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NEONATAL ADMINISTRATION OF β-ENDORPHIN PRODUCES "CHRONIC" INSENSITIVITY TO THERMAL STIMULI[1]

Curt A. Sandman*, Robert F. McGivern*, Chris Berka*, J. Michael Walker+
David H. Coy† and Abba J. Kastin†

*Department of Psychiatry and Human Behavior, University of California Medical Center, Irvine and Fairview Hospital, Costa Mesa
†Veterans Administration Medical Center and
Tulane University School of Medicine
New Orleans, Louisiana 70140
+Mental Health Research Institute, University of Michigan

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SUMMARY

Male and female rat pups were injected with β-endorphin, naloxone or a saline control solution during days 2-7 postnatally. At 90 days of age the rats were tested for analgesia with the tail flick test. Testing was conducted during the first 2 hours of the light and the dark cycle. In both sexes and during both phases of the light cycle rats treated with β-endorphin as infants evidenced a significant elevation in threshold for painful thermal stimuli. Early treatment with naloxone also resulted in elevated threshold for thermal stimuli. Administration of naloxone to these rats as adults did not reverse the analgesic effect. It was concluded that early exposure to β-endorphin results in permanent changes in behavior perhaps by altering the interaction of endogenous opiates with their binding sites during a ciritcal period of opiate receptor development.

The endorphins comprise a class of neuropeptides with striking behavioral properties. Dramatic elevation in pain threshold has been observed after injection with these compounds (1, 2, 3, 4). Among the various endogenous opiates, β-endorphin (the C-fragment of the LPH chain) appears to possess the strongest analgesic influence (4). Although several extra-analgesic responses such as grooming (5) have been reported after peripheral injections of β-endorphin, analgesia is usually observed only after central administration of the molecule. Analgesia (1, 2, 3, 4) and electrocortical effects (7) of the endorphins and morphine are reversible by the opiate antagonist naloxone. In addition naloxone by itself can produce hyperalgesia (8, 9) and distinctive patterns of electrical activity in the brain (7).

1. Please address all correspondence and reprints to Curt A. Sandman, Director of Research, Fairview Hospital, 2501 Harbor Blvd., Costa Mesa, California 92626.

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The rat is not born with a fully developed complement of opiate receptors. A rapid increase in binding develops during the first 3 weeks postnatally and then continues a gradual proliferation for 15-20 weeks (10). Neonatal administration of morphine attenuates morphine-induced analgesia in adulthood (11, 12, 13). Conversely, early treatment with some opiate antagonists such as naltrexone, sensitizes adult rates to the effects of morphine (12). Although there are no reports of chronic changes in nociception after neonatal treatment with other endogenous or exogenous opiates, long term effects on behavior have been reported after neonatal exposure to other neuropeptides such as MSH (14), ACTH fragments (15), Thyroxine (16), and TRH (17). In the present study we report chronic elevation in threshold for painful thermal stimulation in adult rates systemically injected as infants with β-endorphin.

Method

From days 2-7 of age rat pups (N = 75) from multiparous mothers were injected subcutaneously with either β-endorphin (50 µg/rat), naloxone (100 µg/rat), or a vehicle solution. Rats from split-litters (N = 6-10) were randomly assigned to treatment groups and marked for identification. The rats were weaned on day 22 and housed in same sex groups of 4 to 6 animals. The animals were maintained on a 12-hour light, 12-hour dark schedule. Except for routine handling, the rats were not disturbed until they were 90 days old when behavioral testing was initiated.

Each rat was tested with the tail flick test (18) which consisted of 6 trials per session during which the latency of the rat to withdraw its tail from a radiant light source was measured. Since the testing procedure was lengthy each session required several days to complete. Initial calibrations for setting the heat of the lamp were made with separate animals. The intensity was adjusted so that the median latency was 6.0 seconds. Since a clear circadian cyclicity has been observed for pain threshold (8) the rats were tested during the first 2 hours of the light cycle and one week later during the first 2 hours of the dark cycle. In an automated procedure the rat's tail was placed on a platform above the source of heat. A digital timer was activated when the light was turned on. As the rat withdrew its tail from the heat the light engaged a photocell and the timer was turned off. At least 5 minutes elapsed between each of the trials. A cut-off of 10.0 seconds was utilized to protect the rats from the possibility of tissue damage. To ensure stable performance, animals were tested with the entire procedure during both phases of the light and dark cycle before formal measurements were initiated.

A second test of analgesia was conducted to determine the specificity of the influence of neonatal treatment. A shock-jump test was employed with subgroups (N = 4) of males from each treatment group. Rats were placed on an electrified grid in a testing chamber and their response to shock (1-5 ma) (typically a jump) was recorded automatically with a ploygraph. Five shocks of each of the amperage values were delivered in a random sequence.

Results

The tail flick latencies were subjected to a 4-factor analysis of variance with repeated measures. As illustrated in Figure 1, the results indicated that response latencies were significantly affected by peptide treat-

ment ($F_{2,69} = 45.76$ $p < 0.001$), sex of the animal ($F_{1,69} = 56.49$, $p < 0.001$) and the light-dark cycle ($F_{1,69} = 12.33$, $p < 0.001$)[2]. The sequence of observations (trials), did not interact with any of the factors. From Figure 1a it is apparent that infants treated with β-endorphin displayed a long-lasting and perhaps permanent change in pain threshold when tested as adults. The latency for the β-endorphin groups was significantly greater ($p < 0.01$) than both the naloxone and saline treated groups as determined by simple effects tests. Tests of simple effects also indicated that animals treated with naloxone exhibited a greater ($p < 0.05$) threshold for the painful thermal stimulus than the rats given saline. There were no first or second order interactions of drug treatment with sex or light-dark cycles. Since naloxone consistently has been reported to reverse morphine and endorphin induced analgesia (1-4), subgroups (N =4) of each treatment group were injected during the dark cycle with naloxone (100 µg/rat) and tested 15 minutes later for tail flick latency. Analysis of variance indicated that injections of naloxone in adult animals did not alter the tail-flick latency compared with preinjection latency. Thus it appears that neonatal exposure to endogenous opiates and to opiate antagonists administered peripherally can influence permanently the sensitivity to thermal stimuli of male and female animals tested as adults.

As illustrated in Figure 1b males evidenced a significantly higher threshold for pain than females and the threshold during the dark cycle for all animals was significantly greater than for the light cycle (Figure 1c). These findings are in accord with earlier data for sex differences (19) and the influence of dirunal variation (8) on response to painful stimulation. However the hyperalgesia reported after administration of naloxone to adult animals (8) was not observed in animals given naloxone as infants.

The influence of neonatal treatment on the shock-jump test of analgesia was analyzed by analysis of variance. A clear and linear influence on jump amplitude was determined for the different levels of shock. However there was no effect of treatment of β-endorphin or naloxone on the reaction to four levels of shock. These data offer support for the specific influence of endorphins on thermal, but not painful electrical stimulation (9).

Discussion

The results of the present study indicate that early exposure to β-endorphin or an opiate antagonist can have permanent effects on the response to painful thermal stimulation. Since analgesia is not usually observed after peripheral treatment of adults with β-endorphin the effects observed in this study may represent a profound and permanent action of β-endorphin on the brain after peripheral administration during critical stages of development.

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2. Unlike the typical tests of analgesia only between group comparison were possible. Therefore, a second analysis was performed. Since many factors may determine the perception of painful thermal stimulation, including ambient temperature, the data were transformed to account for possible contaminating environmental influences. For each session the latency to respond was transformed into a percentage of the highest value achieved during that session by any animal. The data were then analyzed with the same procedures reported for the latency scores. Since the data transformation was of within-session variability, between-session analyses may not be appropriate. The direction of the effects for this analysis were consistent with the analysis of the latency: during the light cycle session; treatment, $F_{2,69} = 14.09$, $p < 0.001$; Sex, $F_{1,69} = 17.37$, $p < 0.001$; during the dark cycle session; treatment, $F_{2,69} = 8.38$, $p < 0.001$; Sex, $F_{1,69} = 28.26$, $p < 0.001$.

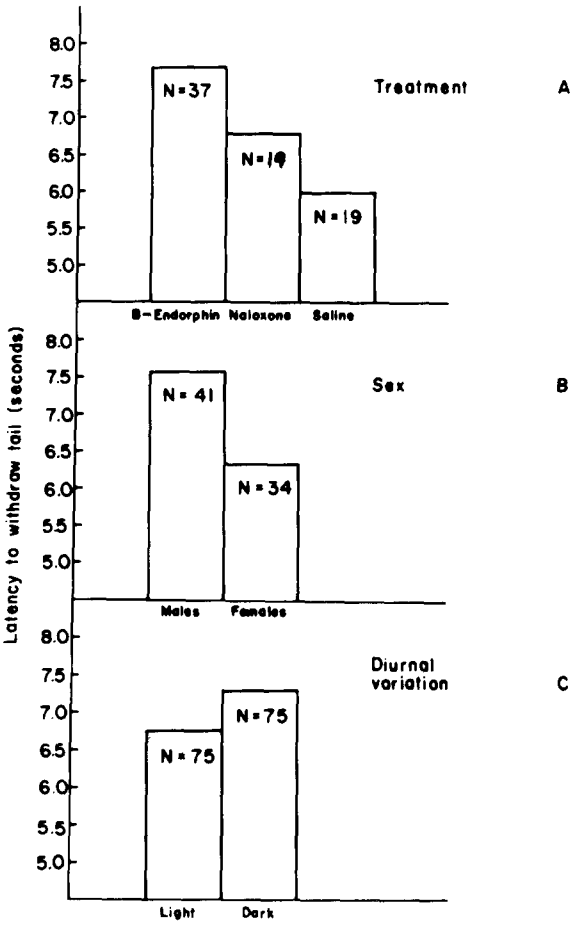

FIG. 1

Latency of response to withdraw tail from thermal stimulus. Panel A: Response of adult rats treated as infants with β-endorphin, naloxone or saline (SD; β-endorphin = 1.19; naloxone = 1.09; saline = 1.02; MS_{error} = 485.99); Panel B: Response of male and female rats (SD; male = 1.26; female = 0.91); Panel C: Response of rats tested during the first 2 hours of the light or dark cycle (SD; light = 1.14; dark = 1.03).

The administration of β-endorphin and naloxone during days 2-7 postnatally corresponds to a highly critical stage in the development of opiate receptors in the rat brain (10). Thus the results of the present study suggest that neonatal exposure to β-endorphin or a potent antagonist may alter the interaction between endogenous opiates and their binding sites. It is conceivable that opiate binding sites proliferate at different rates or in different quantities as a function of early stimulation by β-endorphin or naloxone. It is equally possible that early exposure to these compounds results in persistently elevated levels of endogenous opiates in the brain. The failure of naloxone to reverse the insensitivity to painful stimuli sug-

gests the possibility that these effects are not mediated by opiate receptors. However, it is also possible that putative neurochemical or opiate receptor changes after neonatal exposure to opiates render the "opiate system" resistent to opiate antagonists.

There is active debate concerning the role of the endorphins in behavioral disorders such as schizophrenia (20, 21). The pertinence of our findings to the "schizophrenic-like" behavior of animals raised in isolation (22) or decreases in opiate binding and sensitivity to painful stimuli of mice reared in isolation (10) cannot be determined yet. The eventual significance of these findings may relate to the presence of β-endorphin in amniotic fluid since the concentration appears to coincide with the degree of fetal distress (23). Future studies will determine if fetal exposure to β-endorphin produces behavioral consequences which are similar to those reported here after neonatal injections and whether this neurochemical influence has an effect on the development of maladaptive behavior.

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