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Perinatal Naloxone: When Does Naloxone Affect Hyperalgesia?[1]

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MONDER, H., N. YASUKAWA AND J. J. CHRISTIAN. *Perinatal naloxone: When does naloxone produce hyperalgesia?* PHARMAC. BIOCHEM. BEHAV. 11(2) 235–237, 1979.—Pregnant mice were treated with naloxone via subcutaneous implants, from about 5 days prior to parturition. At birth entire litters were cross-fostered so that groups of offspring were exposed to naloxone treated mothers; before birth, after birth to weaning, from about 5 days prior to birth to weaning, or not exposed to naloxone. When tested on a hot-plate at 50 days of age, females either prenatally treated or treated pre- and postnatally showed hyperalgesia to heat. For males, this effect was not evident. This sex difference may have been induced by the cross-fostering procedure.

Hyperalgesia Naloxone Hot-plate Cross-foster Perinatal

NALOXONE, a potent blocker of opiate receptors, has been used as a tool for the study of systems whose operation is mediated by opiate receptors [7,12]. These systems are involved in emotional and nociceptive functions of the central nervous system. Chronic treatment with naloxone affects either the sensitivity or concentration of naloxone-sensitive opioid receptors on the nerve membrane [15]. The treatment of rodents with naloxone lowers the threshold of perception of pain in hot-plate and tail flinch tests [2, 10, 11, 14]. There is some evidence that naloxone affects some nociceptive systems and not others. For example, naloxone does not affect escape thresholds for foot-shock [13], flinch to shock [8] and tail-pinch response [6]. Examination of other neurochemical systems (i.e., catecholamine and indolamine) have shown that disruption of these systems by drugs, during the development of the animal, can cause long-lasting or permanent changes in concentrations of these neurochemicals [16]. In addition, these changes in chemical balances are often accompanied by behavioral changes. A previous study from this laboratory (Monder *et al.* submitted) demonstrated that the treatment of pregnant mice with naloxone from 3 to 5 days prior to parturition until weaning resulted in offspring with lowered flinch thresholds, when tested on a hot plate at 50 days of age.

The following cross-fostering study was done in order to determine whether the changed threshold is induced by pre- or postnatal exposure to naloxone.

METHOD

Forty nulliparous female mice of an unaggressive heterogenic strain developed at SUNY-Binghamton, were used as dams for this study. They were housed 3 to 5 per cage, and maintained in a vivarium on a 14/10 hr light/dark cycle, with room temperature maintained at approximately 25°C. Food and water were provided ad lib throughout the study.

The females were mated with males of the same strain. The males were removed from the female's cage after 10 days. At 5 to 10 days prior to parturition, the females were implanted subcutaneously with silastic capsules, either empty or containing naloxone (supplied by Dr. A. Rubin, Endo Labs.), using previously described procedures [21]. The implants were designed to release between 20 and 60 μg of naloxone per day.

Within 24 hr of birth, entire litters were cross-fostered to produce litters that had been exposed to naloxone either prenatally, postnatally until weaning, for the entire treatment period (pre- and postnatal), or not exposed to naloxone at all. All litters were cross-fostered. Litters were weighed every other day, from birth to 12 days of age. From 12 to 30 days of age pups were weighed individually. Litters were weaned at 22 days of age.

At 50 days of age the mice were tested for their reaction to heat, using a hotplate maintained at 50°C. Each animal was placed on the hot plate and timed for the first sign of discomfort, i.e., shaking or licking feet, or jumping off the plate. Each animal was given one trial. Animals were tested at between 0900 and 1100 in the morning. This time period was selected since the effects of naloxone on nociceptive thresholds have been shown to be maximal either in the early morning or late afternoon [9].

Statistical differences among the groups were determined by one and two way ANOVAs. Subsequent paired comparisons were made by use of the Duncan method. An alpha level of $p<0.05$ or better was considered as significant.

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RESULTS

There were no differences in body weight among the groups of dams through pregnancy. The number of pups born dead or that did not survive to weaning, and the size of the litters did not differ significantly among the groups. No drug related weight differences developed among the groups for the pups. No developmental differences, such as age at eye opening or vaginal opening developed among the groups of offspring.

The hot-plate test indicated that there were differences among the groups in latency to reaction to heat (Table 1). A treatment by sex analysis indicated that there were significant differencs among the groups relative to naloxone treatment, $F(3,211)=8.95$, $p<0.0001$, and that there was a significant sex by treatment interaction, $F(3,211)=4.15$, $p<0.007$. Subsequent analysis indicated that the offspring of mothers treated with naloxone prenatally or both pre- and postnatally differed from the other two groups, with the prenatally treated animals having significantly shorter latencies on the hot-plate than any of the other groups.

TABLE 1

LATENCY, IN SECONDS FOR SIGNS OF DISCOMFORT ON THE HOT-PLATE FOR MICE TESTED AT 50 DAYS OF AGE. THE HOTPLATE WAS MAINTAINED AT 50 DEGREES C

Naloxone Experiment					
pre	post	Sex	N	Mean	S.E.M.
blank-blank		F	12	3.92	0.97†
		M	18	1.56	0.34
blank-nalox		F	23	2.57	0.33
		M	30	2.80	0.30*
nalox-blank		F	19	1.16	0.45
		M	34	1.06	0.26
nalox-nalox		F	39	1.72	0.26
		M	44	1.75	0.22

*Statistically significant difference from other treatment groups ($p<0.05$). Comparisons are within sex.

†For the females, prenatal controls differed significantly from prenatal naloxone groups ($p<0.05$). Except that the blank-naloxone group did not differ from the naloxone-naloxone group.

In view of the significant sex by treatment interaction, the results for each sex were separately analysed. For the females, the postnatally treated animals did not differ from the controls and the prenatally treated animals did not differ from the animals treated both pre- and postnatally. However, the latter two groups showed significantly shorter latencies on the hot-plate as compared to the former. For the males, the postnatally treated animals had significantly longer latencies on the hot-plate than did the other groups.

DISCUSSION

Our previous study (Monder *et al.* submitted) demonstrated that perinatal exposure to naloxone produces changes in the threshold to heat flinch in a hot-plate test. Thus, some relatively permanent change was induced in the mice by this exposure to the drug. There is evidence that stimulation of opiate receptors affects their sensitivity [15]. One would expect that this effect would be most pronounced if the manipulation of the system with drugs was done during the period that the system was developing. For example, O'Callaghan and Holtzman [18] have shown that perinatal exposure to morphine produces changes in latencies to react on a hot-plate, when rats were tested at 3, 5 and 11 weeks of age.

In addition to drug effects, there are also sex differences in reaction to pre- and perinatal drugs and stress [3, 4, 5, 17, 19, 20]. Our previous work has not demonstrated sex differences in the heat flinch response. The present study indicates that prenatal naloxone reduces latencies to first lick for mice on a hot-plate, but that the effect is sex-dependent. Females were more strongly affected by the drug treatment than were the males. Previous studies, using amphetamine, having shown a greater drug effect in female rats than male rats, with prenatal drug treatment [17].

The differences in response between the males and females may be a function of differential reaction to handling stress. In a previous study (submitted), perinatal naloxone treatment did not result in a significant sex by treatment interaction on the hot-plate test. Therefore, it is possible that the crossfostering procedure may have an effect that interacted with the naloxone treatment, affecting males differently from the females. Ackerman *et al.* [1] reported differences in the survival time of crossfostered rats during food deprivation. A relationship between stress and the hyperalgesic effects of naloxone in some tasks, but not others has been demonstrated [2,8]. Further research is necessary to clarify this point. However, examination of the reactions of the females to the hot-plate indicates the possibility that a system that is most sensitive to naloxone just prior to parturition may be involved in the mediation of nociceptive sensitivity. Further work is necessary to determine if there is a relationship between levels of sex hormones in the blood and reaction to certain nociceptive stimuli. Previous work in our laboratory has demonstrated that prepubertal naloxone treatment results in increased levels of LH in trunk blood of adult mice [21]. We are attempting to determine if there is a correlation between hormone levels and perinatal handling, and the effects of naloxone on behavior.

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