The Journal of Pain, Vol 9, No 10 (October), 2008: pp 962-969
Available online at www.sciencedirect.com

ELSEVIER

Sex Chromosome Complement Affects Nociception and Analgesia in Newborn Mice

Laura Gioiosa, Xuqi Chen, Rebecca Watkins, Elizabeth A. Umeda, and Arthur P. Arnold

Department of Physiological Science and Laboratory of Neuroendocrinology of the Brain Research Institute, University of California, Los Angeles, California.

Abstract: **In animal studies of nociception, females are often more sensitive to painful stimuli, whereas males are often more sensitive to analgesia induced by μ-agonists. Sex differences are found even at birth, and in adulthood are likely caused, at least in part, by differences in levels of gonadal hormones. In this report, we investigate nociception and analgesia in neonatal mice and assess the contribution of the direct action of sex chromosome genes in hotplate and tail withdrawal tests. We used the 4 core genotypes mouse model, in which gonadal sex is independent of the complement of sex chromosomes (XX vs XY). Mice were tested at baseline and then injected with μ-opioid agonist morphine (10 mg/kg) or with the κ-opioid agonist U50,488H (U50, 12.5 mg/kg) with or without the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (0.1 mg/kg). On the day of birth, XX mice showed faster baseline latencies than XY in tail withdrawal, irrespective of their gonadal type. Gonadal males showed greater effects of morphine than gonadal females in the hotplate test, irrespective of their sex chromosome complement. U50 and morphine were effective analgesics in both tests, but MK-801 did not block the U50 effect. The results suggest that sex chromosome complement and gonadal secretions both contribute to sex differences in nociception and analgesia by the day of birth.**

Perspective: ***Sex differences in pain may stem not only from the action of gonadal hormones on pain circuits but from the sex-specific action of X and Y genes. Identification of sex chromosome genes causing sex differences could contribute to better pain therapy in females and males.***

Key words: *Pain, sex difference, hotplate, tail withdrawal, sex chromosomes, neonate.*

In various species including humans, pain perception and sensitivity to analgesic drugs can differ in either sex.Females are generally more sensitive to pain and/or differ from males in neural circuits mediating nociception.[5,18,30] In mice, a variety of *N*-methyl-D-aspartate (NMDA) antagonists block the acute analgesic effects of μ- or κ-agonists or development of tolerance to morphine.[6,12,33,34,37,41] However, specific NMDA antagonists block the morphine analgesia only in females, whereas other NMDA antagonists block analgesia in either sex.[37] Estradiol activates a female-specific mechanism that is absent in males, is insensitive to NMDA antagonists, and is mediated by the melanocortin-1 receptor.[33,34]

In animals including mice, μ-agonists such as morphine are more effective in males than females,[11,12,27,32,35] although the literature is complex and contradictory.[12] Activational and organizational effects of steroids contribute to sex differences in opioid analgesia.[9,10,12,25,32] For example, estradiol can reduce morphine analgesia[12] and testosterone can increase opioid analgesia because of activational effects.[33,42] In addition, treating neonatal female mice with testosterone permanently masculinizes their response to morphine, and castrating males at birth demasculinizes them (organizational effects).[9,25] Analgesia produced by the selective κ-opioid receptor agonist U50,488H (U50) shows sex differences in the same direction as the μ-opioid receptor agonist morphine.[34,40] U50 can be more potent in males than in females, and the U50 effects are blocked specifically in males by MK-801.[34,40] However, organizational and activational effects of gonadal ste-

Received December 21, 2007; Revised May 21, 2008; Accepted June 10, 2008.

Supported by NIH grants NS045966 and NS043196.

Drs. Gioiosa and Chen contributed equally to this work.

Address reprint requests to Dr. Arthur P. Arnold, Department of Physiological Science, UCLA, 621 Charles Young Drive South, Los Angeles, CA 90095-1606. E-mail: arnold@ucla.edu

1526-5900/$34.00

doi:10.1016/j.jpain.2008.06.001

roids do not account for all of the sex differences in nociception and drug-induced analgesia.

Sex differences are also caused by "sex chromosome effects," which are direct actions of X and Y genes on nongonadal cells.[1–3] In this study, we used the "4 core genotypes" (FCG) model to study the biological origins of sex differences in nociception and analgesia in neonatal mice. In this mouse model, the complement of sex chromosomes (XX vs XY) is independent of the gonadal sex (testes vs ovaries) of the mouse.[3,7,8,16,20,21,38]

We recently observed that neonatal XX mice of either gonadal sex have higher expression in brain of prodynorphin mRNA than XY mice (unpublished data). That finding suggested that dynorphin levels might be higher in XX than XY mice, which might alter κ-opioid nociception. μ- and κ-receptors are found in neonatal rodent spinal cord and brain.[39] We therefore asked whether neonatal mice show hormonal and sex chromosome effects on nociception and μ- and κ-mediated analgesia and whether the NMDA antagonist MK801 could block any U50 effect. We found that morphine and U50 were both effective analgesics at this age but that the NMDA antagonist MK-801 did not block the effects of U50 as it does in adults.[40] Groups differing in sex chromosome complement and in gonadal sex showed differences in the 2 assays of nociception.

Methods

Mice

In FCG mice, the testis-determining *Sry* gene is deleted from the Y chromosome, producing the "Y minus" chromosome, Y^-, so that the Y^- chromosome no longer determines gonadal sex.[3,26,28] An *Sry* transgene is inserted onto an autosome, producing XY^- *Sry* males. Breeding these males with XX females produces 4 types of progeny. XX females, XY^- females, XX*Sry* males, and XY^- *Sry* males. We define "male" and "female" according to gonadal sex. Comparing the phenotype of males (XX*Sry* and XY^-*Sry*) and females (XX and XY^-) tests for the effects of presence or absence of *Sry*. The effects of *Sry* are thought to be mediated primarily by sex differences in gonadal secretions, although direct effects of *Sry* on the brain also occur.[17] On the other hand, comparing XX mice (XX females and XX*Sry* males) with XY^- mice (XY^- females and XY^-*Sry* males) tests for the differential effects of sex chromosome complement (XX vs XY). The FCG model tests simultaneously for the effects of gonadal steroids, for sex chromosome effects, and their interaction (eg, hormonal effects that occur only in XY but not XX mice). In the present experiment, we used randomly bred MF1 mice that were kindly provided to us by Paul Burgoyne (National Institute for Medical Research, London, UK), in which the MF1 X chromosome of all mice was invariant (ie, had no allelic variation across animals, a substrain produced by breeding male mice to their XO mothers). The Y^- chromosome derives from strain 129.[26]

Mice were quasi-randomly assigned to groups, attempting to maximize the number of groups represented within each litter. The sex and sex chromosome complement of each mouse was determined by PCR amplifying from genomic DNA a Y chromosome sequence (to detect the presence/absence of the Y^- chromosome), *Sry* (to detect the *Sry* transgene), and a control gene.

Experimental Subjects and Procedures

Experimental subjects were male (n = 175, 91 XX*Sry* and 84 XY^-*Sry*) and female (n = 146, 67 XX and 79 XY^-) FCG MF1 mice, bred in our colony. Animals were tested on the day of birth (P1) between 8 AM and noon. Animals were housed in a light-controlled (L:D 12:12, lights on at 7 AM) and temperature-controlled (21 ± 1°C) environment. XY^-*Sry* males were each housed with pairs of XX females for 2 weeks. Dams were then separated from males and singly housed for the duration of the pregnancy, with no disruption except for the routine cage maintenance. Dams were given free access to standard laboratory food and tap water. As parturition approached, the cages were observed daily for the presence of pups. On the day of birth, pups were removed from the dam, weighed, and placed in individual cardboard containers. The containers were kept on a warming pad for the duration of the testing session. Two tests of nociception were performed as described below. Pups within each litter were randomly assigned to one of the drug conditions. Immediately after hot plate and tail withdrawal baseline assessment, the pup was injected with a drug (see below) and returned to its container. Pups were retested on both pain assays 15, 45, and 90 minutes after injection by an experimenter who was blinded to the drug condition and sex/sex chromosome complement. All procedures were approved by the UCLA Chancellor's Animal Research Committee and conformed to applicable national and international guidelines.

Tests of Nociception

Mice were tested on 2 different behavioral tests of thermal nociception as described by Sternberg et al:[41] the hotplate and tail withdrawal assays, which show diverse sensitivity to drugs and sex. Both the hotplate and tail withdrawal assays have been used extensively in adult rodents and have also been adapted for use in neonates previously.[41]

In the hotplate test, the experimenter held the pup between the thumb and forefinger in an upright position and gently placed one hind foot of the mouse on the surface of the hot plate (52.5°C; AccuScan Instruments, Columbus, OH). Latency to remove the foot from the surface was recorded, with a 15-second cutoff time for nonresponsive animals. The procedure was repeated on the opposite foot after a 10-second interval, and an average of the latencies for both feet was used in the analysis. Foot withdrawal was measured at time t = 0 (before injection) and 15, 45, and 90 minutes later.

In the tail withdrawal test, the pup was held in the same manner, and the distal tip of the tail was lowered into a water bath maintained at 50°C. The latency to