fig. 3). No differences between males and females were found in the decrease in receptor binding for DAMGO at 80 days (data not shown); because an equal number of brains from males and females were combined at 21 days, evaluation of gender response was not conducted at that age.

The results of the above studies revealed that the number of μ opioid receptors were subnormal in 21- and 80-day old rats exposed in utero to NTX. To begin investigating when the changes in the number of μ opioid receptors occurred in NTX-exposed rats, binding analysis of 10-day old animals maternally exposed to an opioid antagonist (i.e., NTX) or saline were conducted. Both the control and NTX-subjected offspring had values for K_d (0.8 ± 0.1 and 0.7 ± 0.1 nM, respectively) and B_{max} (84.4 ± 0.1 and 84.6 ± 0.1 fmol/mg protein, respectively) that were similar (Fig. 3).

DISCUSSION

The effects of maternal opioid antagonist administration on postweaning body weights have received limited attention (7,8,12,18,22). Harry and Rosecrans (7) found no alterations in body weight of 21-day or 28-day offspring from pregnant rats given 1 mg/ml NTX in drinking water throughout pregnancy. Naloxone given to rats by way of minipumps for 7 days beginning on gestation days 11 or 17 had either no effect or resulted in slightly subnormal body weights (i.e., restricted to animals exposed to naloxone beginning on day 17 of pregnancy) in rats examined up to 40 days in age (8). Vorhees (22) found that intraperitoneal administration of naloxone (20 mg/kg twice daily) from gestation days 7 to 20 had no effect on body weight of offspring prior to week 6 but increases were noted for males and females on weeks 6, 8, 10, and 12. Finally, Shepanek and co-workers (18) injected (subcutaneous) pregnant rats with naloxone from days 7 to 20 of gestation and showed that females but not males of dams exposed to 5 or 10 mg/kg naloxone (but not 1 mg/kg) gained weight more rapidly than controls. The present study employed a paradigm of maternal opioid receptor blockade with NTX throughout pregnancy that has no adverse effects on the mother (e.g., weight gain, food intake), and has been confirmed by morphine challenge/nociceptive testing of the dams (12) to invoke a continuous opioid-receptor disruption. Moreover, all pups were crossfostered to untreated lactating females at birth to prevent residual drug exposure and/or problems with females withdrawn from treatment. This investigation also avoided confounding problems in previous studies such as initiating drug administration and/or withdrawing opioid antagonist in the midst of pregnancy, episodic exposure, and withdrawal with the use of improper drug schedules, and variability in drug intake (e.g., oral administration is not monitored for the quantity of drug consumption) that may compromise the value of the data generated. We found that continuous interference with opioid–opioid receptor interfacing throughout gestation resulted in offspring that weighed 8 to 24% more than controls on days 21 and 30. Both male and female progeny of mothers exposed to opioid antagonist were equally affected by opioid receptor blockade. However, these increases in body weights dissipated after 30 days in rats delivered by mothers receiving opioid antagonists during gestation. These results support and extend earlier studies in which body weights from birth to day 21 were reported to be increased in offspring exposed prenatally to NTX (12,13). Thus, continual interruption of opioid–opioid receptor interaction during embryogenesis has long-term repercussions on the acquisition of body weight in both males and females during the early part of the postnatal period which resolve as the animals enter adulthood.

A major focus of this research was to examine the hypothesis that opioid function in the postnatal period is influenced by prenatal development. With respect to nociception, Harry and Rosecrans (7) found less sensitivity to morphine in 90-day-old rats (especially female) exposed to NTX late in gestation, and Hetta and Terenius (8) saw increased sensitivity to morphine in 40-day-old rats exposed to naloxone (0.03 mg/h by minipump) beginning on gestation day 17 and extending to

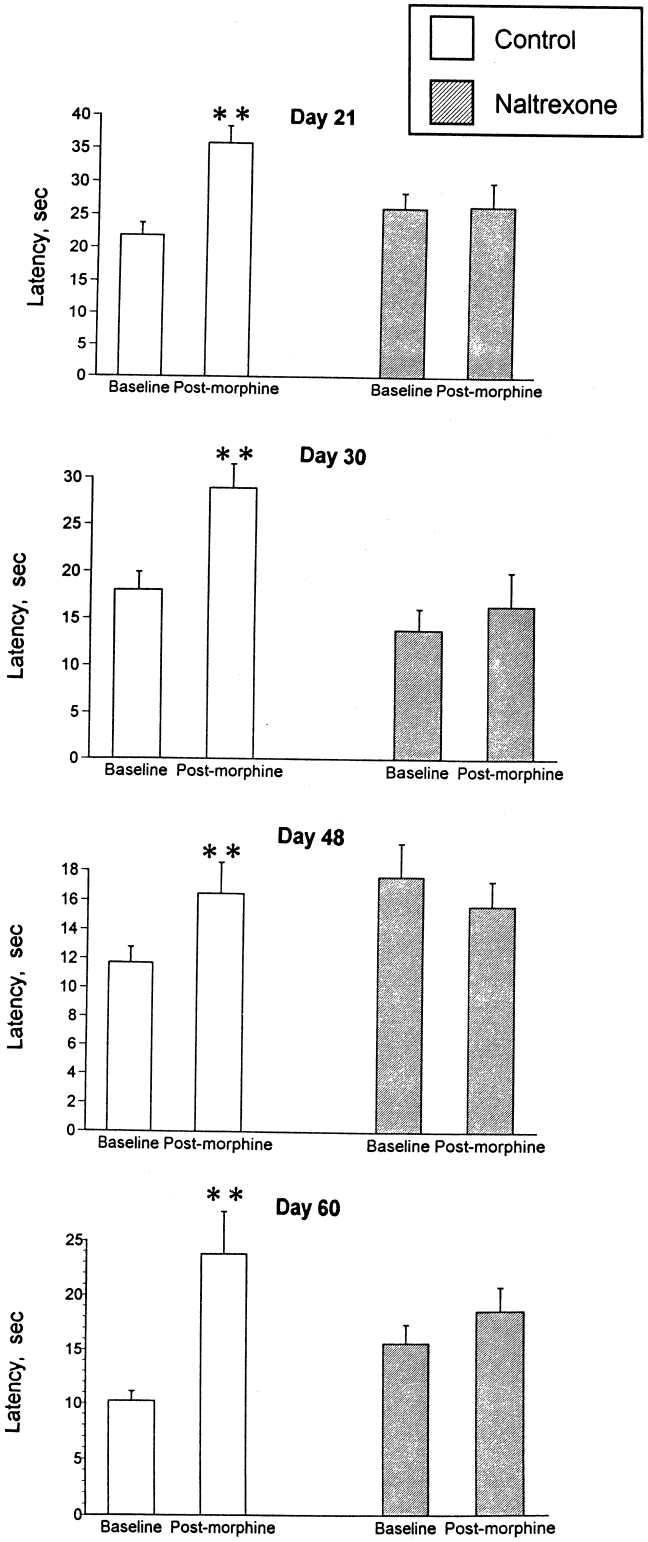

FIG. 1. Latency scores on a hot plate (55°C) in rats maternally exposed to either 50 mg/kg naltrexone (NTX) or saline (controls) throughout gestation; at birth all pups were crossfostered to untreated lactating females. Animals were tested initially to obtain a baseline score, and then received an intraperitoneal injection of either 5 mg/kg morphine at 21 days of age, 10 mg/kg morphine at 30 and 48 days of age, or 12.5 mg/kg morphine at 60 days of age; morphine was given 30 min prior to testing on the hot plate; at least 10 animals/group, with an equal representation of males and females, were utilized at each age. Significantly different from baseline scores at $^{**}p < 0.01$.

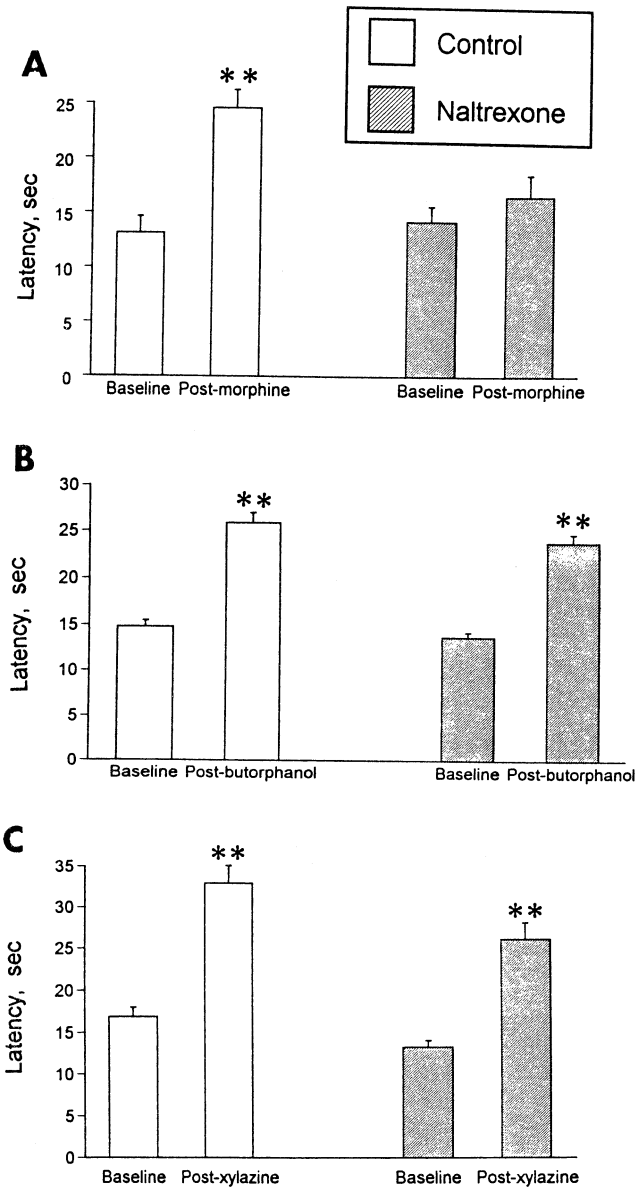

FIG. 2. Latency scores on a hot plate (55°C) in rats (57–63 days of age) maternally exposed to either 50 mg/kg NTX or saline (controls) throughout gestation; at birth all pups were crossfostered to untreated lactating females. Animals were tested initially to obtain baseline scores and then received an intraperitoneal injection of either 12.5 mg/kg morphine, 23 mg/kg butorphanol, or 10 mg/kg xylazine and tested 30 min later; $n = 21$ for NTX and $n = 24$ controls, with approximately equal representation of males and females. Significantly different from baseline scores at $^{**}p < 0.01$.

parturition but not in animals given drug exposure from 11 to 18 days of pregnancy. Using a model of preventing endogenous opioids from interacting with opioid receptors by maternal application of a potent and long-acting opioid antagonist throughout pregnancy, the present results show that sensitivity to the analgesic properties of morphine was impaired at a dosage that produced antinociception in controls; further studies are needed to inquire about nociceptive response to

TABLE 2

RECEPTOR BINDING FOR μ, δ, AND κ OPIOID RECEPTORS IN THE BRAINS OF 21-DAY-OLD RATS EXPOSED TO OPIOID ANTAGONISTS THROUGHOUT GESTATION

	Control		Naltrexone	
	K_d	B_{max}	K_d	B_{max}
μ receptors	1.0 ± 0.1	91.9 ± 4.5	1.2 ± 0.1	72.2 ± 1.0**
γ receptors	3.4 ± 0.3	33.4 ± 1.2	3.3 ± 0.4	33.0 ± 4.4
κ receptors	1.8 ± 0.4	76.4 ± 19.4	2.0 ± 0.4	77.9 ± 20.8

All animals were maternally exposed to 50 mg/kg naltrexone or an equivalent volume of saline daily throughout gestation; offspring were crossfostered to untreated females at birth. Values represent means (K_d = nM; B_{max} = fmol/mg protein) ± SEM for at least two assays performed in duplicate for each ligand; each assay consisted of six brains. Significantly different from the B_{max} values of the controls at $p < 0.01$ (**).

even higher doses of morphine. This irregularity in response did not occur with another opioid, butorphanol, or with a non-opioid compound, xylazine [see (23)]. Thus, interference with the ontogeny of at least one of the endogenous opioid systems during embryogenesis appears to be selectively detrimental to the normal reception of an analgesic drug. The reason(s) for this impairment are unclear at present, however, we did find a decrease in the number of μ, but not δ or κ, opioid receptors using binding studies. Because morphine has a high affinity for μ receptors and low affinity for δ and κ receptors (4), and at least part of the development of these receptors occurs during perinatal life (5,6,19), one may suggest that disruption of opioid–opioid receptor ontogeny has long-term implications on the function of these receptors. In the case of butorphanol, a mixed opioid agonist/antagonist with a higher affinity for μ and κ receptors than δ opioid receptors (4), this drug could have interacted with κ and/or δ receptors (if some or all aspects of μ receptors are damaged) to register analgesia. Therefore, the development of the endogenous opioid systems during prenatal life of a rat has importance to function and expression in the postnatal period.

TABLE 3

RECEPTOR BINDING FOR μ, δ, AND κ OPIOID RECEPTORS IN THE BRAINS OF 80-DAY-OLD RATS EXPOSED TO OPIOID ANTAGONISTS THROUGHOUT GESTATION

	Control		Naltrexone	
	K_d	B_{max}	K_d	B_{max}
μ receptors	1.2 ± 0.1	100.7 ± 5.1	0.7 ± 0.1	80.4 ± 4.7*
γ receptors	4.1 ± 0.8	183.6 ± 27.2	2.5 ± 0.2	182.9 ± 7.4
κ receptors	2.2 ± 0.2	42.6 ± 2.9	1.9 ± 0.1	45.5 ± 1.4

All animals were maternally exposed to 50 mg/kg naltrexone or an equivalent volume of saline daily throughout gestation; offspring were crossfostered to untreated females at birth. Values represent means (K_d = nM; B_{max} = fmol/mg protein) ± SEM for at least two assays performed in duplicate for each ligand; each assay consisted of one brain. Significantly different from the B_{max} values of the controls at $p < 0.05$ (*).

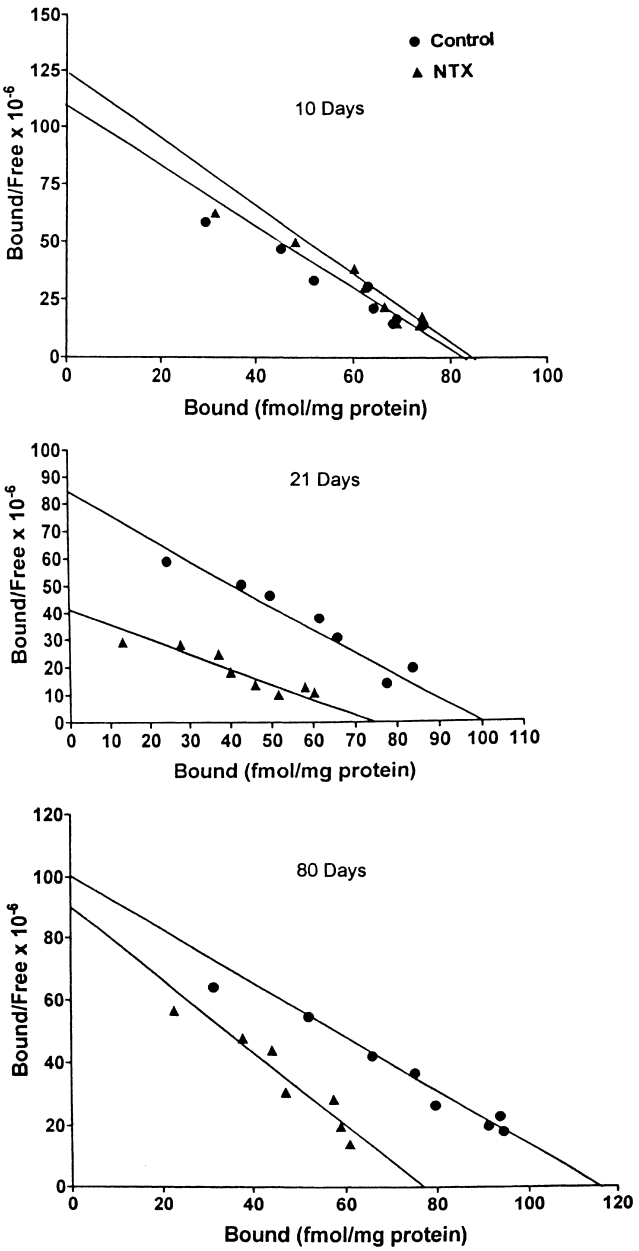

FIG. 3. Representative Scatchard plots of the binding of [^{3}H]-DAMGO to whole brain tissue from 10-day (A), 21-day (B), and 80-day (C) rats maternally exposed to either 50 mg/kg NTX or saline (controls) throughout gestation, and crossfostered at birth to untreated lactating females. See Tables 2 and 3 for K_d and B_{max} values.

Because morphine has a first-pass metabolism in the liver, one could postulate that the lack of antinociception observed in NTX offspring injected with morphine is the result of alterations in liver function. However, the experiments showing butorphanol, also a morphinan congener, showed that this opioid had antinociceptive action in NTX offspring. Additionally, if exposure to NTX in prenatal life was detrimental to the liver and conferred difficulties in viability, it should be noted that previous studies have shown that these NTX offspring have wet and dry weights of the liver at birth and days 10 and 21 that are up to 60% greater than control counterparts. Therefore, it appears that factors other than liver metabolism may be involved with the absence of antinociceptive effect by morphine with respect to rats exposed to NTX in utero.

The offspring of mothers given NTX throughout gestation did exhibit latencies on the hot plate that were comparable to control animals, a result consistent with data of Vorhees (22), Hetta and Terenius (8), and Harry and Rosecrans (7). These observations would suggest that the framework for pain sensation may not be dependent on opioid–receptor interaction during the in utero period. However, Monder and colleagues (15) detected hyperalgesia in female, but not male, mice following maternal treatment with naloxone (minipumps) beginning 5 days prior to parturition. And Shepanek et al. (18) reported increasing sensitivity in male, but not female, rat offspring of mothers receiving 10 mg/kg naloxone from gestation days 7 to 20; animals of either gender exposed to 1 or 5 mg/kg also were normal in nociception. Keshet and Weinstock (10) reported decreased sensitivity in male, but not female, rats of mothers implanted (minipumps) with NTX on day 17 of gestation. The reason for discrepancies between studies as to nociceptive capabilities following prenatal exposure to opioid antagonists remains to be elucidated. As mentioned earlier, methodological issues (e.g., initiating receptor blockade in the course of pregnancy) in previous reports may lead to obfuscating interpretation of drug action.

The mechanism(s) underlying the selective changes in nociception and opioid receptor number detected in the present data require resolution. It should be kept in mind that all of the animals investigated were last exposed to opioid antagonist by the maternal route from 3 to 11 weeks previously. Also, the dosage of 50 mg/kg NTX utilized is 2.5% of the LD_{50} (3) and is known to block opioid receptors for 24 h in rats (24). Furthermore, this dosage has been shown to have no adverse effects (e.g., weight gain, food intake) in pregnant rats (12). Pharmacokinetic data in the rat shows that this opioid antagonist disappears from the plasma after 24 h (14). Moreover, previous studies have determined that treatment with opioid antagonists increases opioid receptor binding (11,17,21) and produces a supersensitivity to morphine (17,20,21), effects not observed herein. Thus, residual drug is not suspected of producing the effects observed. Some opioids and opioid receptors are present during embryogenesis in the rat (2,5,6, 16,19). Therefore, it may be postulated that maternal exposure to the opioid antagonist interferes with the normal interfacing of the endogenous opioid system, particularly with respect to μ-related opioids and receptors, and this disruption has profound ramifications on development and wiring in the nervous system related to nociception and opioid receptors in postnatal life.

These data raise an intriguing question of clinical significance. Opioid receptor blockade using NTX has been in place for treatment of opioid addiction as well as alcoholism. In the event that NTX is consumed by pregnant women, our findings suggest that such exposure to an opioid antagonist during development could lead to alterations in the perception of pain in later life when confronted with particular analgesics such as morphine. Further research into the long-term consequences of maternal consumption of opioid antagonists on offspring are warranted if data on rodents in this study can be extrapolated to the human condition.

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