Neonatal Animal Models of Opiate Withdrawal

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Abstract

The symptoms of opiate withdrawal in infants are defined as neonatal abstinence syndrome (NAS). NAS is a significant cause of morbidity in term and preterm infants. Factors, such as polysubstance abuse, inadequate prenatal care, nutritional deprivation, and the biology of the developing central nervous system contribute to the challenge of evaluating and treating opiate-induced alterations in the newborn. Although research on the effects of opiates in neonatal animal models is limited, the data from adult animal models have greatly contributed to understanding and treating opiate tolerance, addiction, and withdrawal in adult humans. Yet the limited neonatal data that are available indicate that the mechanisms involved in these processes in the newborn differ from those in adult animals, and that neonatal models of opiate withdrawal are needed to understand and develop effective treatment regimens for NAS. In this review, the behavioral and neurochemical evidence from the literature is presented and suggests that mechanisms responsible for opiate tolerance, dependence, and withdrawal differ between adult and neonatal models. Also reviewed are studies that have used neonatal rodent models, the authors’ preliminary data based on the use of neonatal rat and mouse models of opiate withdrawal, and other neonatal models that have been proposed for the study of neonatal opiate withdrawal.

Key Words: abstinence syndrome; addiction; animal model; dependence; neonate; opiate withdrawal

General Overview

Opiates have been used since antiquity for medicinal (analgesics) and recreational (euphorics) purposes. These drugs exert their agonistic effects at opioid receptors, which are located on the surfaces of cells. Among the three main types of opioid receptors (mu [μ], delta [δ], and kappa [κ]), the μ-opioid receptor is the most relevant to dependence and withdrawal. In humans and experimental animals, exposure to μ-opioid receptor agonists (e.g., morphine, methadone, and heroin) leads to counteradaptive alterations at the cellular, molecular, psychological, and behavioral levels (Clouet and Iwatsubo 1975; Nestler 1992; Nestler and Aghajanian 1997). These counteradaptive alterations are known as tolerance, physical dependence, and withdrawal (Koob and Bloom 1988; Nestler et al. 1993). Tolerance is defined as attenuated physiological or cellular responses to repeated exposure to opiates. Physical dependence is manifested indirectly as a myriad of physiological disturbances and physical symptoms that occur when opiates are withdrawn. Opiate withdrawal, the behavioral manifestation of physical dependence, is the constellation of somatic and autonomic symptoms that occurs upon abrupt cessation of an opiate agonist or administration of an opiate antagonist.

An adequate understanding of the mechanisms involved in chronic opiate use is required to determine the appropriate steps for the proper maintenance of an already-established drug addiction. Opiate addiction has been recognized as having a neural basis that involves the development of complex behaviors such as opiate tolerance, dependence, and withdrawal. Several laboratories have focused efforts on elucidating the molecular and cellular basis of these processes using animal models of opiate exposure. Although many studies have focused on the adult animal model of opiate exposure, only a few studies have explored the neonatal model. This review highlights some of the published literature relevant to newborn animals and the importance of their use in characterizing the behavioral, anatomical, cellular, and molecular responses involved in opiate dependence and withdrawal in the human neonate.

Opiate Exposure in Neonates

There is a large population of opiate-addicted childbearing-aged women in the United States, and each year unknown numbers of infants are born to these women (Zhu and Barr 2000). Moreover, improvements in technology for the treatment of critically ill infants have led to increased use of opiates for analgesia and sedation (Suresh and Anand 2001). These improvements have resulted in a greater number of infants displaying clinical symptoms of opiate withdrawal within 24 to 72 hrs after birth or rapid cessation of the opiate (Suresh and Anand 2001). Opiate withdrawal in newborn infants is also known as...
neonatal abstinence syndrome (NAS). NAS is characterized by disturbances in the central and autonomic nervous systems that result in hyperexcitability, high-pitched cry, tremor, diarrhea, tachypnea, feeding intolerance, and, in severe cases, seizures (Barr and Jones 1994; Blinick et al. 1976; Dashe et al. 2002; Kaltenbach and Finnegan 1992; Suresh and Anand 1998). Furthermore, opiate-exposed infants have an increased incidence of sudden infant death syndrome (Finnegan 1991; Kandall et al. 1993; Ward et al. 1986). Management of opiate tolerance and NAS in opiate-exposed infants remains a major medical challenge. Thus, basic and clinical research that targets the cellular and molecular mechanisms underlying the development of opiate dependence and withdrawal in the infant is needed (Suresh and Anand 2001).

**Animal Models of Opiate Exposure and Withdrawal**

Animal models of “acute” (i.e., a single use) and “chronic” (i.e., long-term use) exposures have been useful in understanding opiate tolerance and dependence (De Vries and Shippenberg 2002; Harris and Gewirtz 2005; Nestler 1996; Schulteis and Koob 1996). Exposure to opiates in these models occurs via osmotic minipumps, implanted pellets, or daily injections. Acute opiate exposure has been defined as the administration of an opiate in single or multiple dosages over 1 to 2 days, whereas chronic opiate exposure has been defined as administration of an opiate for a minimum of 5 days. Acute opiate exposure may be useful in understanding the early stages of opiate dependence and addiction, and chronic opiate exposure studies are performed to determine the counteradaptive cellular and molecular mechanisms that underlie neural plasticity induced by chronic opiate exposure. Both paradigms of exposure are used to correlate the symptoms of opiate withdrawal with cellular responses (Aston-Jones et al. 1999; Georges et al. 2000; Laorden et al. 2002; Stornetta et al. 1993).

Two experimental paradigms of opiate withdrawal are used to study opiate dependence: spontaneous and precipitated withdrawal. Spontaneous opiate withdrawal occurs after the sudden discontinuation or the rapid tapering of chronically administered opiates. Precipitated opiate withdrawal occurs after the use of an opioid receptor agonist, which displaces the opioid receptor agonist. Both spontaneous and precipitated withdrawal syndromes are accompanied by symptoms of abstinence; however, the onset is faster with precipitated opiate withdrawal. Naloxone and naltrexone are the most common competitive μ-opioid receptor antagonists used to precipitate opiate withdrawal. The major difference between these two compounds is that naltrexone, which is orally active, has a much longer duration of action than naloxone.

In addition to using animal models to investigate the behavioral aspects of opiate dependence and withdrawal, investigators commonly measure the cellular expression of immediate-early genes (IEGs) to identify activated cells and neurons involved in the adaptive and counteradaptive processes that mediate opiate tolerance, dependence, and withdrawal. Although many other IEGs (e.g., c-jun [Beckmann et al. 1995; Couceyro and Douglass 1995], Jun-B [Couceyro and Douglass 1995; Frankel et al. 1999], and neuronal activity-regulated pentraxin (Narp) [Reti and Baraban 2003]) are affected by opiate exposure, the most common IEG used in opiate withdrawal studies is cellular-Fos (C-Fos), which produces a protein of the same name: c-Fos. The c-Fos proto-oncogene product is a nuclear protein that can bind to DNA and regulate the transcription of specific cellular genes (Verma and Graham 1987). c-Fos has been used in numerous studies as a marker of increased physiological activity in the central nervous system (CNS) (Sagar et al. 1988). In response to naloxone-precipitated opiate withdrawal, c-Fos immunoreactivity is commonly used as a marker to (1) map neuronal networks involved in opiate withdrawal, and (2) correlate the activation of specific neuronal networks with somatic withdrawal or aversive behavior in adult and neonatal rats (Aston-Jones et al. 1999; Benavides et al. 2003; Georges et al. 2000; Hayward et al. 1990; Laorden et al. 2002; Maeda et al. 2002; Stornetta et al. 1993). Use of this technique has revealed that at least 20 regions are involved in mediating the symptoms of opiate dependence in adult rats (Le Guen et al. 2003). These regions include the rostral ventrolateral medulla, nucleus tractus solitarius, Kollikker-Fuse nucleus, area postrema, locus coeruleus (LC), periaqueductal gray (PAG), paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis (BNST), supramammillary nucleus, and central nucleus of the amygdala (Le Guen et al. 2003).

In addition to the use of c-Fos-like immunoreactivity, biological changes due to opiate withdrawal have been measured by several biochemical and electrophysiological changes. For example, the measurements of 3-methoxy-4-hydroxy-phenylethylenglycol (MHPG), the principal metabolite of brain norepinephrine (NE) (Ahtee et al. 1989; Crawley et al. 1979; Fuertes et al. 2000; Funada et al. 2001; Swann et al. 1982), and 2-deoxyglucose have been used (Bell et al. 1988; Kimes et al. 1998; Kraus et al. 1996). The measurement of MHPG in brain regions during opiate withdrawal has been correlated with withdrawal behaviors in rats as well as humans (Anderson et al. 1985; Charney et al. 1984; Swann et al. 1982). An enhancement in the levels of MHPG is an indicator of noradrenergic activity in the brain. MHPG measurements have been used to assess neurochemical data related to noradrenergic hyperactivity during...
opiate withdrawal in adult rats (Taylor et al. 1988) and to evaluate its role in neonatal opiate abstinence in young rats (Little et al. 1996).

The development of the 2-(14C)deoxyglucose method (Sokoloff 1981; Sokoloff 1982; Sokoloff et al. 1977) provides a method to map functional neural pathways simultaneously in all anatomical components of the CNS. This method identifies functionally active neural circuits that are affected as a result of behavioral or pharmacological manipulation (i.e., naloxone-precipitated withdrawal) by utilizing a radioactive-labeled analogue of glucose, 2-(14C)deoxyglucose, which is transported into neurons, phosphorylated by hexokinase (followed by no further metabolism), and trapped within the cells. A physical representation of the relative rates of glucose utilization throughout the entire brain and the measurement of actual rates of glucose utilization in individual brain regions are assessed by quantitative autoradiography. By measuring glucose utilization in different regions of the brain, it is possible to estimate the level of functional activity within that structure. For example, noradrenergic structures as well as those structures receiving noradrenergic inputs have displayed increased metabolic activity as evidenced by the enhancement of 2-[14C]deoxyglucose utilization after opiate withdrawal in adult rats (Geary and Wooten 1985; Kelsey et al. 1990; Kimes et al. 1990, 1998; Kraus et al. 1996).

**Opiate Withdrawal Differs Between Neonates and Adults**

**Behavioral Evidence**

Opiate withdrawal behaviors in the neonate differ from those behaviors seen in the adult. A major obstacle to developing an animal model of NAS is the difficulty in demonstrating a characteristic constellation of behavioral symptoms resulting from opiate exposure and precipitated withdrawal in neonatal animals (Barr et al. 1998). The CNS of a newborn rat is approximately equivalent to a 24-wk human fetus at birth, a full-term neonate at 7 days, and a toddler at 3 wk; and the newborn rat CNS is mature at 28 days (Marsh et al. 1997). The opioid system of the human newborn (and presumably the rat) is structurally and functionally different from that of the adult, and significant changes in opioid actions occur before and after birth (Marsh et al. 1997). In the rat, the earliest age at which adult-like withdrawal symptoms (e.g., wet dog shakes, burrowing, teeth chatter, diarrhea, and weight loss) occur is between 42 and 52 postnatal days (Fanselow and Cramer 1988; Jones and Barr 1995; and Windh et al. 1995).

Abstinence behavior in opiate-treated, naltrexone-precipitated rat pups has been shown to be age appropriate (Table 1; Jones and Barr 1995). Thus, neonatal rats develop tolerance, dependence, and withdrawal; however, the somatic symptoms in the youngest animals can be quite subtle, which may represent incomplete myelination of the peripheral nervous system, limiting the expression of overt behavioral signs of withdrawal as described in the adult animal. A variety of other potential explanations beyond myelination may also explain the subtle expression of somatic signs in neonates. Possible explanations include changes in the function of the endogenous opioid system (i.e., differential development of CNS μ-opioid receptors) (McPhie and Barr 2000), activation of different brain regions (i.e., age-dependent activation varying by maturation of neuronal groups), extent of participation of certain neurotransmitters/pathways in the expression of withdrawal, or even developmental changes in effector physiology (Windh et al. 1995). Changes in somatic signs have been demonstrated not only between neonates and adults (Blasig et al. 1973; Jones and Barr 1995; Wei et al. 1993) but also between neonates and weanling rats (Windh et al. 1995).

**Neurochemical Evidence**

Nonopioid (e.g., noradrenergic, cholecystokinin, choliner- gic, dopaminergic, GABAergic, glutamatergic, purinergic, serotoninergic, nitric oxide) neuronal networks are also involved in mediating the development and expression of opiate tolerance, dependence, and withdrawal (Robinson et al. 1991; Vaccarino and Kastin 2000, 2001; van Ree et al. 1999). The discussion below is limited to what is currently known about the role of the glutamatergic and noradrenergic systems in the neonate versus the adult.

**Glutamatergic System**

Glutamatergic neurotransmission via the ionotropic N-methyl-D-aspartate (NMDA1) and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA1) receptors and metabotropic glutamate receptors is involved in opiate-

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**Table 1 Opioid withdrawal behaviors in young rats**

<table>
<thead>
<tr>
<th>Young rat behavioral repertoire</th>
<th>Descriptions</th>
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<tbody>
<tr>
<td>Head shakes</td>
<td>Lateral and rotary motions of head</td>
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<tr>
<td>Straub tail</td>
<td>Erect tail (45 or 90°)</td>
</tr>
<tr>
<td>Rolling</td>
<td>Turning body over at least one full rotation</td>
</tr>
<tr>
<td>Wall climbing</td>
<td>Placing both forepaws on the cage wall</td>
</tr>
<tr>
<td>Stretching</td>
<td>Elongation of extremities</td>
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<tr>
<td>Moving paws/fluttering</td>
<td>Continuous movement of paws toward face</td>
</tr>
<tr>
<td>Vocalization</td>
<td>Audible sounds</td>
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*Adapted from Barr and Wang 1992; Ceger and Kuhn 2000; Fanselow and Cramer 1988; Jones and Barr 1995; and Windh et al. 1995 (see text).
induced neural and behavioral plasticity (Jackson et al. 2000; Trujillo 2000; Zhu and Barr 2004); the specific role of each of these glutamatergic receptors in opiate-induced neuroplasticity may be developmentally regulated. In adult animals, the binding of an agonist to the μ-opioid receptor results in calcium (Ca^{2+}) influx via NMDA receptors, with subsequent activation of Ca^{2+}-dependent second messengers such as nitric oxide (NO), which is synthesized by nitric oxide synthase (NOS). Not surprisingly, opiate tolerance and dependence can also be modulated by the NMDA/NO cascade (Özek et al. 2003; Pasternak et al. 1995; Trujillo 2000). In adult animals, the general consensus is that NMDA receptor antagonists inhibit the development, but not the expression, of opiate tolerance, dependence, and withdrawal (Trujillo 2000). An anatomical study was performed (Jones and Barr 2001) to determine whether the neural circuitry underlying adult opiate withdrawal (i.e., the LC, PAG, and the amygdala to a lesser extent) is similar in the neonate. It was demonstrated that injections of methylhaloxonium, a hydrophobic opioid receptor antagonist, into the LC and PAG, but not the amygdala, precipitated morphine withdrawal in neonatal rats at postnatal day (PND) 7. It was concluded that the neural circuitry mediating opiate withdrawal behaviors is similar in neonates and adult rats, but the behaviors expressed are age specific.

In contrast to what has been demonstrated in the adult rat, NMDA receptor antagonists are ineffective in suppressing the development or expression of tolerance, dependence, and withdrawal in neonatal animals (Zhu and Barr 2000, 2004). Moreover, co-chronic treatment with MK-801 and morphine did not attenuate the development of opiate tolerance or dependence in electrophysiological studies using the neonatal rat isolated spinal cord preparation or in behavioral studies using the adult rat (Bell and Beglan 1995a,b). NOS inhibitors block opiate withdrawal in the neonate in a manner similar to what is observed in the adult animal (Rasmussen et al. 1990). Although the effects of opiates rely on similar second messenger systems (e.g., NO) as in the adult, the factors that activate these systems differ in neonates (Zhu and Barr 2004).

In adult animals, other glutamate receptors (i.e., AMPA and metabotropic) may play an auxiliary or parallel role to that of the NMDA receptor in the development and expression of opiate tolerance, dependence, and withdrawal (Kest et al. 1997; McLemore et al. 1997). In the neonate, AMPA and metabotropic glutamate receptor antagonists may be more effective therapeutic agents for the treatment of opiate tolerance, dependence, and withdrawal than NMDA receptor antagonists (Fitzgerald et al. 1996; Fundytus and Coderre 1997; Jang et al. 2000; Popik et al. 2000; Vandergriff and Rasmussen 1999; Vekovischeva et al. 2001). The developmental profile of the AMPA receptor, in contrast to that of the NMDA receptor, suggests that the Ca^{2+}-permeable AMPA receptor may play a dominant role in these processes in the neonate (Zhu and Barr 2004). Several lines of evidence indicate that AMPA receptors, rather than NMDA receptors, are indispensable for opiate withdrawal during development (Fitzgerald et al. 1996; Jakowec et al. 1995a,b; Mahanty and Sah 1998; Ozawa et al. 1998; Washburn et al. 1997). In neonatal rats, the AMPA receptor plays the dominant role in gating Ca^{2+} influx. During the second postnatal week, the AMPA receptor matures rapidly and loses Ca^{2+} permeability. The NMDA receptor gains Ca^{2+} permeability during development and, thus, acquires its dominant adult role (Zhu and Barr 2001).

**Noradrenergic System**

Opiate withdrawal induces a state of neuronal hyperexcitability in the brain that is associated with changes in several neurotransmitters. For example, the noradrenergic system has an important role in the expression of opiate withdrawal symptoms in adults and neonates as a result of neuronal activation (Benzadíes et al. 2005; Ceger and Kuhn 2000; Maldonado 1997; Nestler and Aghajanian 1997; Williams et al. 2001). Upon abrupt discontinuation of chronic opiate exposure, multiple counteradaptive cellular and molecular effects occur (including superactivation of adenyl cyclase with subsequent elevation of cAMP), which lead to excessive NE release followed by binding of NE to noradrenergic receptors on cells and neurons. This excessive NE release leads to the overactivation of the autonomic nervous system (Bozarth 1994; Maldonado et al. 1992; Przewlocki 2004). α2-Adrenergic and μ-opioid receptors are frequently co-localized on neurons and cells that are believed to be the neuroanatomical substrates for the sympathtic symptoms of opiate withdrawal (Freedman and Aghajanian 1985; Williams et al. 2001). Clonidine, a central α2-adrenergic receptor agonist, decreases the hyperactivation of LC neurons during opiate withdrawal and is used clinically to attenuate abstinence symptoms in humans (Aston-Jones et al. 1997; Bozarth 1994; Gold et al. 1978; Maldonado et al. 1992). Clonidine reduces hyperactivity of noradrenergic neurons in the midbrain and brainstem, as measured by changes in cAMP and NE levels as well as c-Fos gene and protein expression in response to precipitated opiate withdrawal in adult animals (Delfs et al. 2000; Maldonado 1997).

The noradrenergic neurons of the LC are important mediators of the somatic signs of opiate withdrawal. Noradrenergic neurons of the LC contain μ-opioid receptors on presynaptic nerve endings (Delfs et al. 2000; Mansour et al. 1988). During antagonist-precipitated withdrawal, there is increased firing of NE neurons in the LC resulting in increased NE release, which correlates temporally with the behavioral signs of opiate withdrawal (Bozarth 1994; Maldonado et al. 1992; Przewlocki 2004; Rasmussen 1991). This hyperactivation results in part from opiate-induced alterations in μ-opioid receptor coupling and density, and from second messengers within these noradrenergic neurons (Bailey and Connor 2005). These findings suggest that the noradrenergic neurons within the LC mediate some of the somatic signs of opiate withdrawal; however, these neurons do not exclusively mediate opiate withdrawal.
The BNST is another important neuronal target for noradrenergic inputs, and NE release is significantly elevated in this region during opiate withdrawal (Fuentealba et al. 2000). Projections from the A1/A2 noradrenergic neurons of the caudal medulla to the BNST and the central nucleus of the amygdala are crucial for the aversive response to opiate withdrawal (Aston-Jones and Harris 2004; Aston-Jones et al. 1999). The BNST and the medial and central nuclei of the amygdala are closely related and are thus considered a single functional system, collectively known as the “extended” amygdala. The extended amygdala, which is involved in anxiety and fear responses, may be an important neuronal region that mediates anxiety-induced drug seeking after protracted withdrawal (Aston-Jones and Harris 2004). The neural network that mediates drug reinforcement, reward, and craving of opiates also includes the ventral tegmental area, prefrontal cortex, and nucleus accumbens (Koob 1992; Koob et al. 1992; Przewlocki 2004; Samson and Harris 1992; Wise and Bozarth 1987).

**Preliminary Data Based on Use of the Neonatal Rat Model**

Our laboratory is interested in determining the involvement of the noradrenergic system, specifically the role of the BNST and central α2-adrenergic receptors, in opiate withdrawal. In preliminary studies, we have used the newborn rat model of in utero opiate exposure and precipitated withdrawal to determine the effect of tapering morphine therapy alone or in combination with clonidine both on the behavioral signs of opiate withdrawal and on the correlation between somatic signs of opiate withdrawal and c-Fos protein expression in the BNST. Osmotic mini-pumps containing methadone or vehicle were placed in pregnant dams on gestation day (GD) 15. At PND 8, methadone- or vehicle-exposed pups were cross-fostered onto naïve dams and treated with tapering doses of morphine alone or in combination with a standard dose of clonidine. After naloxone-precipitated opiate withdrawal on PND 10 and 11, behaviors were videotaped and subsequently scored. The brains were processed for c-Fos expression via immunohistochemistry or Western blot analysis.

Our experimental paradigm of opiate exposure produced physical dependence in rat pups at PND 10 and 11, as evidenced by the behavioral signs of opiate withdrawal, which correlated with an increase in c-Fos immunoreactive cells in the BNST of these animals. Behavioral signs and c-Fos expression were more prominent in response to precipitated opiate withdrawal in newborn rat pups treated with morphine plus clonidine versus morphine alone after 2 days of treatment. The mechanisms associated with the heightened expression of somatic symptoms in the morphine plus clonidine group are not known. However, we hypothesize that our findings are related to the duration of therapy. Treatment of the animals for at least 3 days with morphine plus clonidine decreased the behavioral signs of opiate withdrawal. Our preliminary data suggest that clonidine is effective in reducing opiate withdrawal symptoms in opiate-dependent newborn rats. To our knowledge, these preliminary data are the first to demonstrate a direct correlation between the behavioral and cellular markers of opiate withdrawal in the BNST of newborn rats.

**Preliminary Data Based on Use of the Neonatal Mouse Model**

In an effort to exploit the power of genetically manipulated mice and to elucidate the specific genes and proteins that are involved in opiate tolerance and dependence during prenatal and early postnatal development, we have developed a model of chronic opiate exposure and withdrawal using the neonatal C57BL/6 mouse, a commonly used background strain. Our preliminary data (Yohay et al. 2005) describe the behavioral repertoire of symptoms associated with opiate withdrawal in this mouse model. Osmotic mini-pumps that delivered methadone or vehicle for 4 wk were placed in pregnant dams 1 wk before parturition. Thus, mouse pups were transplacentally exposed to methadone for 1 wk and then exposed to methadone via breast milk for 7 to 21 days. Opiate withdrawal was precipitated with naloxone at PND 7 and 21. The animals were videotaped for 15 min after naloxone challenge. Comparisons were made between opiate-exposed and control animals. We compared individual withdrawal behaviors and whether the mice were active or quiescent, and we found no significant differences in behavioral repertoire between withdrawn and control mice at PND 7 or 21 (Yohay et al. 2005). We observed an increase in c-Fos protein expression in brainstem homogenates of opiate-exposed versus control mouse pups at PND 7 and 21. Our preliminary data suggest that despite the lack of a robust and reliable behavioral repertoire associated with opiate withdrawal in the newborn mouse, c-Fos expression in key neuronal groups may be a sensitive outcome variable for the study of the cellular and molecular processes underlying opiate dependence and withdrawal in the newborn mouse.

**Overview of Neonatal Rodent Models**

The rat is the most commonly used neonatal model of chronic opiate withdrawal in the literature. Chronic opiate withdrawal in other neonatal and fetal animals has not been well studied. The use of other neonatal animal models is important for corroborating data obtained in the rat and utilizing powerful genetic tools, such as knock-out mice. In addition to our preliminary data in the neonatal mouse described above, another group has reported on the neurochemical effects of opiates in the newborn mouse. Chronic opiate withdrawal induces neuronal excitation, resulting in elevated intracellular Ca2+ ([Ca2+]i) levels, which are toxic to neurons and eventually cause death. The pathological
elevation of [Ca$^{2+}$], is buffered by Ca$^{2+}$ binding proteins such as parvalbumin (PV$^1$). Because increased expression of PV is associated with excessive neuronal excitation related to pathogenesis, it is significant that maternal morphine exposure augments the expression of PV in specific regions of the cortex of the neonatal mouse brain (Maharajan et al. 2000). Thus, an increase in the Ca$^{2+}$ binding proteins may reflect increased [Ca$^{2+}$], levels due to opiate exposure (Maharajan et al. 2000). Neither spontaneous nor precipitated withdrawal was examined in these studies.

Other Animal Models of NAS

Guinea Pig

Since the mid-1990s, the effects of in utero chronic opiate exposure on respiration have been reported based on use of the neonatal guinea pig model (Gray et al. 2001; Hunter et al. 1997; Matsuda and Olsen 2001; Murphey and Olsen 1994b, 1995; Olsen et al. 1988; Smith et al. 1999a,b, 2004). Chronic intermittent opiate exposure in utero during the last half of gestation, followed by spontaneous opiate withdrawal, has been shown to result in increased locomotor activity and minute ventilation during the first postnatal week (Hunter et al. 1997). Presumably, in utero opiate exposure followed by precipitated withdrawal leads to behavioral and cellular changes that are consistent with chronic opiate withdrawal in guinea pigs.

Although the previously cited studies have not used the guinea pig to study chronic opiate withdrawal specifically, their work highlights the potential use of the guinea pig as a model of human NAS. The guinea pig was chosen primarily for the similarity of its placenta to that of the human. Specifically, like humans, guinea pigs have hemomonochorial placentas (i.e., there is only a single layer of trophoblastic cells, which will eventually divide into the chorion and the placenta, between the maternal and fetal circulations). The guinea pig and human placentas are similarly permeable to hydrophilic drugs such as morphine (Murphey and Olsen 1994a; Olsen et al. 1989). Furthermore, morphine-6-beta-D-glucuronide, a metabolite of morphine with opioid activity in adults, is metabolized similarly in guinea pigs and humans (Murphey and Olsen 1994b; Smith et al. 1999a, b). In addition, the guinea pig at birth is more mature than either the rat or mouse at birth; hence, the guinea pig is a better model of the human infant at term. However, increased maturity at birth limits the use of the guinea pig as a model of NAS: the long gestation period of 59 to 72 days and the relatively small litter size (approximately 2-5 guinea pigs/litter).

Chicken

During the 1980s, fetal and neonatal chickens were used as models of NAS. Chicken fetuses at GD 12 demonstrated increased motility, a sign of opiate withdrawal, after a single dose of methadone followed by a naloxone challenge (Bronson and Sparber 1989a). In addition, a single dose of methadone followed by naloxone challenge caused withdrawal symptoms in neonatal chickens at PND 1 (Bronson and Sparber 1989b). A partial increase in motility of a chicken fetus at GD 14 was demonstrated after chronic methadone infusion followed by precipitated withdrawal with naloxone (Seran and Sparber 1988). To our knowledge, no additional reports of opiate withdrawal studies in fetal or neonatal chickens have appeared in the literature after the 1980s.

Lamb

The fetal lamb is frequently used to study the effects of chronic opiate exposure on respiratory and sleep-wake cycles (Hasan et al. 1990; Olsen et al. 1988; Szeto et al. 1988; Toubas et al. 1985; Umans and Szeto 1983). In opiate-infused fetal lambs, a characteristic opiate withdrawal syndrome was observed, which included increased movement and neck tone, eye movements, tachypnea, bradycardia, hypertension, and passage of meconium (stool) into the amniotic fluid followed by naloxone-precipitated withdrawal (Umans and Szeto 1985). Studies correlating cellular alterations with behavioral signs of opiate withdrawal have yet to be performed in the fetal lamb model. Opiate withdrawal in newborn lambs has not been studied. The gestation period of the sheep is approximately 147 days, which is relatively long and possibly limits the utility of this model. Extensive experience has been reported from physiological experiments using chronically catheterized fetal sheep (e.g., Craft et al. 1982; Cuffler et al. 1985; Raye et al. 1980), and there is a large body of knowledge on brain development and cerebral blood flow in fetal sheep (e.g., Brooks et al. 1992; Olsen et al. 1983). Thus, developing the fetal sheep as a model of NAS could contribute significantly to our understanding of the autonomic effects of chronic opiate exposure with respect to tolerance, dependence, and withdrawal.

Conclusions and Perspectives

Infants exposed to opiates in utero usually become physically dependent and, after parturition, suffer withdrawal symptoms defined as NAS. NAS is characterized by CNS, gastrointestinal, and respiratory dysfunction. The information gained from the use of animal models may provide information to ensure well-managed pregnancies, healthy births, and the alleviation of withdrawal symptoms in infants afflicted with NAS. The ultimate goal is to develop treatment strategies with clear superiority over strategies currently used for maintenance therapy of opiate-dependent women and their newborn infants, especially with regard to the attenuation of the symptoms of NAS. However, the literature has a limited number of reports on neonatal models of opiate dependence and withdrawal.
Many questions involving the neurobiological mechanisms of opiate dependence and withdrawal in the neonate remain to be explored and answered. Because cellular and molecular mechanistic experiments cannot be performed in human infants for obvious ethical reasons, animal models of NAS are an absolute necessity. Neonatal animal models are beginning to be used to elucidate the neurobiological mechanisms of opiate dependence and withdrawal as well as to define the behavioral sequelae of chronic opiate withdrawal. No one animal is the perfect model; however, depending on the research question, certain attributes of each model may be beneficial. The benefits of animal models of NAS are countless. The use of neonatal animal models will help investigators address specific questions (Table 2) relating to the effects of opiate exposure on the developing CNS. With these models, we can better

1. Define opiate withdrawal behaviors and the physiological consequences of opiate withdrawal;
2. Examine the effects of opiate exposure on long-term developmental outcomes;
3. Explore the neurobiological basis for addiction with respect to determining how opiate exposure in utero may affect addictive tendencies in adults;
4. Determine how opiate dosing predicts NAS;
5. Examine the interactions of other drugs (e.g., cocaine, tobacco, marijuana, alcohol) with opiates; and
6. Assess the efficacy of various treatment strategies (e.g., clonidine) in conjunction with or in lieu of opiate weaning treatment.

Table 2 Questions addressed by the use of neonatal animal models (NAS)

- What are the long-term neurodevelopmental effects of maternal opiate exposure?
- Is there a correlation between maternal opiate dosing and the severity of the NAS?
- What influences do polydrug abuse, inadequate prenatal care, poor nutrition and mental illness have on the outcome of NAS?
- Is there a correlation between NAS and opiate levels in the blood, urine, and/or amniotic fluid that may be used to develop prognostic assays, which would be the basis of pharmacotherapy?
- Which are the most efficacious treatment modalities to reduce neonatal morbidity and mortality due to maternal opiate exposure?
- What are the neurobiological mechanisms of drug dependence and withdrawal?
- What are the toxicological consequences of opiate withdrawal in the neonate?
- Can the effects of prenatal opiate exposure be studied in the absence of maternal complications?
- Are there correlations between the brain regions purported to mediate NAS and gene expression and neurotransmitter release?

As with any model, the animal model of NAS has limitations (Table 3). Because human studies are scarce, extrapolating animal data to humans is extremely difficult. For example, in humans, opiate (e.g., morphine, methadone, heroin) dosing is generally intermittent, with inherent and often unpredictable peaks and troughs in plasma blood levels. In many of the current animal studies, opiates are administered continuously via osmotic minipumps or implanted pellets, which does not reflect the usage pattern of humans and may affect chronic opiate withdrawal at both the cellular and behavioral levels. Animal models do not account for psychosocial factors that may heavily influence NAS in human infants. Animals and humans may metabolize opiates differently and thereby affect extrapolations of animal data to humans. Perhaps the major concern about opiate exposure in the human fetus and infant is the unknown potential for long-term effects such as developmental delay, decreased IQ, learning disabilities and behavioral problems.

It is likely that we will not be able to resolve all of the important issues with current NAS animal (rat and mouse) models. However, a primate model of NAS, although prohibitively expensive, would likely allow investigators to explore the long-term effects of NAS. Despite the vast imperfections inherent in the use of models, the future of basic research and the foundation on which our clinical studies of opiate withdrawal will be based rest heavily on the ethical and sound scientific use of animal models of NAS. The exploration of neonatal animal models of NAS, although in its infancy, shows promise for future understanding and treatment of NAS.

Table 3 Limitations of neonatal animal models

- Difficulty defining long-term outcomes of perinatal opiate exposure
- Difficulty understanding genetic predisposition to opiate abuse
- Difficulty examining the global effects of perinatal opiate exposure with respect to confounding maternal complications (e.g., HIV/AIDS and polysubstance abuse)
- Difficulty examining the effects of perinatal opiate exposure with respect to psychological and social factors (i.e., conditioned environmental cues associated with opiate use)
- Difficulty elucidating the processes by which acute effects of opiates are altered into long-term adaptations in specific brain regions that result in addiction
- Difficulty understanding the role that stress plays in the addicted state
- Difficulty in determining how the brain compensates for opiate-induced counteradaptations
- Difficulty elucidating the role that race may play in opiate addiction
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