

Opiate withdrawal during development: are NMDA receptors indispensable?

Hongbo Zhu and Gordon A. Barr

Despite decades of research, the mechanisms that underlie opiate tolerance, dependence and withdrawal remain elusive. Evidence accumulated over the past ten years suggests that the NMDA receptor plays a central role in mediating the neuroplasticity induced by chronic opiate administration in adult animals. Yet, during ontogeny, the NMDA receptor complex undergoes qualitative developmental changes, which renders some of the basic assumptions for a role of the NMDA receptor in opiate withdrawal invalid in infants. Recent data indicate that NMDA receptor antagonists are not effective in blocking morphine tolerance, dependence and withdrawal in the neonatal rat. Roles for other glutamate receptor types (e.g. metabotropic glutamate receptors) have also been proposed recently. In this article, the latest evidence that characterizes the dynamic roles of glutamate receptors in these phenomena during ontogeny will be discussed.

The potent pharmacological effects of opiates have been recognized by humans since ancient times. Today, opiates are still used in medical settings for their analgesic virtue and abused as 'street' drugs for their recreational properties. Chronic exposure to opiates leads to dramatic behavioral and neural changes known as tolerance and physical dependence. Tolerance represents a reduced effect following repeated exposure to the opiate. Physical dependence describes an altered physiological state caused by repeated opiate exposure. Cessation of drug administration then leads to a withdrawal syndrome that is characterized by severe physiological disturbances and a plethora of withdrawal signs¹. These phenomena probably represent adaptive changes in neural systems to chronic opiate exposure and are prominent examples of neural plasticity^{2,3}.

The NMDA receptor has a well-established role in neuronal plasticity [e.g. long-term potentiation (LTP)⁴] and is also involved in opiate-related neural plasticity. Indeed, the past ten years have seen an explosion of evidence to indicate that the NMDA receptor plays a key role in opiate tolerance, dependence and withdrawal^{2,3,5-7}. Specifically, the opioid system interacts with NMDA receptors such that activation of the mu opioid receptor results in Ca²⁺ influx through the NMDA receptor channel. The subsequent activation of various Ca²⁺-dependent second messenger system cascades, particularly the activation of nitric oxide synthase (NOS) [the enzyme responsible for the production of nitric oxide (NO)], plays a central role in these phenomena^{2,7}. Although this hypothesis is

supported by broad and persuasive evidence, the data on which it is based have been obtained exclusively from the adult animal. Two emerging lines of research suggest that a different picture might exist early in life.

Ontogeny and modulation of opiate withdrawal

It has long been known that human infants suffer deleterious effects as a result of maternal opiate use and, until recently, the only detailed description of opiate abstinence syndrome in neonates was for humans⁸. But the complexity of the human setting makes it difficult to separate those factors that are due to opiate use and those that are due to the abuse of other drugs, poor prenatal care, poor nutrition or other complications experienced by the mothers of these children. To overcome the confounding maternal effects in human infants, various animal models have been developed⁸.

Recent animal studies have shown that infant rat pups, and even the fetal rat, experience opiate tolerance and/or dependence if the dams are exposed to opiates during their pregnancy or the pups are treated directly with opiates⁹⁻¹⁵. On the basis of these data, two major conclusions can be drawn: (1) when the behavioral repertoire appropriate to the age of the animal is examined, infant rat pups experience opiate tolerance and withdrawal if the dams are exposed to opiates during their pregnancy or the pups are treated directly with opiates; and (2) the withdrawal syndrome changes during development to the adult form following weaning (Box 1).

'...during early life, receptors other than NMDA receptors might link to the same second messenger system as in adult life...'

In adult animals, a variety of NMDA receptor antagonists have been reported to inhibit not only the development or acquisition of opiate tolerance and dependence but also the expression of established opiate dependence – opiate withdrawal^{15,6}. Various drugs that act on the NMDA receptor or Ca²⁺-dependent second messenger systems have been studied recently in the infant rat. In contrast to data obtained in adults, NMDA receptor antagonists are neither effective in blocking the development of opiate tolerance or dependence¹⁶⁻¹⁸, nor effective in suppressing the expression of opiate withdrawal^{18,19} in neonatal rats. However, in agreement with the data from studies in adults, NOS inhibitors suppress withdrawal in the infant¹⁹. Thus, in the infant, although opiate actions rely on the same second messenger systems as in the adult, the factors that activate these systems differ. Of particular interest is the recent finding that the NMDA receptor antagonist MK801 is ineffective in blocking both the

Hongbo Zhu
Biopsychology Doctoral Program, Hunter College and the Graduate Center, City University of New York, 695 Park Avenue, New York, NY 10021, USA.
Gordon A. Barr*
Dept of Psychology, Biopsychology Doctoral Program, Hunter College and the Graduate Center, City University of New York, 695 Park Avenue, NY 10021, USA, and Division of Developmental Psychobiology, New York State Psychiatric Institute, College of Physicians and Surgeons, Columbia University, 1051 Riverside Drive, NY 10032, USA.
*e-mail: gbarr@hunter.cuny.edu

Box 1. Ontogeny of opiate withdrawal

Until recently, the only description of an opiate abstinence syndrome in neonates was for human infants. Indeed, early work suggested that infants were 'immune' from opiate withdrawal^a. However, more recently, three research groups described an opiate withdrawal syndrome in the infant rat, which included behaviors such as increased separation-induced vocalizations, head swaying, stretching, rolling and an inability to stay quiet^{b-h}. Aspects of this syndrome are observed even in the fetusⁱ, and the withdrawal syndrome slowly changes during development to reach, around puberty, the classic constellation of withdrawal behaviors so often described for the adult animal^c. These behaviors are specific for opiate withdrawal because they do not occur in the absence of an opiate receptor agonist and antagonist. Opiate withdrawal in the infant can also be demonstrated in the isolated spinal cord of the infant rat^{j,k}. This syndrome occurs following administration of a variety of opiate receptor agonists, including fentanyl, methadone and morphine, given either directly to the pup or to the dam (and transferred to the fetus and pup via the placental blood supply and milk, respectively). The negative affective state associated with abstinence appears in the infant rat, but develops later, at ~14 days of age, dissociating the unconditioned 'physical' withdrawal signs from the dysphoric aspects of abstinence.

The withdrawal syndrome in the pup consists of behaviors that are qualitatively distinct from those of the adult, and lacks autonomic signs. At least two possibilities might explain why the infant and adult behaviors differ. First, it is possible that the neural circuitries that mediate withdrawal in the infant and adult are fundamentally different. Thus, the differing behaviors are a result of a developmentally unique physiological change following chronic opiate treatment. Second, the 'higher' brain circuits that mediate withdrawal might be the same but the output systems might differ. This is true for the autonomic system, which is immature in the preweaning rat, and for many of the complex motor behaviors observed in the adult during withdrawal. In one experiment, the periaqueductal gray and locus coeruleus, two structures that mediate withdrawal in the adult, also mediated withdrawal in the infant^l. Similarly, the spinal cord is involved in withdrawal in both the adult and the infant^{j,k}. These data argue for developmental continuities in the neural circuits that mediate withdrawal. By contrast, there are fundamental differences in the pharmacological underpinnings of withdrawal in the 1-week-old pup compared with the adult.

References

- a Fanselow, M.S. and Cramer, C.P. (1988) The ontogeny of opiate tolerance and withdrawal in infant rats. *Pharmacol. Biochem. Behav.* 31, 431–438

- b Barr, G.A. and Wang, S. (1992) Tolerance and withdrawal to chronic morphine treatment in the week-old rat pup. *Eur. J. Pharmacol.* 215, 35–42
- c Jones, K.L. and Barr, G.A. (1995) Ontogeny of morphine withdrawal in the rat. *Behav. Neurosci.* 109, 1189–1198
- d Barr, G.A. *et al.* (1998) Neonatal withdrawal following pre- and postnatal exposure to methadone in the rat. *Pharmacol. Biochem. Behav.* 60, 97–104
- e Windh, R.T. *et al.* (1995) The ontogeny of mu opiate tolerance and dependence in the rat: antinociceptive and biochemical studies. *J. Pharmacol. Exp. Ther.* 273, 1361–1374
- f Ceger, P. and Kuhn, C.M. (2000) Opiate withdrawal in the neonatal rat: relationship to duration of treatment and naloxone dose. *Psychopharmacology* 150, 253–259
- g Thornton, S.R. and Smith, F.L. (1997) Characterization of neonatal rat fentanyl tolerance and dependence. *J. Pharmacol. Exp. Ther.* 281, 514–521
- h Thornton, S.R. *et al.* (1997) Characterization of neonatal rat morphine tolerance and dependence. *Eur. J. Pharmacol.* 340, 161–167
- i Jones, K.L. and Barr, G.A. (2000) Opiate withdrawal in the fetal rat: a behavioral profile. *Pharmacol. Biochem. Behav.* 66, 419–424
- j Bell, J.A. and Beglan, C.L. (1995) Co-treatment with MK-801 potentiates naloxone-precipitated morphine withdrawal in the isolated spinal cord of the neonatal rat. *Eur. J. Pharmacol.* 294, 297–301
- k Bell, J.A. and Beglan, C.L. (1995) MK-801 blocks the expression but not the development of tolerance to morphine in the isolated spinal cord of the neonatal rat. *Eur. J. Pharmacol.* 294, 289–296
- l Jones, K. and Barr, G.A. (2001) Injections of an opioid antagonist into the locus coeruleus and periaqueductal gray but not the amygdala precipitates morphine withdrawal in the 7-day-old rat. *Synapse* 39, 139–151

development of morphine dependence and the expression of morphine withdrawal in the 7-day-old rat but is partially effective in the 14-day-old rat and fully effective in the 21-day-old rat¹⁸. Thus, there is a transition age, around the second postnatal week, when MK801 becomes effective in suppressing morphine withdrawal¹⁸. Preliminary experiments have extended these findings to the establishment of tolerance^{20,21}. Neither of the two NMDA receptor antagonists (MK801 and dextromethorphan) is effective in blocking the development of morphine tolerance in the 7-day-old rat, yet both of them are fully effective in the 21-day-old rat. Therefore, from a developmental perspective, during early life, receptors other than NMDA receptors might link to the same second messenger system as in adult life and, at a later time, either confer this role to NMDA receptors or recede to an auxiliary role¹⁸.

The AMPA receptor, another member of the glutamate receptor family, seems to fit such a role for

several reasons. First, the fact that the AMPA receptor is colocalized with the NMDA receptor in many cell types²², including NOS-containing neurons²³, suggests that the AMPA receptor, similar to the NMDA receptor, is involved in neural circuitry that is activated during opiate withdrawal. Indeed, chronic morphine treatment elevates levels of GluR1 (a subunit of the AMPA receptor) in the rat ventral tegmental area²⁴ and morphine withdrawal increases both the level of AMPA receptor mRNA expression and [³H]AMPA binding in various brain sites in the rat²⁵. Second, in adult mice or rats, LY293558, a competitive AMPA receptor antagonist, attenuates and reverses morphine analgesic tolerance^{26,27}, inhibits the development of acute morphine dependence²⁷ and decreases behavioral signs of morphine withdrawal²⁸. Furthermore, LY293558 effectively suppresses morphine withdrawal-induced firing of locus coeruleus neurons, whereas NMDA receptor antagonists do not²⁸. Thus, the AMPA

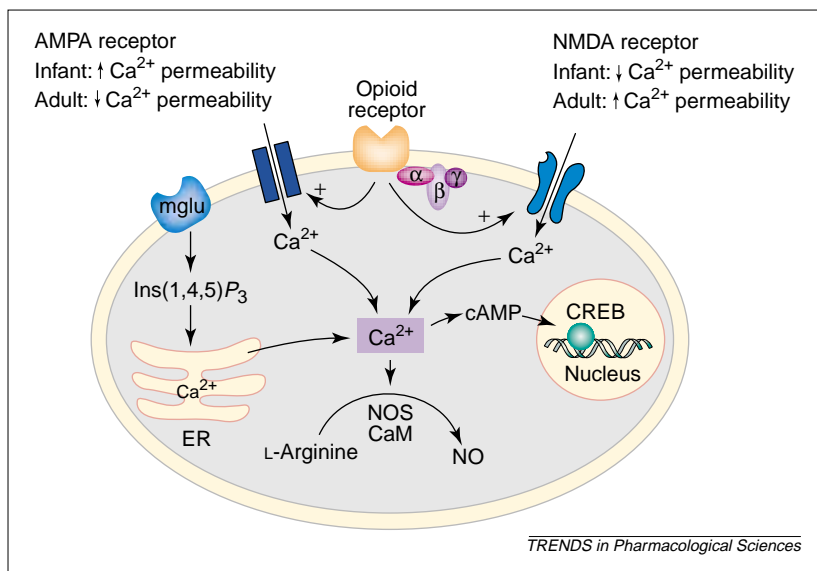


Fig. 1. Dynamic roles of glutamate receptors in opiate tolerance, dependence and withdrawal. In both neonatal and adult animals, chronic opiate exposure could lead to the activation (by phosphorylation) of both AMPA receptors and NMDA receptors. However, in the adult rat, the AMPA receptor has low Ca^{2+} permeability, and thus Ca^{2+} influx through the NMDA receptor channel is the major trigger responsible for subsequent biochemical cascades that follow exposure to opiates. In the neonatal rat, Ca^{2+} -permeable AMPA receptors, which could have no or a very low degree of desensitization²³, are abundant on neurons involved in opiate actions. Activation of these AMPA receptors results in a marked increase of intracellular Ca^{2+} . The NMDA receptor might not be a crucial component in the neonates because: (1) the immature NMDA receptors have low Ca^{2+} permeability; and (2) Ca^{2+} entry through Ca^{2+} -permeable AMPA receptors desensitizes colocalized NMDA receptors⁵⁰. The steep Ca^{2+} gradient that results from influx of Ca^{2+} through NMDA or AMPA receptors activates Ca^{2+} -dependent second messengers, such as nitric oxide synthase (NOS), which results in further long-lasting effects. Other glutamate receptor types [such as the metabotropic glutamate (mglu) receptors] could also be involved in both the neonates and the adult because these receptors can activate various second messenger systems by other mechanisms. Abbreviations: CaM, calmodulin; CREB, cAMP response element-binding protein; ER, endoplasmic reticulum; Ins(1,4,5) P_3 , inositol (1,4,5)-trisphosphate; NO, nitric oxide.

receptor has at least a role that is auxiliary, if not parallel and independent, to the role of the NMDA receptor in opiate tolerance and withdrawal in adult animals. In addition, data show that the competitive AMPA receptor antagonist NBQX [2,3-dihydro-6-nitro-7-sulfamoyl-benzo(*f*)quinoxaline] is effective in suppressing morphine withdrawal and in inhibiting the development of morphine tolerance in rats as young as 7 days of age^{20,21}, in sharp contrast to the ineffectiveness of NMDA receptor antagonists. Last, but most importantly, the developmental profile of the AMPA receptor, in contrast to that of the NMDA receptor, suggests that the AMPA receptor (rather than the NMDA receptor) might play the dominant role in these processes in younger animals.

Ontogeny of NMDA and AMPA receptors

Both NMDA and AMPA receptors are heteromeric receptors composed of multiple subunits. During ontogeny, the composition of these receptors undergoes dramatic differential change, resulting in functionally different receptors.

Native NMDA receptors comprise subunits that include NR1, NR2A–2D and NR3A. The various subunits of the NMDA receptor complex undergo differential maturation during the first few postnatal weeks of the animal²⁹, resulting in considerably

different functions of the NMDA receptor³⁰. Specifically, brain levels of NR1 and NR2A subunits are lowest shortly after birth, increase to reach peak levels by 14 or 21 days of age, and subsequently decrease at 28 days of age. By contrast, the NR2B subunit levels are highest during early development and decrease as the animal ages. The NR2C subunit is restricted to the cerebellum and its presence is undetectable until the second postnatal week³¹. The expression of the NR3A subunit begins just before or during the first postnatal week³². Thus, in the neonatal brain, the predominant NMDA receptor subunits are NR1, NR2B, NR2D and NR3A. Consequently, NMDA-receptor-mediated Ca^{2+} fluxes can be rather weak immediately after birth, and the magnitude of these responses can increase dramatically during the first to second postnatal weeks³³. Furthermore, it has been suggested that there are two types of NMDA receptor in the rat^{34,35}, an immature type and a mature type, and that a temporal switching of the NMDA receptor from the immature form to the adult mature form occurs around the second postnatal week³⁵.

'...in the infant very different therapeutic approaches might be necessary for the treatment of the clinically adverse effects of opiates from those used in the adult.'

Native AMPA receptors are also heteromeric and are composed of subunits GluR1–4. Like NMDA receptors, AMPA receptors also undergo major developmental changes in subunit expression, leading to the acquisition of the adult pattern between postnatal day (P) 7 and P21 (Refs 36,37). However, unlike the immature NMDA receptor, which has low Ca^{2+} permeability depending on the region in the CNS, the immature AMPA receptor has high Ca^{2+} permeability⁴ and its activation produces a marked increase (comparable to that triggered by normal NMDA receptors) in free Ca^{2+} in the cytoplasm^{4,38}. In contrast to the initial thought that NOS was functionally coupled through NMDA receptors to Ca^{2+} influx but poorly linked to other Ca^{2+} pools³⁹, Ca^{2+} entry through Ca^{2+} -permeable AMPA receptors activates downstream Ca^{2+} -dependent intracellular events, such as the activation of NOS and NO production²³. Indeed, the Ca^{2+} -permeable AMPA receptors can mediate virtually all major events that the NMDA receptor is capable of mediating, including the induction of LTP (NMDA receptor independent) in the hippocampus⁴⁰ and amygdala⁴¹, activation of the calmodulin–NOS cascade²³, phosphorylation of cAMP response element-binding protein (CREB)⁴² and neurotoxicity⁴. Two observations that are particularly supportive of a

role for the Ca^{2+} -permeable AMPA receptor in opiate withdrawal during ontogeny are: (1) the Ca^{2+} -permeable AMPA receptor is strongly expressed at early ages at some of the major sites of opiate action, such as the amygdala⁴¹ and spinal cord^{36,37,43}; and (2) chronic morphine treatment elevates GluR1 but not GluR2 expression in the rat ventral tegmental area²⁴ – elevated expression of GluR1 relative to GluR2 results in an AMPA receptor complex that is more permeable to Ca^{2+} (Refs 4,36,37,44).

Dynamic roles of glutamate receptors in opiate actions
Several models have hypothesized a role for glutamate in opiate tolerance, dependence and withdrawal in the past few years^{2,7,45}. These models position the NMDA receptor, Ca^{2+} influx through this receptor and the subsequent activation of Ca^{2+} -dependent second messenger systems as indispensable steps for the establishment of opiate tolerance and dependence³. Although the role of the NMDA receptor in opiate tolerance and dependence in adult rodents has been firmly supported, these models might need to be expanded to take into consideration the fact that the infant acquires and expresses withdrawal in the absence of fully mature NMDA receptors. On the one hand, opiate tolerance, dependence and withdrawal can be induced in the infant rat; on the other hand, NOS inhibitors, but not NMDA receptor antagonists, are effective in suppressing these processes. Thus, we hypothesize that the essence of the existing models, which focus on the role of the second messenger system, including the influx of Ca^{2+} into the cell and the production of NO, might still hold in the infant. However, in younger animals, the Ca^{2+} -permeable AMPA receptor plays the dominant role in gating Ca^{2+} influx, and later in development (in the rat, during the second postnatal week) the AMPA receptor matures rapidly, loses Ca^{2+} permeability and confers this role to the NMDA receptor (which gains Ca^{2+} permeability) (Fig. 1).

Given that opiate tolerance, dependence and withdrawal are complicated phenomena and involve adaptive changes that encompass cellular, anatomical, systemic, behavioral and psychological levels, multiple mechanisms for these processes might exist. Indeed, it is unlikely that a single mechanism works in isolation. Thus, the present model does not exclude the possible roles of other glutamate receptor types, such as the kainate receptor and the metabotropic glutamate receptors, nor does it exclude the possible roles of other neurotransmission systems in these phenomena. These receptors and systems might interact with various second messenger systems and directly or indirectly lead to the long-lasting plasticity that is characteristic of opiate tolerance and dependence. For example, Crain and Shen have proposed a model that highlights the importance of sustained activation of sensitized excitatory G_s -coupled, GM1 ganglioside-regulated opioid receptor functions in opiate

tolerance, dependence and withdrawal⁴⁶. Unfortunately, there are no data that address the interaction of the stimulatory opioid mechanisms described in their model and the role of any glutamate receptor in the chronic effects of opiates; nor do we know whether or not there is differential developmental regulation of G_i - and G_s -coupled opioid receptors. Of course, the seminal work on the G_s -coupled opioid receptors by Crain and Shen was developed in fetal explants, which are probably in an immature state. Thus, age differences in opiate actions, particularly the existence of a transition period for NMDA receptor antagonists in inhibiting both dependence¹⁸ and tolerance^{20,21}, cannot be explained easily by any possible G_s -coupled opioid receptor differences between different ages. The interaction between glutamate receptor functions and excitatory G_s -coupled opioid receptor functions, which might also account for the age differences in opiate actions outlined above, represents an unexplored field and requires further study⁴⁷. Finally, the model described in this article might apply to opiate withdrawal induced by chronic administration (chronic withdrawal) instead of a single injection (acute withdrawal) of opiates because chronic and acute opiate administration have opposing effects on second messenger systems¹. Thus, not surprisingly, although NMDA receptor antagonists have been found to be ineffective in attenuating chronic opiate withdrawal, they suppress acute morphine withdrawal in the infant rat^{48,49}.

Concluding remarks

Although the role of the NMDA receptor in the development and expression of opiate tolerance, dependence and withdrawal is well established in the adult (e.g. mice deficient in NMDA receptors do not show tolerance), very different mechanisms might exist in the infant. Although the establishment of opiate tolerance and dependence, and the expression of withdrawal might rely on the same second messengers (e.g. Ca^{2+} and NOS) during development, different glutamate receptor types are linked to these cascades during ontogeny. Consequently, in the infant very different therapeutic approaches might be necessary for the treatment of the clinically adverse effects of opiates from those used in the adult. Determination of the dynamic roles of glutamate receptors in opiate tolerance, dependence and withdrawal will significantly further our understanding of these phenomena as a whole. It is expected that our understanding of the role of glutamate-mediated neurotransmission in opiate tolerance and withdrawal will benefit from two lines of research: (1) the development of simpler model systems (e.g. the dorsal horn of the spinal cord), where multiple levels of analysis can be carried out (e.g. behavioral, anatomical, cellular and molecular); and (2) the use of various glutamate receptor subunit knockout animals in developmental studies on opiate actions.

Acknowledgements

We thank Charles E. Inturrisi of the Weill Medical College of Cornell University for his helpful comments on this article. Our research is supported by National Institute on Drug Abuse (NIDA) grants DA 06600 and DA 00325, and by funds from the Biopsychology Doctoral Program of the City University of New York (CUNY).

References

- 1 Nestler, E.J. (1996) Under siege: the brain on opiates. *Neuron* 16, 897–900
- 2 Mao, J. (1999) NMDA and opioid receptors: their interactions in antinociception, tolerance and neuroplasticity. *Brain Res. Rev.* 30, 289–304
- 3 Trujillo, K.A. (1999) Cellular and molecular mechanisms of opioid tolerance and dependence: progress and pitfalls. *Pain Forum* 8, 29–33
- 4 Ozawa, S. *et al.* (1998) Glutamate receptors in the mammalian central nervous system. *Prog. Neurobiol.* 54, 581–618
- 5 Herman, B.H. *et al.* (1995) The effects of NMDA receptor antagonists and nitric oxide synthase inhibitors on opioid tolerance and withdrawal. Medication development issues for opiate addiction. *Neuropsychopharmacology* 13, 269–293
- 6 Inturrisi, C.E. (1997) Preclinical evidence for a role of glutamatergic systems in opioid tolerance and dependence. *Semin. Neurosci.* 9, 110–119
- 7 Mayer, D.J. *et al.* (1999) Cellular mechanisms of neuropathic pain, morphine tolerance, and their interactions. *Proc. Natl. Acad. Sci. U. S. A.* 96, 7731–7736
- 8 Barr, G.A. and Jones, K. (1994) Opiate withdrawal in the infant. *Neurotoxicol. Teratol.* 16, 219–225
- 9 Barr, G.A. and Wang, S. (1992) Tolerance and withdrawal to chronic morphine treatment in the week-old rat pup. *Eur. J. Pharmacol.* 215, 35–42
- 10 Barr, G.A. *et al.* (1998) Neonatal withdrawal following pre- and postnatal exposure to methadone in the rat. *Pharmacol. Biochem. Behav.* 60, 97–104
- 11 Jones, K.L. and Barr, G.A. (1995) Ontogeny of morphine withdrawal in the rat. *Behav. Neurosci.* 109, 1189–1198
- 12 Jones, K.L. and Barr, G.A. (2000) Opiate withdrawal in the fetal rat: a behavioral profile. *Pharmacol. Biochem. Behav.* 66, 419–424
- 13 Thornton, S.R. and Smith, F.L. (1997) Characterization of neonatal rat fentanyl tolerance and dependence. *J. Pharmacol. Exp. Ther.* 281, 514–521
- 14 Thornton, S.R. *et al.* (1997) Characterization of neonatal rat morphine tolerance and dependence. *Eur. J. Pharmacol.* 340, 161–167
- 15 Windh, R.T. *et al.* (1995) The ontogeny of mu opiate tolerance and dependence in the rat: antinociceptive and biochemical studies. *J. Pharmacol. Exp. Ther.* 273, 1361–1374
- 16 Bell, J.A. and Beglan, C.L. (1995) Co-treatment with MK-801 potentiates naloxone-precipitated morphine withdrawal in the isolated spinal cord of the neonatal rat. *Eur. J. Pharmacol.* 294, 297–301
- 17 Bell, J.A. and Beglan, C.L. (1995) MK-801 blocks the expression but not the development of tolerance to morphine in the isolated spinal cord of the neonatal rat. *Eur. J. Pharmacol.* 294, 289–296
- 18 Zhu, H. and Barr, G.A. (2001) Inhibition of morphine withdrawal by the NMDA receptor antagonist MK-801 in rat is age dependent. *Synapse* 40, 282–293
- 19 Zhu, H. and Barr, G.A. (2000) Naltrexone-precipitated morphine withdrawal in infant rat is attenuated by acute administration of NOS inhibitors but not NMDA receptor antagonists. *Psychopharmacology* 150, 325–336
- 20 Barr, G.A. and Zhu, H. Ontogeny of AMPA and metabotropic glutamate receptor mediated morphine withdrawal in the rat. *Soc. Neurosci.* (in press)
- 21 Zhu, H. and Barr, G.A. Ontogeny of NMDA receptor mediated morphine tolerance in the rat. *Soc. Neurosci.* (in press)
- 22 Gu, J.G. *et al.* (1996) Synaptic strengthening through activation of Ca²⁺-permeable AMPA receptors. *Nature* 381, 793–796
- 23 Fedele, E. and Raiteri, M. (1999) *In vivo* studies of the cerebral glutamate receptor/NO/cGMP pathway. *Prog. Neurobiol.* 58, 89–120
- 24 Fitzgerald, L.W. *et al.* (1996) Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *J. Neurosci.* 16, 274–282
- 25 Jang, C.G. *et al.* (2000) An autoradiographic study of [³H]AMPA receptor binding and *in situ* hybridization of AMPA sensitive glutamate receptor A (GluR-A) subunits following morphine withdrawal in the rat brain. *Brain Res. Bull.* 52, 217–221
- 26 Kest, B. *et al.* (1997) The competitive α -amino-3-hydroxy-5-methylisoxazole-4-propionate receptor antagonist LY293558 attenuates and reverses analgesic tolerance to morphine but not to delta or kappa opioids. *J. Pharmacol. Exp. Ther.* 283, 1249–1255
- 27 McLemore, G.L. *et al.* (1997) The effects of LY293558, an AMPA receptor antagonist, on acute and chronic morphine dependence. *Brain Res.* 778, 120–126
- 28 Rasmussen, K. *et al.* (1996) A selective AMPA antagonist, LY293558, suppresses morphine withdrawal-induced activation of locus coeruleus neurons and behavioral signs of morphine withdrawal. *Neuropsychopharmacology* 15, 497–505
- 29 Danysz, W. and Parsons, A.C.G. (1998) Glycine and *N*-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. *Pharmacol. Rev.* 50, 597–664
- 30 Colwell, C.S. *et al.* (1998) Postnatal development of glutamate receptor-mediated responses in the neostriatum. *Dev. Neurosci.* 20, 154–163
- 31 Guilarte, T.R. and McGlothan, J.L. (1998) Hippocampal NMDA receptor mRNA undergoes subunit specific changes during developmental lead exposure. *Brain Res.* 790, 98–107
- 32 Das, S. *et al.* (1998) Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature* 393, 377–381
- 33 Barrios, M. and Liljequist, S. (1996) Developmental changes in glutamate receptor-activated translocation of protein kinase C in cerebellar granule neurons. *Dev. Brain Res.* 94, 22–30
- 34 Hrabetova, S. *et al.* (2000) Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction. *J. Neurosci.* (Online) 20, RC81
- 35 Sircar, R. (2000) Developmental maturation of the *N*-methyl-D-aspartic acid receptor channel complex in postnatal rat brain. *Int. J. Dev. Neurosci.* 18, 121–131
- 36 Jakowec, M.W. *et al.* (1995) Quantitative and qualitative changes in AMPA receptor expression during spinal cord development. *Neuroscience* 67, 893–907
- 37 Jakowec, M.W. *et al.* (1995) *In situ* hybridization analysis of AMPA receptor subunit gene expression in the developing rat spinal cord. *Neuroscience* 67, 909–920
- 38 Carriedo, S.G. *et al.* (1998) Rapid Ca²⁺ entry through Ca²⁺-permeable AMPA/kainate channels triggers marked intracellular Ca²⁺ rises and consequent oxygen radical production. *J. Neurosci.* 18, 7727–7738
- 39 Contestabile, A. (2000) Roles of NMDA receptor activity and nitric oxide production in brain development. *Brain Res. Rev.* 32, 476–509
- 40 Jia, Z. *et al.* (1996) Enhanced LTP in mice deficient in the AMPA receptor GluR2. *Neuron* 17, 945–956
- 41 Mahanty, N.K. and Sah, P. (1998) Calcium-permeable AMPA receptors mediate long-term potentiation in interneurons in the amygdala. *Nature* 394, 683–687
- 42 Perkinson, M.S. *et al.* (1999) Ca²⁺-permeable AMPA receptors induce phosphorylation of cAMP response element-binding protein through a phosphatidylinositol 3-kinase-dependent stimulation of the mitogen-activated protein kinase signaling cascade in neurons. *J. Neurosci.* 19, 5861–5874
- 43 Engelman, H.S. *et al.* (1999) The distribution of neurons expressing calcium-permeable AMPA receptors in the superficial laminae of the spinal cord dorsal horn. *J. Neurosci.* 19, 2081–2089
- 44 Washburn, M.S. *et al.* (1997) Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. *J. Neurosci.* 17, 9393–9406
- 45 Fundytus, M.E. and Coderre, T.J. (1999) Opioid tolerance and dependence: a new model highlighting the role of metabotropic glutamate receptors. *Pain Forum* 8, 3–13
- 46 Crain, S.M. and Shen, K.F. (1998) Modulation of opioid analgesia, tolerance and dependence by G_s-coupled, GM1 ganglioside-regulated opioid receptor functions. *Trends Pharmacol. Sci.* 19, 358–365
- 47 Crain, S.M. and Shen, K. (2000) Enhanced analgesic potency and reduced tolerance of morphine in 129/SvEv mice: evidence for a deficiency in GM1 ganglioside-regulated excitatory opioid receptor functions. *Brain Res.* 856, 227–235
- 48 Jones, K.L. *et al.* (1998) The NMDA receptor antagonist, LY235959, attenuates behaviors and c-fos expression during acute morphine withdrawal in the neonatal rat. *Soc. Neurosci.* 24, 1739
- 49 Jones, K.L. *et al.* (1999) Dextromethorphan attenuates behaviors and c-fos expression during acute morphine withdrawal in the neonatal rat. *Soc. Neurosci.* 25, 34
- 50 Kyzozis, A. *et al.* (1995) Calcium entry through a subpopulation of AMPA receptors desensitized neighbouring NMDA receptors in rat dorsal horn neurons. *J. Physiol.* 485, 373–381

Chemical name

LY293558: (3*S*,4*aR*,6*R*,8*aR*)-6-[2-(1(2*H*-tetrazole-5-yl)ethyl)] decahydroisoquinoline-3-carboxylic acid