Changing Mechanisms of Opiate Tolerance and Withdrawal during Early Development: Animal Models of the Human Experience

Gordon A. Barr, Anika McPhie-Lalmansingh, Jessica Perez, and Michelle Riley

Abstract

Human infants may be exposed to opiates through placental transfer from an opiate-using mother or through the direct administration of such drugs to relieve pain (e.g., due to illness or neonatal surgery). Infants of many species show physical dependence and tolerance to opiates. The magnitude of tolerance and the nature of withdrawal differ from those of the adult. Moreover, the mechanisms that contribute to the chronic effects of opiates are not well understood in the infant but include biological processes that are both common to and distinct from those of the adult. We review the animal research literature on the effects of chronic and acute opiate exposure in infants and identify mechanisms of withdrawal and tolerance that are similar to and different from those understood in adults. These mechanisms include opioid pharmacology, underlying neural substrates, and the involvement of other neurotransmitter systems. It appears that brain circuitry and opioid receptor types are similar but that NMDA receptor function is immature in the infant. Intracellular signaling cascades may differ but data are complicated by differences between the effects of chronic versus acute morphine treatment. Given the limited treatment options for the dependent infant patient, further study of the biological functions that are altered by chronic opiate treatment is necessary to guide evidenced-based treatment modalities.

Key Words: dependence; glutamate; infant; morphine; opiate; protein kinase; rodent model; tolerance; withdrawal

Introduction

Opiate tolerance to and withdrawal from opium and its derivatives have long been recognized, and reports of the deleterious effects of opiates on infants are over 100 years old (Happel 1892; Tate 1899). Today, infants may be exposed to opiates for medical reasons; for example, iatrogenic induction of opiate dependence is not uncommon in preterm infants in the neonatal intensive care unit (Anand et al. 2010). In addition, opiate use by pregnant women can result in dependence in the fetus. The rates of such use are difficult to ascertain and estimates vary widely, but some are as high as 2–4% of pregnant women (Keegan et al. 2010; Lester et al. 2001; Shannon et al. 2010).

Most of the research on mechanisms underlying tolerance and dependence has used animal, and especially rodent, models, so we provide comparisons of the developmental stages of rats and humans. These comparisons are at best estimates and clearly depend on the metric compared.

In terms of overall rates of protein synthesis in the brain, the rat is altricial and at birth is roughly equivalent to an early-third-trimester human fetus (Dobbing 1981); birth in the human would be equal to a 7-day-old pup (Figure 1). A more fine-grained analysis (Clancy et al. 2007) describes a range of developmental equivalencies depending on brain site. In this model, the birth of the rat infant "translates" to the human as the start of the second trimester for noncortical and limbic structures and the early third trimester for cortical structures. This places the rat at about 18 days of postnatal age when the human is born.

In this review we focus on mechanisms of tolerance and withdrawal after repeated or acute exposure of infants to opiates (mostly morphine), building on the work of others who have addressed similar topics (Noda and Nabeshima 2004; Richardson et al. 2006). (For reviews of the long-term effects of in utero and neonatal opiate exposure, see Lester and Lagasse 2010; Schempf 2007; Vathy 2002; and Yanai et al. 2003.)

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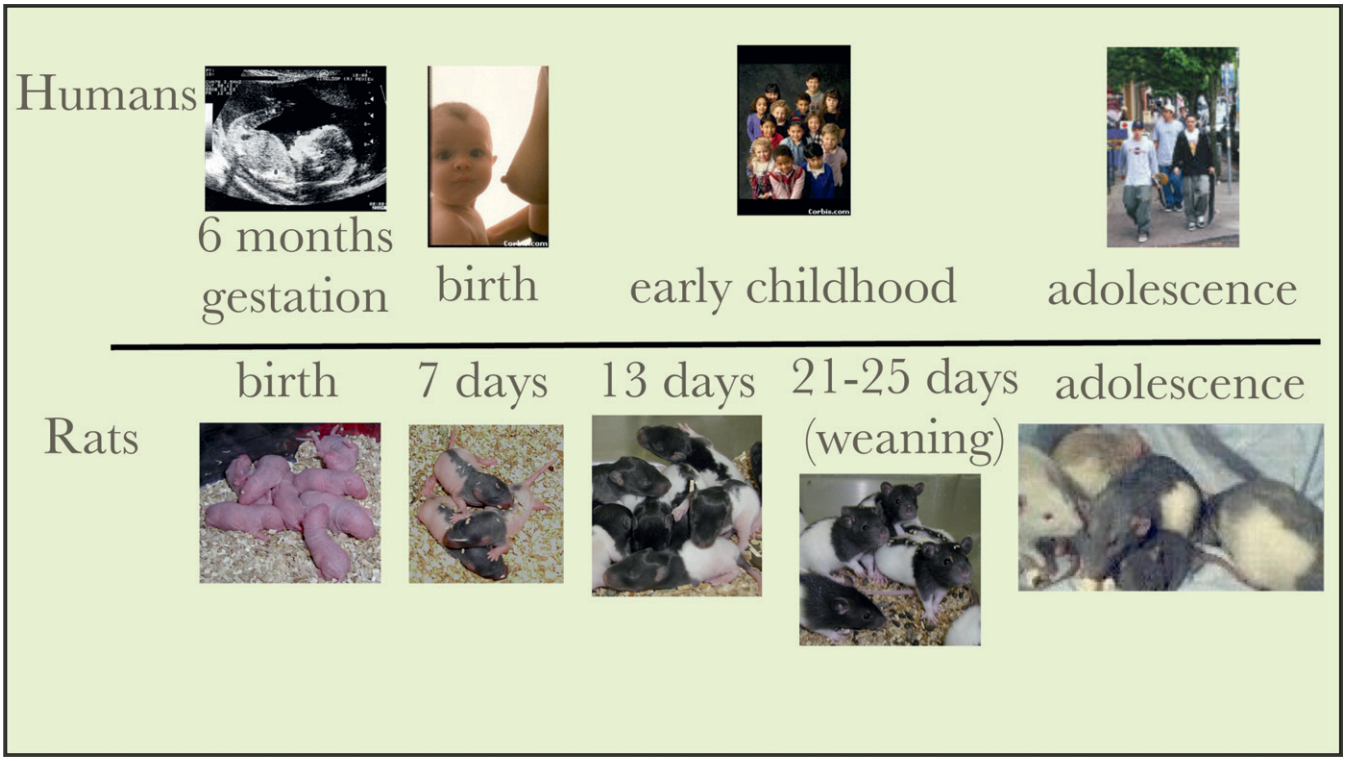

Figure 1 Illustration of rough equivalent ages for a rat and human based on rate of protein synthesis in the brain (Dobbing 1981). Other schemes, based on other criteria, show slightly different age equivalencies (Clancy et al. 2007) and individual brain regions and physiological functions develop at individual rates. Despite slight differences the rat is altricial and is developmentally similar at birth to the human fetus at 6 months.

Effects of Chronic Opiate Exposure in the Infant

Tolerance

Tolerance is defined as a decreased response to a drug after administration of or exposure to the drug; empirically it is defined as a shift to the right in the dose-response curve with an increased effective dose (ED_{50}) or effective concentration (EC_{50}) (Figure 2). The degree of tolerance may differ for different drug effects. It usually follows multiple administrations of the drug but can occur after a single injection (acute tolerance or tachyphylaxis). The mechanisms of tolerance to acute (single-dose) and chronic (repeated) exposure to the drug may differ. The maximum effect can also be reduced in some cases.

Numerous studies have shown that human and nonhuman infants become tolerant to the analgesic effects of opiates (Anand et al. 2010; Barr et al. 1986; Barr and Wang 1992; Ceger and Kuhn 2000; Richardson et al. 2006; Tempel et al. 1988; Thornton and Smith 1997; Thornton et al. 1997; Windh et al. 1995; Zissen et al. 2007). The degree to which tolerance develops is less for infants than that for adults for reasons that are not known but are likely to be pharmacokinetic and pharmacodynamic.

In addition to tolerance to the drug's analgesic actions, a variety of species (humans, rodents, sheep, and guinea pigs) exhibit tolerance to sedation, arousal changes in sleep state, and EEG and respiratory depression (Choe and Smith 2000; Eaton et al. 1992; Szeto et al. 1988, 1990). In contrast, there is no tolerance to morphine-induced suppression of distress vocalizations (Barr and Wang 1992). We are not aware of any data on the development of tolerance to the gastrointestinal effects (e.g., constipation) of morphine. Tolerance can also occur to drugs that target kappa-opioid receptors (Barr et al. 1986).

There are no data suggesting that the age of the human infant influences the magnitude or rapidity of tolerance to opiates. Indeed, tolerance in the infant rat is typically of a lesser degree than that of the older pup or adult rat (van Praag and Frenk 1991; van Praag et al. 1993; Windh et al. 1995; Zhu and Barr 2003).

Studies of acute tolerance, in which the test dose of the opiate takes place shortly after an initial dose, are rare but show that it does occur in the older preweaning rodent (Huidobro and Huidobro 1973). In one study a single injection of morphine to 2-day-old rat pups resulted in tolerance revealed by testing 3 weeks later (Bardo and Hughes 1981) but not when the initial injection was at 5, 9, or 13 days of age. This approach is not a test of "acute tolerance" in the strict sense, but it demonstrates long-term effects of opiate treatment in the infant and can indicate whether there is a critical period for the development of long-term consequences, although to our knowledge this critical period has not been clearly defined.

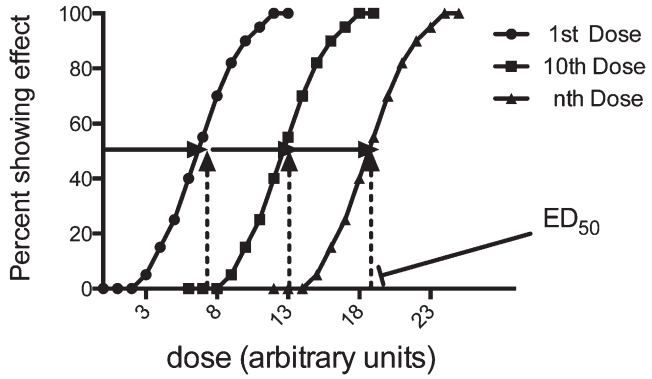

Figure 2 Schematic demonstrating tolerance. When a drug that can induce tolerance is given repeatedly, the dose response curve shifts to the right. (Sensitization, not shown, would be a shift to the left.) The dose that affects 50% of the subjects (ED_{50}) is then increased.

Dependence and Withdrawal

Dependence and withdrawal can be physical or psychological or both. Their intensity is a function of the drug, duration of use, dose, and the kinetics of the drug in addition to the age of the subject.

Physical dependence entails the presence of physical signs when the drug is withdrawn or when an antagonist to the drug's action is given. Such signs can be minor (e.g., a caffeine withdrawal headache) or life threatening (e.g., seizures from barbiturate or alcohol withdrawal). Physical signs of opiate withdrawal in human and nonhuman adults include flu-like symptoms such as muscle aches, runny nose, abdominal pain and diarrhea, dilated pupils, and nausea and vomiting.

Psychological dependence is a need to continue drug use and does not require physical signs. It can last well beyond the resolution of physical withdrawal signs. Humans have also described a state of dysphoria that exceeds in severity the actual physical symptoms.

Unconditioned Behaviors

The mechanisms by which opiates produce withdrawal in adults are well studied if not fully understood (e.g., Frenois et al. 2005a; Mao 1999; McClung 2006; Nestler et al. 1993). We focus here on mechanisms specific to the infant.

As mentioned above, very early studies demonstrated that a pregnant woman's use of opiates can have deleterious effects on the infant (Happel 1892; Shute and Davis 1933; Tate 1899), who may become passively dependent through placental transfer and experience abrupt withdrawal at birth. Withdrawal can also occur in neonates that have received opiates for pain management.

Upon withdrawal the infant experiences behavioral and state regulation disturbances, called the neonatal abstinence syndrome (NAS), which includes increased irritability, increased movements and activity, sucking and swallowing disturbances, sleep deprivation, and disorganized and fragmented sleep-wake states (Franck and Vilardi 1995; Gewolb et al. 2004; Hutchings 1990; O'Brien and Jeffery 2002). Among infants whose dependence is due to maternal opiate use there is no strong relationship between the type or dose of opiate and the severity and duration of withdrawal (Coghlan et al. 1999; Kuschel et al. 2004). In infants treated medically with opiates, withdrawal is measured by a recently validated psychometric tool that takes into account the presence and intensity of multiple withdrawal symptoms (Franck et al. 2008).

Animal models of opiate withdrawal have been developed only in the past 30 years. Part of the difficulty of defining withdrawal in infants is that its manifestations are fundamentally different from those of the adult. Opiate withdrawal in human or nonhuman adults includes, among other signs, activation of the sympathetic nervous system, but in the infant these processes are immature (Myers et al. 1992; Quigley et al. 1996); thus classic signs in the adult rodent, such as teeth chattering, jumping, diarrhea, "wet-dog shakes," and ptosis (which cannot occur in infant rodents, whose eyes have not yet opened), do not occur between 14 and 21 days of age in the rat (Jones and Barr 1995).

Early rodent studies reported that infants in withdrawal show altered activity and sleep-wake rhythms (Hutchings et al. 1979, 1980; Kirby 1981). Subsequent work demonstrated that, in addition to increased behavioral activation, there are clearly defined behaviors for the NAS in rodent infants, postweanlings, and indeed throughout development (Figure 3; Barr et al. 1998; Jones and Barr 1995; Thornton and Smith 1997; Thornton et al. 1997; Windh et al. 1995). Infant behaviors include increased ultrasonic vocalizations upon separation from the dam and littermates, head swaying, paw movement, and rolling (Table 1; Barr et al. 1998; Jones and Barr 1995; Thornton and Smith 1997; Thornton et al. 1997; Windh et al. 1995). Administration of naloxone after a single opiate injection induces similar withdrawal signs (Jones et al. 2002; Perez-Saad et al. 1996). Some withdrawal behaviors also occur in the fetal rat after precipitated withdrawal when the dam has been treated with morphine (Ceger and Kuhn 2000; Jones and Barr 2000; Kirby 1981).

Sensitization

Hyperalgesia (heightened sensitization to pain) can occur during either chronic administration or withdrawal, but there is no consensus about the frequency or circumstances of its occurrence (for reviews, Bannister and Dickenson 2009; Bekhit 2010). In animal models only one laboratory has examined sensitization after opiate treatment in infants (Sweitzer et al. 2004a,b; Zhang and Sweitzer 2008; Zissen et al. 2007). Withdrawal induced mechanical allodynia and thermal hyperalgesia in rat pups as early as 7 days of age and in some cases lasted weeks (Zhang and Sweitzer 2008). The sensitization occurred after acute, chronic, or intermittent morphine treatment, although there were differences among

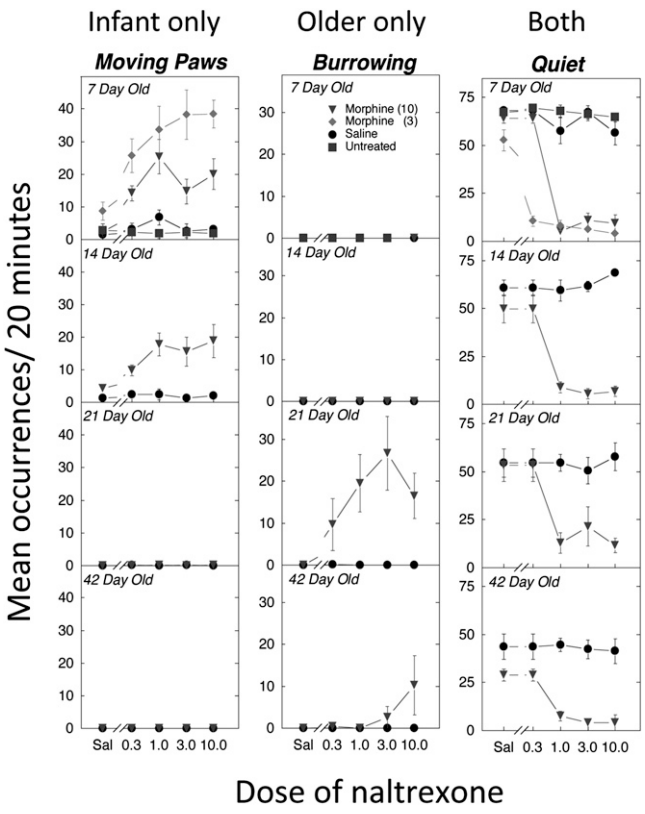

Figure 3 Examples of behaviors that are unique to infant rats (7 days old), occur only in older rats (21+ days old), or occur throughout the lifespan (adapted from Jones and Barr 1995). See Table 1 for definitions of the behaviors. Numbers after morphine are the chronic doses (3 or 10 mg/kg 2x/day) begun 7 days before testing. Sal, saline

the treatment regimens (Sweitzer et al. 2004a; Zissen et al. 2006, 2007).

Sensitization may be the result of central or peripheral processes, and there is enhanced nociception during the second phase of the formalin test (a measure of central and peripheral sensitization; Zissen et al. 2006) and enhanced slow ventral root potential (sVRP[1]), an electrophysiological correlate of nociception in an ex vivo spinal cord preparation (Sweitzer et al. 2004b).

Affective Consequences

In adult humans and animals, opiate withdrawal is associated with a strong negative affect (Fendt and Mucha 2001; Handelsman et al. 1992; Kanof et al. 1993; Koob et al. 1989; Mucha et al. 1986). In the human infant, the interpretation of negative affective consequences of withdrawal is drawn from increased fussiness, increased crying, and a decreased ability to be soothed. With opiates that have a short half-life (e.g., heroin, fentanyl), it is possible that the human fetus undergoes withdrawal after exposure to the opiate. It is not known whether withdrawal induces a negative affective state in the fetus (Handelsman et al. 1992; Kanof et al. 1993). If so, the fetus, which can learn in utero (e.g., DeCasper and Fifer 1980; Smotherman 2002; Stickrod et al. 1982; for review, Moon and Fifer 2000), could associate maternal cues (e.g., odors) with that aversive state. That association might affect later attachment to the mother, although to our knowledge there are no data that bear on this speculation.

The question of whether opiate withdrawal in the infant is aversive has been the subject of animal studies. In the infant rodent, withdrawal from a variety of dysphoric drugs increases ultrasonic vocalizations, a behavior normally expressed under stressful conditions such as cold ambient temperature. At 7 days of age, pups spontaneously withdrawn from chronic morphine cry more than controls 6 hours after the last injection and show altered ultrasonic vocalization patterns 3 days later (Barr and Wang 1992).

It is not clear, however, that the aversive properties of withdrawal can be conditioned in infant rats, in part because, unlike older pups and adults, they are resistant to learning to associate cues with aversive stimuli (Sullivan et al. 2009): in a conditioned odor aversion paradigm, pups in precipitated withdrawal did not learn to avoid an odor associated with withdrawal at 7 days of age, but did so at 14 days (Barr and Goodwin 1997). This is not due to an inherent inability to learn aversions since younger pups can learn these associations under certain circumstances (Barr et al. 1994; reviewed by Sullivan et al. 2009). Thus, although conditioned aversions are not learned early (7 days of age), unconditioned responses associated with an aversive state are present quite early and show the negative affective component of opiate withdrawal.

Mechanisms of Opiate Tolerance and Withdrawal in the Infant

There are multiple possible reasons for different withdrawal syndromes in the neonate and the adult. Given the immaturity of the central nervous system (CNS) in the infant, it is likely that the neural mechanisms that mediate tolerance and withdrawal in the infant differ from those of the adult in at least two ways. First, there may be age-related differences in cellular mechanisms that mediate withdrawal, including changes in receptor populations, their ability to be internalized, intracellular messengers, and/or transcription factors. Second, different neural circuitry may mediate withdrawal in the infant and the adult. In their most simple form, the CNS circuits may be similar across ages, whereas the output mechanisms—the autonomic nervous system, for example—may differ. As discussed in the following sections, the literature suggests that the anatomical circuits are at least similar and that the opioid receptor involved is the same. In contrast, the role of intracellular signals, in particular those related to glutamate *N*-methyl-D-aspartate (NMDA[1]) receptors, appears to differ.

[1]Abbreviations that appear ≥3x throughout this article: NMDA, *N*-methyl-D-aspartate; PAG, periaqueductal gray of the midbrain; PK, protein kinase; PND, postnatal day; sVRP, slow ventral root potential

Table 1 Precipitated withdrawal behaviors in the infant rat

Behavior	Definition
Burrowing	Sliding the body under shavings in the observation chamber
Head swaying	Lateral and/or rotary motion of the head
Paw movement	Continuous movement of the hind paws without walking
Quiet	"Sedated" appearance without movement
Rolling	Turning the body over at least one full rotation
Ultrasonic vocalization	Vocalizations in the ultrasonic range (typically ~40 kHz) that imply distress in infant rodents
Together	Bodily contact with one or more littermates
Walking	Moving forward at least one step
Wall climbing	Putting both forepaws on the wall of the observation chamber, typically with movement

Adapted from Zhu and Barr (2004).

Opiate Receptor Development

Because opioid receptors develop at different stages (Leslie and Loughlin 1993)—for example, in rats the delta opioid receptor does not appear until the second week of life, comparable to early childhood in humans (De Vries et al. 1990; Leslie et al. 1982; Spain et al. 1985)—it is possible that different classes of opioid receptors mediate withdrawal in infants and adults. Indeed, studies have shown that the effects of chronic morphine treatment on receptor dynamics are age dependent. When pups are treated with morphine starting at postnatal day (PND[1]) 1, there is a downregulation of mu opioid receptor numbers at PND 4 that is not seen at PND 8 or older, even with continued treatment (Stoller et al. 2002; Tempel 1991; Tempel et al. 1988). This downregulation may be because of unique properties of opioid receptors shortly after birth, but what those properties may be is not known.

Withdrawal is mediated by similar opioid receptor types: delta (DOR), kappa (KOR), and mu (MOR). In young pups (less than a week old) mu opioid receptors are the major receptor type involved in withdrawal (McPhie and Barr 2000), although the animals may develop tolerance to drugs that prefer the kappa opioid receptor (Barr et al. 1986). Antagonists to the delta or kappa opioid receptor did not precipitate behavioral withdrawal in the 7-day-old pup, whereas an antagonist to the mu opioid receptor did; similarly, in adults, mu opioid receptors regulate morphine-induced tolerance and dependence (Dumas and Pollack 2008; Raehal and Bohn 2005). In pups treated from PND 14 to PND 17 with morphine, the kappa agonist U50,488 is less effective than in controls, suggesting that chronic morphine exposure alters kappa receptor function (Stoller et al. 2007). Although the mu opioid receptor density and affinity are unaltered in the older pups, they still exhibit tolerance and withdrawal (Stoller et al. 2002; Tempel et al. 1988).

Changing Neural Circuitry

One possible reason for differences in the withdrawal behaviors of infants and adults is differential involvement of neural circuits: the periaqueductal gray (PAG[1]), locus coeruleus (LC), amygdala, ventral tegmental area, nucleus accumbens, hypothalamus, and spinal cord (for adult circuitry see Chieng et al. 1995; Druhan et al. 2000; Frenois et al. 2005b; Maldonado et al. 1992). It appears, however, that similar neural circuits are involved at both stages of life. Direct injection of an opiate antagonist into either the PAG or the LC precipitated withdrawal in a morphine-dependent 7-day-old pup, whereas injections into the amygdala did not (Jones and Barr 2001). This latter finding is not necessarily inconsistent with the adult literature. The amygdala mediates the aversive properties of opiate withdrawal in the adult (Maldonado et al. 1992) but learned aversions to opiate withdrawal are not present in the infant rat at PND 7 (Barr and Goodwin 1997).

Late maturation of the ability to learn a conditioned aversion is not limited to opiate withdrawal as the immaturity of amygdala function limits it ability to regulate other learned aversions (Sullivan et al. 2009). Moreover, withdrawal activates the same brain circuits—in the olfactory bulb, nucleus accumbens, hypothalamus, PAG, LC, medulla oblongata, and spinal cord (assessed by Fos protein or messenger RNA expression)—in both the infant and the adult (Maeda et al. 2002; McPhie and Barr 2009). However, there are developmental differences in activation patterns between the infant and adult medulla (Maeda et al. 2002).

Role of Glutamate Neurotransmission

NMDA Receptors: Chronic Opiate Exposure

In the adult animal, NMDA blockers reduce the development and expression of opiate withdrawal and tolerance

(Noda and Nabeshima 2004; Trujillo 2000), whereas in the infant these blockers are less effective in alleviating either tolerance or withdrawal until the animal begins weaning (for review, Noda and Nabeshima 2004; Zhu and Barr 2001).

Tolerance and withdrawal can be defined by behavioral changes or by changes in the sVRP after dorsal root simulation in the isolated spinal cord (Yanagisawa et al. 1984). One advantage of the in vitro spinal cord preparation is that it bypasses changes that might be due to other physiological systems.

In pups younger than 8 days of age, coadministration of the NMDA antagonists MK-801 or dextromethorphan with morphine does not reduce tolerance or withdrawal but rather can exacerbate both (Bell and Beglan 1995a,b; Zhu and Barr 2000, 2003). In older animals NMDA blockers become effective in reversing behavioral tolerance or withdrawal—they are somewhat effective at PND 14 and fully effective at PND 21 in reducing both tolerance and withdrawal (Zhu and Barr 2000, 2003). In pups 12 to 17 days of age, 3 days of twice-daily morphine treatment downregulated glutamate transporter activity and may thus have increased NMDA receptor activation because of the resulting higher levels of extracellular glutamate (Thomson et al. 2006). Further research is necessary to determine whether this mechanism occurs in younger animals, when NMDA antagonists do not have the ability to prevent withdrawal.

Acute treatment with MK-801 affects the expression (but not the development) of withdrawal in older animals but is mostly ineffective in the 6- to 7-day-old pup. Although it decreased head moves, it increased walking, wall climbing, and overall locomotor activity (Zhu and Barr 2000). In contrast, it reduced behavioral tolerance in older pups (14 days of age), reduced the sVRP in the isolated spinal cord preparation (likely by a synergistic action with morphine; Bell and Beglan 1995b), and inhibited excitatory postsynaptic currents (EPSCs) in voltage-clamped cells in spinal slices (Zeng et al. 2006).

Fos expression in the olfactory bulb, hypothalamus, and medulla of the infant rat is stimulated during withdrawal (Maeda et al. 2002). This heightened level of expression was reduced by concurrent treatment with MK-801 and morphine from PND 2 to PND 7 in the olfactory bulb and hypothalamus but not in the medulla (Maeda et al. 2002).

In a different approach, we used two strains of mice to assess the role of the NMDA glutamate receptor in tolerance to morphine during early development. Adult mice of the 129 strain display little or no analgesic tolerance to morphine, whereas other strains such as CD-1 and Swiss-Webster mice become tolerant (Crain and Shen 2000; Kest et al. 2002; Kolesnikov et al. 1998; Liang et al. 2006). It has been hypothesized that the mouse strains differ in functional NMDA receptor function (Kolesnikov et al. 1998) and that deficiencies in GM1 ganglioside regulate excitatory opioid receptor function (Crain and Shen 2000). Thus the 129 strain either lacks or has an impaired receptor.

If the NMDA receptor is *not* important in tolerance in the infant then the lack of its functionality should have no consequence—the 129 and CD-1 infant mice should respond the same to chronic morphine exposure; in older mice, for which the NMDA receptor is important, the strains should differ. To assess this hypothesis we injected CD-1 and 129S6 pups with morphine starting on either PND 2 or PND 16 for 7 days and tested for analgesic tolerance. At PND 8, both types of mice showed tolerance in a tail flick test, whereas by PND 22 the 129S6 pups no longer did (Figure 4). The data are consistent with adult data showing that there is no role for the NMDA receptor in tolerance in the 8-day-old mouse but a necessary involvement at PND 21 (Perez and Barr, unpublished).

NMDA Receptors: Acute Opiate Exposure

A single injection of morphine can induce tolerance and establish dependence, and a subsequent injection of an NMDA blocker reduces withdrawal, tolerance, and Fos expression (Jones et al. 2002). Acute morphine treatment does not induce many of the neuroplastic changes—for example on receptors or second messenger systems (for review, Zhang et al. 2009)—that chronic treatment would. In the first week of life in the rat, these changes, induced by chronic morphine exposure, are not NMDA dependent. NMDA antagonists are effective, however, when given either after or with chronic treatment with morphine after PND 21.

Non-NMDA Glutamate Receptor Effects

We are aware of only one study that examined non-NMDA glutamate receptor effects after chronic opiate exposure during early development (Zhu and Barr 2004). Use of both a behavioral model and the isolated spinal cord preparation showed that an acutely administered group II metabotropic glutamate agonist and/or an AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) blocker reduced withdrawal in 7-day-old rat pups. Whether they would block neuroplastic changes if given concurrently with morphine is not known.

Nitric Oxide

The production of nitric oxide via activation of the NMDA receptor may facilitate the development and expression of morphine-induced tolerance and dependence (Elliott et al. 1995; Inturrisi 1997; Thorat et al. 1994; Trujillo 1995; Vaupel et al. 1995a,b). Very little is known about nitric oxide and its effects on withdrawal in infants but the one existing study showed that acute inhibition of nitric oxide synthase by either L-NAME (NG-nitro-L-arginine methyl ester) or 7-nitroindazole blocked withdrawal behaviors (Zhu and Barr 2000).

Other Neurosubstrates: Substance P

There is a single study on the role of substance P (SP) in tolerance in infants (Thomson et al. 2008). The data, from 12- to 17-day-old pups, show a downregulation of the neurokinin-1 (NK1) receptor and a loss of SP effects in lamina 1 of the spinal cord, but not in the dorsal root ganglia, after twice-daily morphine treatment (Thomson et al. 2008). This

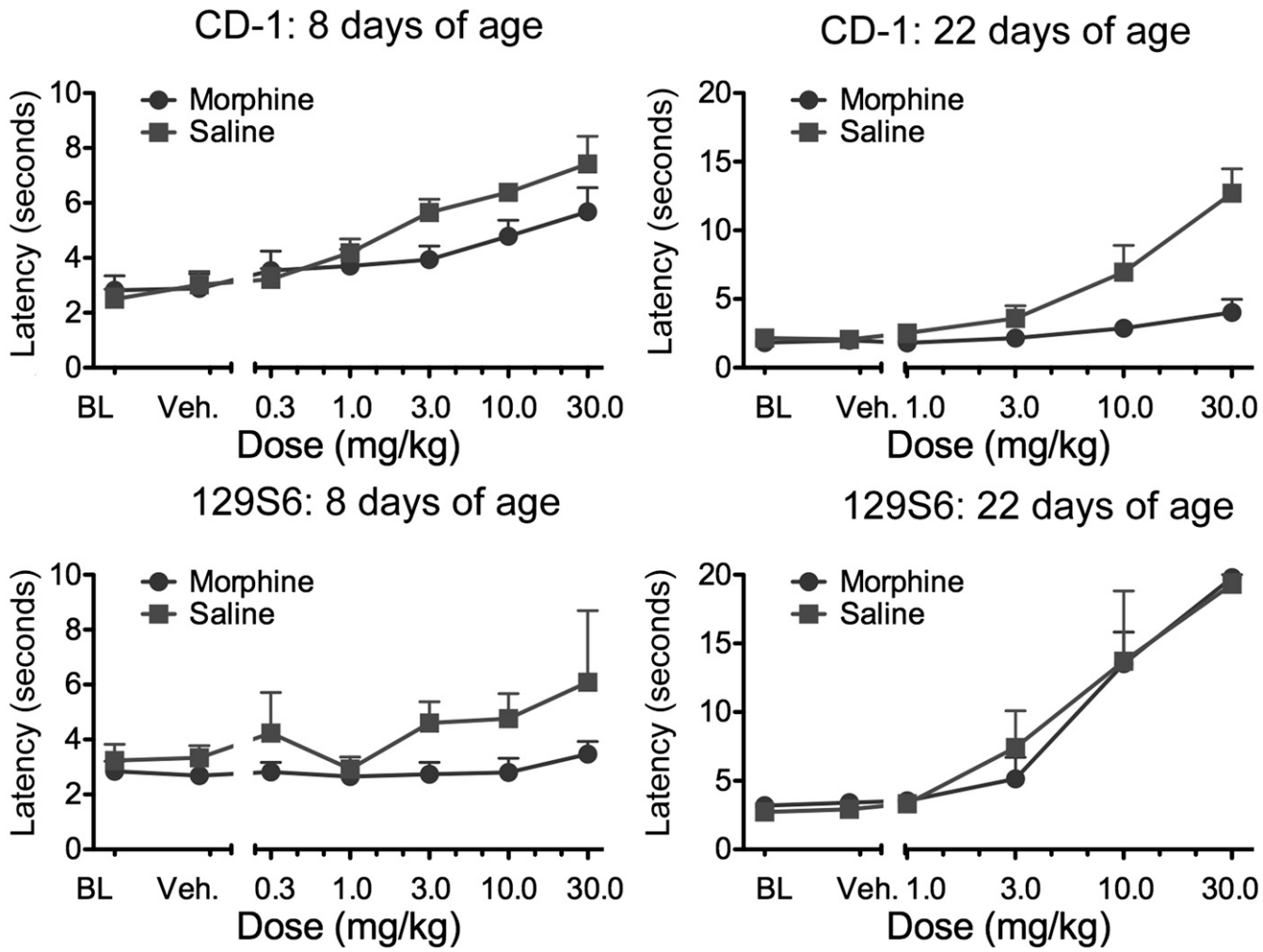

Figure 4 The development of tolerance in CD-1 and 129S6 mice. Pups were injected twice daily for 6½ days starting at either 1 or 15 days of age (N = 3–9 per condition). At 8 or 22 days of age, respectively, they were tested for analgesia in a cumulative dose response paradigm with morphine using the tail immersion test. At 8 days of age, both strains showed tolerance, but at 22 days only the CD-1 mice were tolerant. Thus the deficit in the 129S6 mouse—whether a lack of functional NMDA receptors or deficiencies in GM1 ganglioside–regulated excitatory opioid receptor function—has no influence on tolerance in the infant. In the older pup, the deficit has functional consequences (Perez and Barr, unpublished data). BL, baseline; NMDA, *N*-methyl-D-aspartate; veh, vehicle

result is the opposite of that found in adults, in which continuously infused morphine increased NK1 receptor internalization (King et al. 2005). Thomson and colleagues attributed the results mostly to differences in the treatment protocol (twice daily injection vs. continuous infusion), but they may also be age dependent because SP has a late-developing role in nociception in the infant (King and Barr 2003; King et al. 2000a,b). More research is necessary to improve understanding of the role of substance P in opiate tolerance.

Protein Kinase Signaling Cascades

Withdrawal from Acute Morphine Exposure

In the adult animal, morphine alters intracellular signaling pathways (Figure 5; reviewed by Chen and Sommer 2009; Zhai et al. 2008; Zhang et al. 2009), but few studies have examined these cascades in early development. We summarize those here.

A broadly acting protein kinase (PK[1]) C antagonist blocked spontaneous or precipitated withdrawal after a single injection of morphine both in vivo in rats and in vitro (sVRP in isolated rat spinal cord) at PND 7 (Sweitzer et al. 2004b). Calcium-independent PKC antagonists (but not calcium-dependent PKCγ antagonists) blocked precipitated thermal hyperalgesia and the increased sVRP response; antagonists to both PKCs blocked spontaneous withdrawal (Sweitzer et al. 2004b). Sweitzer and colleagues (2003) also examined the role of PKCγ and PKCε in naloxone-precipitated withdrawal, which induced both allodynia and withdrawal behaviors at 7 and 21 days of age. When withdrawal was precipitated shortly after acute morphine (30 minutes), PKCε but not PKCγ contributed to withdrawal at PND 7. Both isoforms

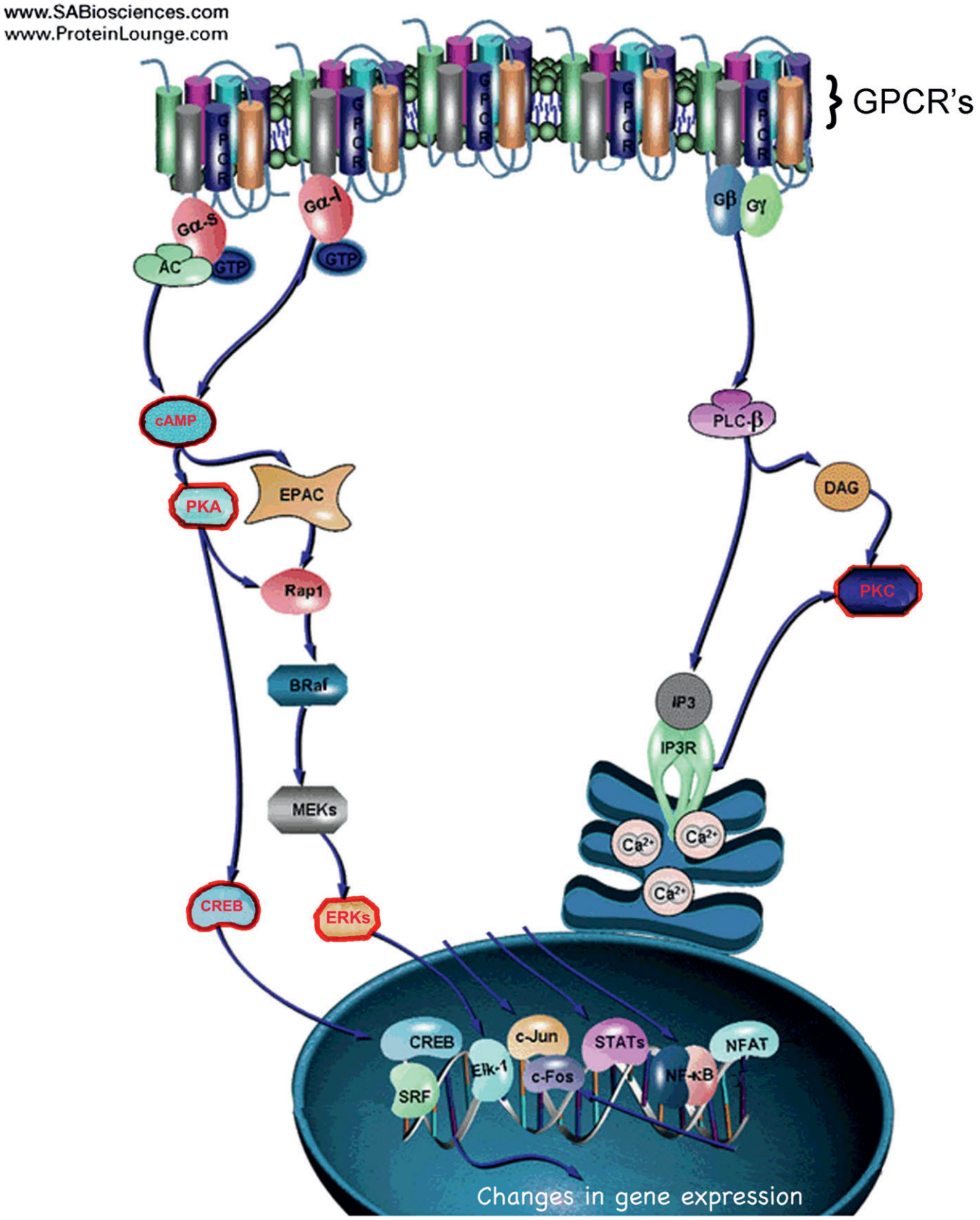

Figure 5 Schematic diagram of protein kinase (PK) A and PKC intracellular signaling pathways by which G protein–coupled receptors (GPCRs) activate cAMP and other signaling molecules and thus affect gene expression. Adapted from SABiosciences/Protein Lounge.

were involved at PND 21. With later withdrawal (120 minutes after morphine administration), both isoforms contributed to withdrawal at both ages.

Because PKCε is located in dorsal root ganglia and PKCγ is concentrated in the spinal cord, both of which are immature at PND 7, these effects point to the need to consider age-related anatomical changes in the circuits that regulate the effects of morphine.

Withdrawal from Chronic Morphine Exposure

In experiments with rodents that examined the role of PKC and PKA, we found little evidence for their involvement in either tolerance or withdrawal at PND 7 after repeated twice-daily morphine treatment (Figure 6; McPhie and Barr, unpublished). Acute injection of a general PK blocker or of specific PKC or PKA antagonists did not reduce tolerance after 13 twice-daily morphine injections (administered over 6½ days; Figure 6). These drugs also do not reduce behavioral withdrawal signs (data not shown). Concurrent blockade of protein kinases during the establishment of tolerance and dependence might be effective but those experiments have not been conducted.

In a different set of experiments, we examined changes in levels of other signaling molecules. These protein kinases—pAkt, pERK,[2] pCREB,[3] and pCaMKIIa[4]—are regulated by opioid signaling and also modulate neuronal plasticity, transcription, and cell survival in the adult. We injected pups twice daily starting at PND 1 or PND 14 and assayed them either 4 hours after the last morphine injection (to assess tolerance) or after precipitated withdrawal. We then performed immunohistochemistry and counted cells stereologically in the PAG and spinal cord; we present the PAG data here (Riley and Barr, unpublished). Fos expression in both groups of animals was increased by chronic morphine and augmented further after the administration of naltrexone to precipitate withdrawal (data not shown). pAkt and pERK were increased by chronic morphine but not further enhanced in withdrawal (Figure 7). pCREB was unaltered in the PAG (Figure 7) but enhanced in the spinal cord (not shown).

These differences in intracellular signaling molecules do not easily map to age-dependent differences in behavior. Perhaps the levels of activated phosphorylated proteins are less important than the dynamics that stabilize their absolute levels even as their functional activity is altered. For example, basal activity of the isoforms PKCα and PKCγ remains unchanged by prenatal heroin exposure, whereas the cholinergic receptor–induced translocation and activation of PKCγ and PKCβII were lost (Shahak et al. 2003; Yaniv et al. 2004). Unfortunately, the only studies on PK isoforms are on acute withdrawal, so their role in the longer-term effects of chronic morphine is not known.

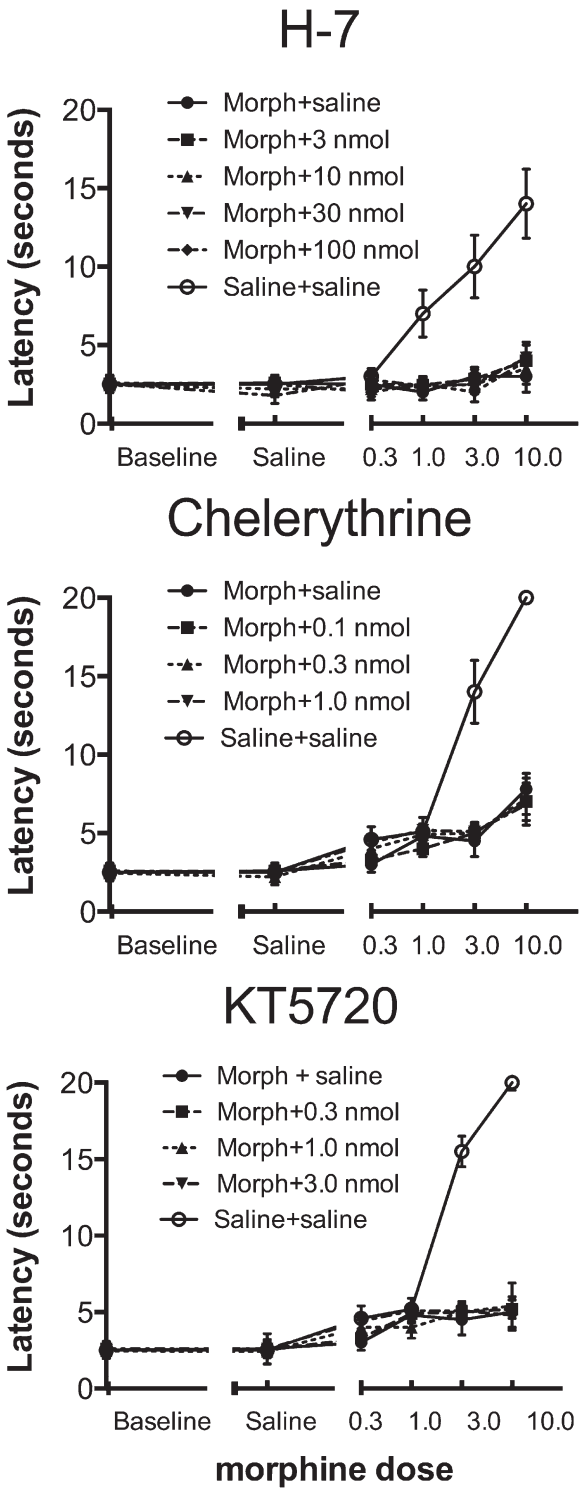

Figure 6 Rat pups were made tolerant to morphine by 13 twice-daily injections (10 mg/kg) from postnatal day (PND) 1 to PND 7, after which we used a cumulative dose-response paradigm to test for tolerance to the drug's analgesic effect. We injected H-7, chelerythrine, and KT5720—broadly acting protein kinase (PK), PKC, and PKA antagonists, respectively—before the tolerance test (a thermal tail immersion test of nociception). None of these drugs reduced tolerance at any dose (McPhie-Lalmansingh and Barr, unpublished data). Morph, morphine; nmol, nanomole

[2]pERK, phosphorylated extracellular-signal-regulated kinase

[3]pCREB, phosphorylated cyclic adenosine monophosphate (cAMP) response element binding [protein]

[4]pCaMKII, calcium (Ca^{2+})/calmodulin-dependent protein kinase II

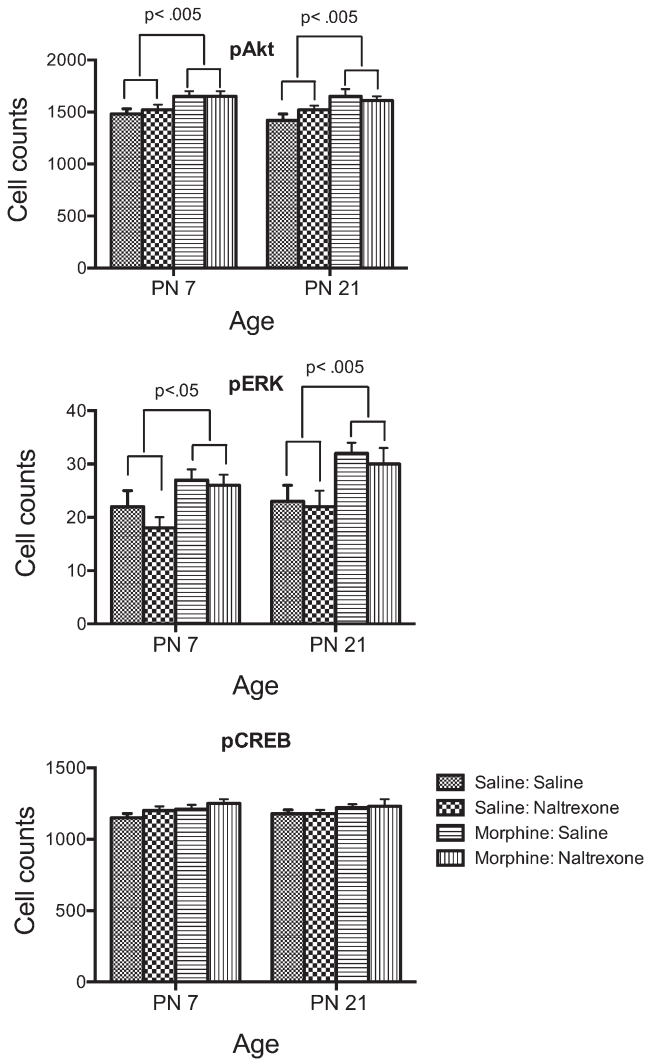

Figure 7 pAkt, pCREB, and pERK in the periaqueductal gray. Rat pups were treated as described in Figure 6. At postnatal day (PND) 7 and 21, pAkt and pERK were enhanced by chronic morphine but not further increased in withdrawal (Riley and Barr, unpublished data). There were no changes in pCREB at either age for any treatment. pCREB, phosphorylated cyclic adenosine monophosphate (cAMP) response element binding [protein]; pERK, phosphorylated extracellular-signal-regulated kinase

Endothelin

Endothelins are strong vasoconstrictors. Endothelin-1 is released at the site of tissue injury, interacts with its receptors, and enhances pain in both adults and infants (McKelvy et al. 2007; McKelvy and Sweitzer 2008). Endothelin-1 injection in a rat's rear paw in infancy, on PND 7 or PND 11, decreased morphine-induced analgesia (i.e., tolerance) at PND 21 (McKelvy and Sweitzer 2009) in a sex-dependent manner, and the treatment on PND 7 reduced mu opioid receptor expression in the hindpaw skin (McKelvy and Sweitzer 2009).

Acetylcholine

Cholinergic neurotransmission has been suggested to play a role in morphine withdrawal in the infant rat. Acute withdrawal (head shaking) precipitated in 9-day-old pups by naloxone or nalorphine can be blocked by spiroperidol, clonidine, and scopolamine (Perez-Saad et al. 1996). However, only scopolamine shifted dose-response curves without altering maximum effect, showing that it is specific and the others are not. The authors argue for a specific role of cholinergic neurotransmission in morphine withdrawal in the infant rat. Unfortunately, we are not aware of any follow-up studies to confirm or extend these data.

Treatment

There is no strong evidence of effectiveness for any treatments of human neonatal abstinence syndrome other than the use of opiates for tapering (for recent thorough reviews on treatment of infants for opiate dependence, Osborn et al. 2010a,b). Environmental manipulations such as dimming lights or reducing noise are typically unsuccessful. The careful administration of opiates likely reduces both the time to regain lost birth weight and the duration of required supportive care, although such use may lengthen hospital stays.

Opiates are also likely superior to clonidine, phenobarbitone, and diazepam in reducing infant withdrawal syndrome. Osborn and colleagues (2010a,b) recommend initial opiate treatment for NAS infants but point to methodologic limitations in most studies and suggest that further research is needed to address many questions, including, for example, the effects of adding barbiturates or clonidine to opiates.

Summary

It is clear that human and nonhuman infants can become tolerant to opiates and experience withdrawal. The pattern of withdrawal differs and tolerance is less profound than in adults, likely because of the immaturity of neural systems that mediate both experiences. Although the nature of that immaturity is not known, there is reason to believe that it includes glutamate neurotransmission. The mechanisms underlying acute and chronic tolerance and dependence differ, and the latter probably involve neuroplastic changes that are not associated with acute tolerance or dependence. However, there have been leads not followed; for example, the roles of acetylcholine, substance P, and the endothelins remain to be clarified.

Further research in all these areas will enhance understanding of the mechanisms underlying opiate-induced neural changes.

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