

Research report

The role of opioid receptors in morphine withdrawal in the infant rat

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Abstract

Exposure to opiates such as morphine can lead to psychological and physical dependence in both adult and infant humans. Infant rats experience opiate withdrawal behaviors that are qualitatively different from the withdrawal behaviors displayed by adult rats. In the adult, withdrawal is largely mediated by the μ -opioid receptor. We sought to understand more about what role each opioid receptor (μ , κ , and δ) plays in the display of the physical withdrawal in the infant rat. Beginning on postnatal day 1, infant rats were injected with morphine sulfate twice a day for 6.5 days. On the afternoon of the seventh day the infant rats were given an i.c. injection of a vehicle, the μ -opioid receptor antagonist CTOP, the κ -opioid receptor antagonist nor-BNI, or the δ -opioid receptor antagonist naltrindole. CTOP precipitated withdrawal behaviors in the 7-day-old rat in a dose-dependent manner. Neither nor-BNI nor naltrindole induced any significant changes in the frequency of the withdrawal behaviors. These data suggest that in the infant rat control of certain behavioral withdrawal signs is modulated primarily by the μ -opioid receptor, as is the case in the adult rat. © 2000 Elsevier Science B.V. All rights reserved.

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Topic: Drugs of abuse: opioids and others

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1. Introduction

Despite the various synthetic and semi-synthetic opioids that have been produced, morphine remains the most commonly used opiate drug in clinical settings for the relief of acute or chronic pain in both adult and infant humans. However, one major disadvantage of morphine is that repeated administration (chronic exposure) often leads to dependence. When dependence occurs, the slow or abrupt (through the administration of an antagonist) cessation of the opiate causes disruption of the basic functioning of the nervous system on different levels [10,17,28]. For instance, at the behavioral level physical withdrawal signs occur; at the physiological level there are changes in body temperature; at the affective level aversive affective states

occur; and at the neuronal level there are changes in receptor function.

Human infants, exposed to opiates after in utero or postnatal chronic exposure, experience an array of withdrawal behaviors different from those seen in adults. These behaviors include altered sleep patterns [5], high-pitched crying, respiratory and gastrointestinal dysfunction, and irritability and tremors [19]. In the short-term, dependence and the manifestation of these withdrawal behaviors can result in strained mother–child bonding and increased morbidity or mortality of the infant [7]; the long-term consequences of infant exposure to opiates are still unknown.

In adult rats, opiates exert their pharmacological actions through three opioid receptor classes, μ , κ , and δ , which are phylogenetically conserved. Advances in gene cloning and related techniques have shown that each receptor has its own unique pharmacological profile (with differing degrees of affinity to the endogenous and synthetic opioids), anatomical distribution throughout the central and

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peripheral nervous system, developmental profile, and function.

In the adult rat, the μ -opioid receptor has been shown to play a vital role in eliciting physical withdrawal. The direct blockade of the μ -opioid receptor, by a specific antagonist or a specific agonist after a non-specific antagonist challenge, results in withdrawal behaviors that are quantitatively greater than those seen from similar experiments with the δ - or κ -opioid receptors [3,13,14,23]. Withdrawal behaviors associated with the blockade of the δ - and κ -opioid receptors are either less dramatic or are not seen at all [3,13,14,23,30].

Early experiments focusing on the ability of infant rats to experience withdrawal and dependence to morphine, did not detect any behavioral changes before the age of 30 days [6]. However, more recent research has shown that infant rats do in fact experience withdrawal behaviors [11,29]. Most of these behaviors change throughout the ontogeny of the rat until the behaviors seen in adulthood are displayed. For instance, behaviors that are commonly seen in adults, like wet dog shakes, grooming, jumping, diarrhea, and ptosis [11,27,29] do not appear until 21-days of age, whereas at 7-days of age behaviors like paw movements, head movements, and rolling are common [11].

The display of withdrawal behaviors that are qualitatively different throughout the ontogeny of the rat may be due, in part, to the different development of the CNS μ -, κ -, and δ -opioid receptors. The present study was designed to determine whether the unique withdrawal behaviors seen in the 7-day-old infant rat are in fact due to receptor classes distinct from those involved in the display of physical withdrawal behaviors in the adult rat.

In order to determine the specific participation of each opioid receptor type in the expression of morphine abstinence, we quantified the occurrence of a series of different physical withdrawal behaviors precipitated by the administration of the selective μ -opioid receptor antagonist CTOP, the selective κ -opioid receptor antagonist nor-BNI, and the selective δ -opioid receptor antagonist naltrindole. We hypothesized that, as is the case in the adult rat, the μ -opioid receptor plays the greatest role in modulating the withdrawal behaviors seen in the 7-day-old rat and is therefore the predominant receptor modulating this behavior throughout the ontogeny of the rat.

2. Materials and methods

2.1. Subjects

The subjects were the offspring of Long-Evans Hooded rats mated in our laboratory. The parents (and offspring) were housed in plastic tubs with beta chips used as bedding. The colony room was maintained at a temperature of 22–24°C under a light cycle of 12 h, with the lights

turned on at 08:00 h. Food (Lab Diet) and water were available ad libitum. Tubs were checked twice daily (~09:00 and 17:00 h) and any new pups found at either time were recorded as 0 days old. The experiment began on the day that the pups became 1 day of age, and at that time the litter was culled to eight pups, without regard to sex.

2.2. Morphine dependence

The following procedures for this study were conducted in accordance with the guidelines set forth by the National Institutes of Health and approved by the Institutional IACUC. At 1 day of age pups were removed from the dam and transferred to a plastic container, with beta chips for bedding, before being placed in an incubator. Using India ink, each pup was permanently tattooed for purposes of identification [8]. Each pup was weighed and injected with 10 mg/kg of morphine sulfate, i.p. (a volume of 0.01 ml/g). After the injection of the final animal, all pups were placed in the incubator once again for 45–60 min before being returned to the dam. The weighing was repeated once a day for 7 days, and the injections were given twice daily for 6 days and once more on the morning of the 7th day.

2.3. Withdrawal/behavioral observation

Four hours after the morning morphine injection on the 7th day, the pups were removed from the dam and placed in a plastic container in an incubator where the temperature was maintained at 32°C. One pup was randomly chosen and removed from its littermates; it was weighed, sexed, and marked on its back for identification during the behavioral observation. Pups were injected with either saline or one of four doses (0.1, 0.3, 1.0, 3.0 μ g/4 μ l, intracisternal (i.c.)) of D-Phe-Cys-Trp-D-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP), naltrindole hydrochloride H₂O (Naltrindole), or nor-binaltorphimine (nor-BNI). The i.c. injection procedure was adapted from Carden et al. [2]. The doses were assigned randomly, and the experimenter was blind to the dose. The pup was returned to the container in the observation chamber, a little distance from the remainder of its littermates and its behavior was recorded. After the behavior of the target pup was observed and its rectal temperature was taken, it was anesthetized with sodium pentobarbital (so it would remain quiet), and returned with its littermates. In this manner, five pups were used.

Specific physical withdrawal behaviors of opiate exposed rats at 7-, 14-, 21-, and 42-days of age were defined previously, when chronically morphine exposed rats were injected with the non-specific opioid antagonist naltrexone. Head moves, moving paws, quiet, rolling, separated, stretching, walking, and wall climbing occurred in the 7-day-old infant rat [11]. Behavioral definitions of these behaviors can be found in Table 1.

Table 1
Behavioral definitions^a

Behavior	Definition
Head moves	Lateral and rotary motion of head
Moving paws	Continuous movement of hindpaws without walking
Quiet	Sedated appearance with no movement
Rolling	Turning body over at least one full rotation
Stretching	Extension or dorsal flexion of the trunk, causing a lengthening of the body
Together	Seeking physical contact with littermates
Twisting	Twisting of the body without a complete rotation
Walking	Taking more than one step forward

^a Abbreviated from Jones and Barr [11].

2.4. Data analysis

A factorial analysis of variance (ANOVA) was conducted for each behavior. Wall climbing and separated were omitted because they rarely occurred. Each pup was observed for 15 min, which was then divided into three time periods, consisting of 5 min each. Each time period was treated as a within-subjects variable. No significance occurred across the time periods; thus that factor was not included in the analysis. All four doses were injected within a single litter and the drug dose effect was treated as a repeated measures variable.

3. Results

3.1. CTOP

The i.c. administration of the μ -opioid receptor antagonist CTOP caused withdrawal in the 7-day-old rat. Of the observed behaviors, there was a significant increase in the amount of time spent stretching $F(4,28)=3.2$, $P<0.05$; twisting $F(4,28)=3.7$, $P<0.05$; and walking $F(4,28)=2.9$, $P<0.05$; and a decrease in the amount of time spent being quiet $F(4,28)=4.4$, $P<0.05$. The frequency of each of these significant behaviors was dose dependently increased by CTOP (or decreased, in the case of the quiet behavior). The three remaining observed behaviors, head moves, moving paws, and together showed no significant change in the number of occurrences (Fig. 1).

3.2. Naltrindole and nor-BNI

Neither the i.c. administration of the specific δ -opioid receptor antagonist naltrindole nor the specific κ -opioid receptor antagonist nor-BNI caused withdrawal behaviors

in the 7-day-old rat (see Figs. 2 and 3, respectively). There was no significant change in the frequency of occurrences of each of the observed behaviors by either one of the antagonists.

4. Discussion

The i.c. administration of the specific μ -opioid receptor antagonist CTOP caused withdrawal in the 7-day-old rat, significantly increasing the occurrence of the stretching, twisting, and walking behaviors, while decreasing the occurrence of the quiet behavior. CTOP did not significantly increase the occurrence of the head moves, moving paws, and together behaviors in the infant rats. The i.c. administration of the specific δ -opioid receptor antagonist naltrindole and the specific κ -opioid receptor antagonist nor-BNI, however, caused no significant changes in the occurrence of any of the observed behaviors in the 7-day-old rat.

4.1. The μ -opioid receptor

These results are in accordance with published pharmacological findings about the role of the μ -opioid receptor in the withdrawal behaviors displayed by adult rodents. The administration of CTAP (a derivative of the antagonist CTOP), naltrindole, and nor-BNI to morphine-dependent adult rats, resulted in the display of withdrawal signs from all three drugs [14]. However, the number of signs that were significantly increased by CTAP was far greater than the number of signs increased by either naltrindole or nor-BNI. In addition, when CTAP was combined with either naltrindole or nor-BNI, the degree to which the withdrawal behaviors significantly increased in appearance was no different from the degree to which the behaviors were elicited by CTAP alone [14]. Similar results were found with rats that were chronically administered the agonists DAMGO, DPDPE, and U-50,488H specific to the μ , δ , and κ (respectively) receptor classes. Upon administration of the nonselective opioid antagonist naloxone, the μ -opioid receptor agonist DAMGO yielded the highest abstinence scores [3].

MOR (morphine opioid receptor) lacking mice showed none of the usual physical withdrawal signs (jumping, teeth chattering, wet dog shakes, etc.) and no conditioned place aversion, after the administration of naloxone [15]. Despite these distinct behavioral differences, the mutant mice showed no difference in the total number and brain distribution of the δ - and κ -opioid receptors, or the expression of mRNA for the endogenous peptide genes, proenkephalin, prodynorphin, and proopiomelanocortin [15]. Thus the present data are consistent with the adult data.

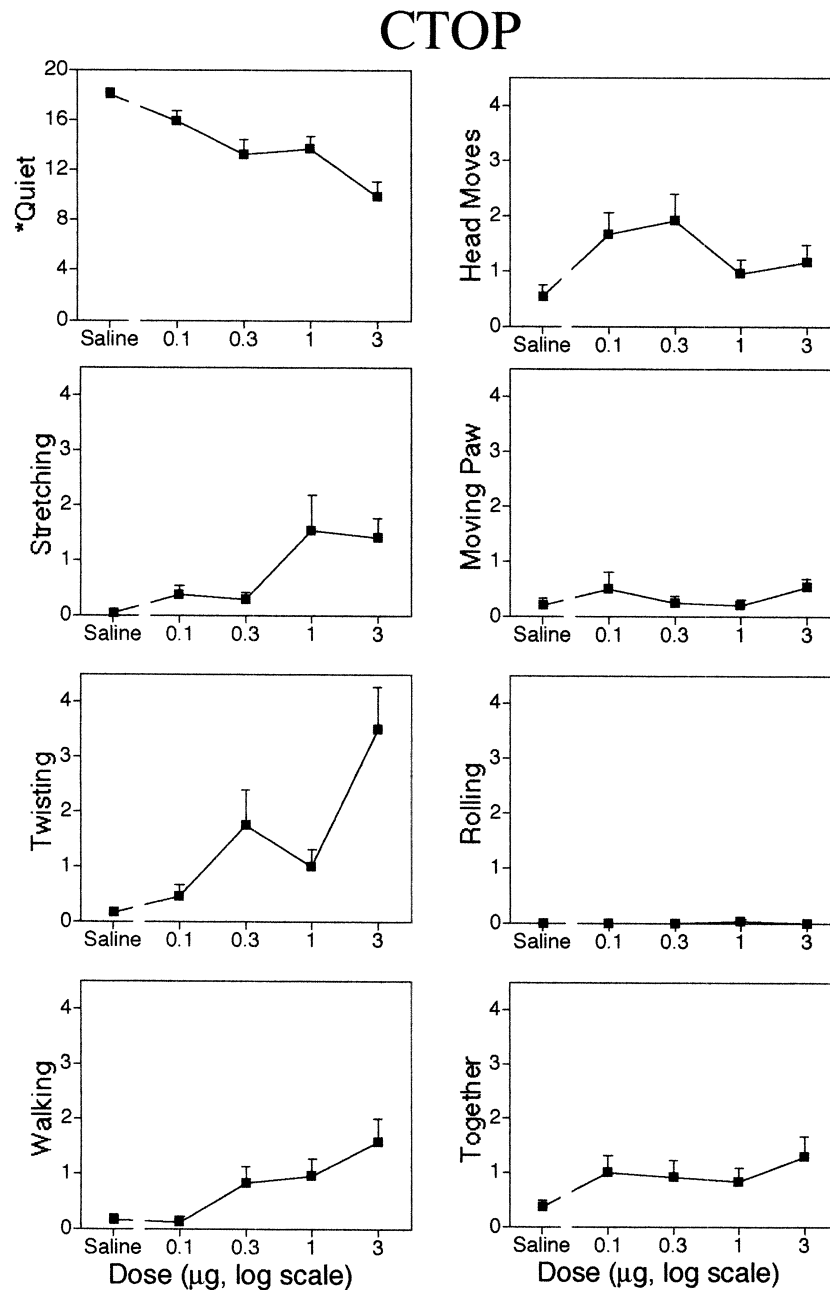


Fig. 1. Dose effect of CTOP. This figure shows the mean number (+S.E.M.) of occurrences, in 15 min, of various morphine withdrawal behaviors in the 7-day-old rat. The left column depicts behaviors that showed significant dose effects ($P < 0.05$). The right column depicts behaviors that showed no significant dose effects. *Quiet is presented on a different scale.

4.2. The κ -opioid receptor

As with the μ -opioid receptor, the κ -opioid receptor is detectable and functional (as measured by its ability to release dopamine) by gestational day 17 [4] and its mRNA is present by gestational day 13 [9] in the rat. In the mouse, binding [20] has been found as early as embryonic day 11.5, as well as the presence of mRNA for the κ -opioid receptor [31]. In the P1 (postnatal day 1) rat it is present in high concentrations and increases to reach its adult levels by age 30 [1,12,16]. Therefore the finding that

the blockade of the κ -opioid receptor did not result in any level of increase in the appearance of withdrawal behaviors in the 7-day-old rat was unexpected.

The role of the κ -opioid receptor in physical withdrawal behaviors in the adult is less clear. Pharmacological studies have found a more robust physical withdrawal syndrome in morphine-dependent adult rats pretreated with a single dose of nor-BNI, as compared to rats pretreated with saline [22,25]. In addition, the co-administration of U-50,488H and morphine in adult mice reduced the display of morphine induced locomotor activity and straub tail [24].

Naltrindole

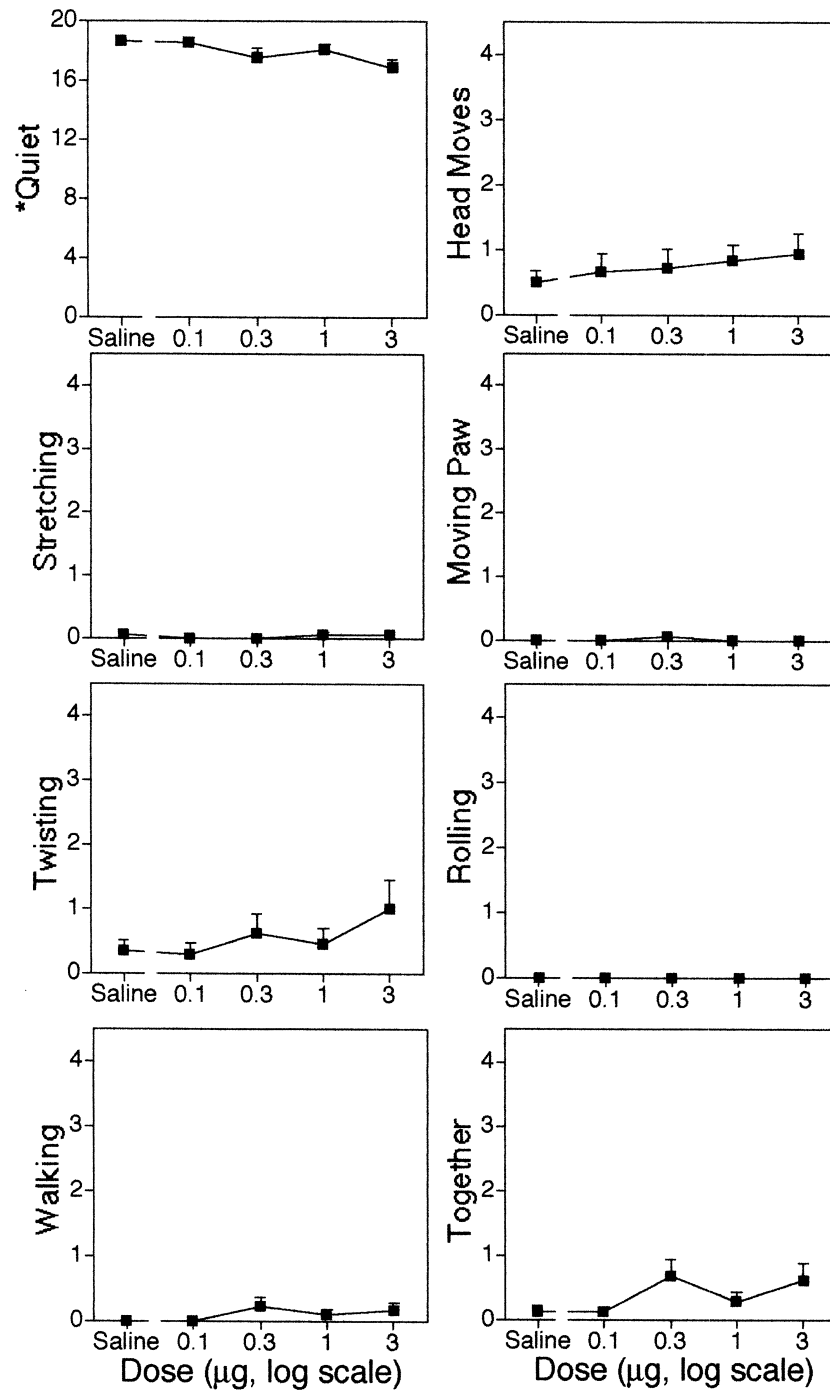


Fig. 2. Dose effect of naltrindole. This figure shows the mean number (+S.E.M.) of occurrences, in 15 min, of various morphine withdrawal behaviors in the 7-day-old rat. No behaviors showed any significant dose effects. *Quiet is presented on a different scale.

These findings suggest that one of the roles of the κ -opioid receptor in withdrawal may be to antagonize some of the effects of the μ -opioid receptor; although it is not clear if this is also the case in infant rats.

Genetic studies have found the absence of the κ -opioid receptor to play a more direct role in adult withdrawal. In

morphine-treated KOR (kappa opioid receptor)-deficient mice, the occurrence of physical withdrawal signs was significantly lower than that seen in wild-type mice [21]. As is the case with the MOR-deficient mice, no difference was found among KOR-deficient, and wild-type mice in terms of their expression of mRNA for the endogenous

nor-BNI

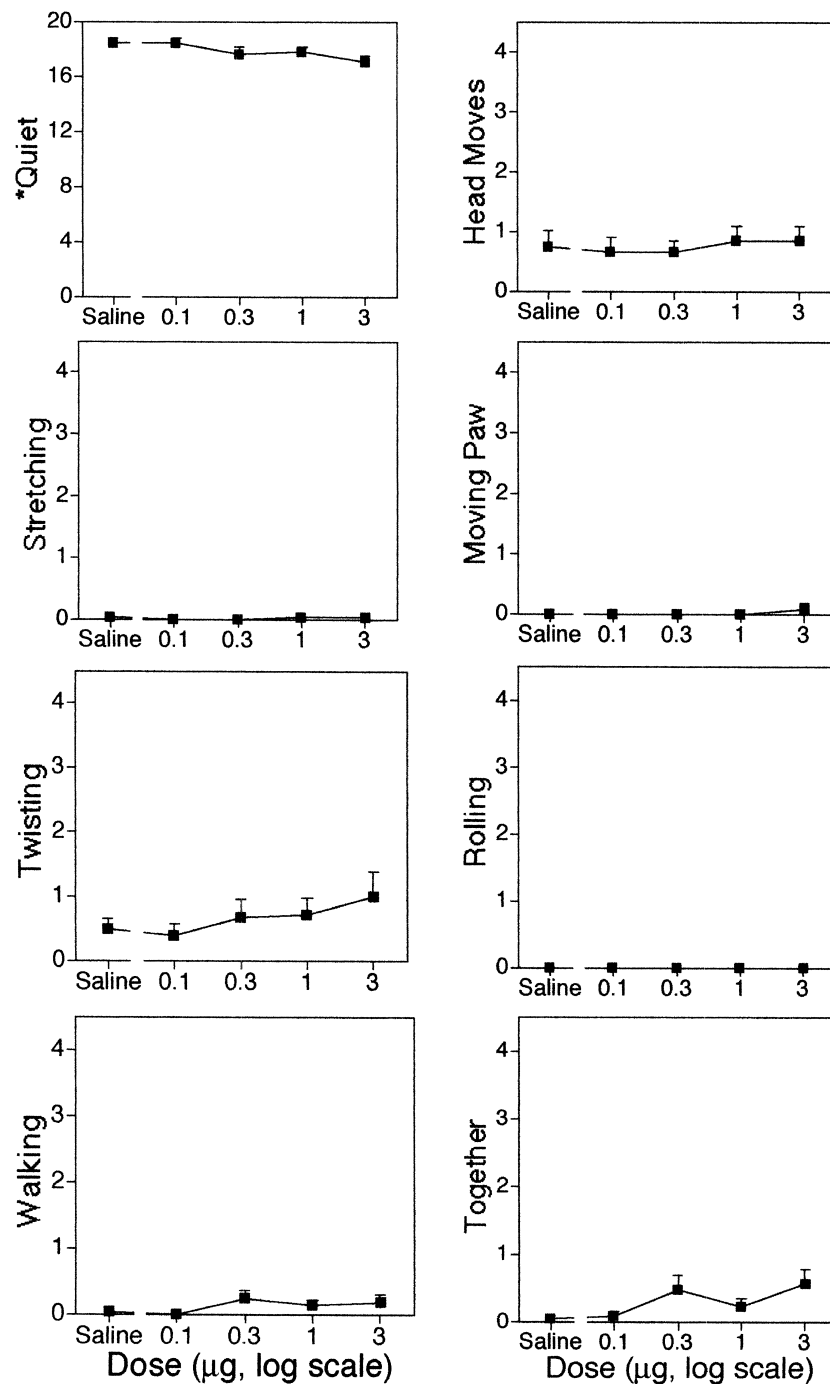


Fig. 3. Dose effect of nor-BNI. This figure shows the mean number (+S.E.M.) of occurrences, in 15 min, of various morphine withdrawal behaviors in the 7-day-old rat. No behaviors showed any significant dose effects. *Quiet is presented on a different scale.

opioid peptide genes, and the number and distribution of the δ - and μ -opioid receptors. Thus, the role of the κ -opioid receptor, in the morphine abstinence syndrome, is less clearly defined in both the adult and infant rat.

4.3. The δ -opioid receptor

The δ -opioid receptor protein is not present until at least postnatal days 5–7 [4,18,26] in the rat, although its mRNA

is detectable by gestational day 13.5 in the mouse [31]. Therefore, it was expected to play no role in the abstinence syndrome of the 7-day-old rat, as was the case in this study.

5. Conclusion

As was hypothesized, the μ -opioid receptor plays the greatest role in modulating the withdrawal behaviors seen in the 7-day-old rat and is therefore the predominant receptor modulating this behavior throughout the ontogeny of the rat. The appearance of behavioral withdrawal signs like paw movements, head movements, and rolling in the 7-day-old rat that are eventually replaced by behaviors like wet dog shakes, grooming, diarrhea, and ptosis, are mediated by the μ -opioid receptor as they are in the adult. However, it is apparent that there are mechanisms secondary to the μ -opioid receptor that play a significant role in the display of the behaviors that are unique to the infant. The nature of these secondary mechanisms is an area that requires exploration. The exact role of the κ - and δ -opioid receptors in dependence and the display of physical withdrawal behaviors is less clear, but the present evidence suggest that the δ - and κ -opioid receptors play no role in the display of the physical opiate withdrawal behaviors in the young rat.

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