The development of analgesic, pro- and anti-convulsant opiate effects in the rat

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Summary. - Evidence indicates that the neonate is capable, if not perceiving nociception, then at least reacting to noiceptive stimuli. These responses can be inhibited by opiates such as morphine. The analgesic potency of morphine in rat pups increases with maturatation, due to (a) the proliferation of opiate receptors and (b), the maturation of supraspinal descending inhibition which becomes functional at 3 weeks post-natally. Tolerance to repeated injections of morphine in pups is less pronounced than in adults since it is masked by several processes, it has been demonstrated to occur within the first two weeks of life. Toxic effects of morphine in the neonate, as can be demonstrated both in behavior and EEG, differ from those in adults. Thus, convulsions induced by morphine which have been reported to occur in adults, were absent in pups. Excitatory effects of morphine in behavior develop in 3 different stages. During the first week morphine caused behavioral activation which is not mediated by specific opiate receptors. In the second week morphine produces EEG spikes in a dose-dependent fashion, but at this age these spikes were not reversable by opiate antagonists. Opiate specific EEG spikes and other opiate specific excitor effects start to predominate during the third week of life.

Keywords: pups, development, analgesia, opiates, convulsant effects.

Riassunto (Sviluppo degli effetti analgesici, pro-convulsivanti e anti-convulsivanti indotti dagli oppioidi nel ratto). - I risultati sperimentali indicano che l'animale neonato è in grado di reagire a stimoli nocicezzi, anche se non è chiaro se i neonati percepiscano un vero e proprio senso di dolore. Queste reazioni possono essere inibite da oppioidi come la morfina. La potenza analgesica della morfina nei ratti neonati cresce con la maturazione, sia per: a) proliferazione dei recettori oppioidi, sia per: b) maturazione della inibizione soprassspinale discendente, che diventa funzionalmente attiva a tre settimane dopo la nascita. La tolleranza a iniezioni ripetute di morfina nei neonati è meno evidente che negli adulti, dal momento che essa è mascherata da altri processi, ma è stato dimostrato che si manifesta entro le prime due settimane di vita. Gli effetti tossici della morfina del neonato, come può essere dimostrato sia attraverso il comportamento che l' EEG, differiscono da quelli evidenziati nell'adulto. Infatti le convulsioni indotte da morfina sono state evidenziate nell'animale adulto, ma sono assenti nel neonato. Gli effetti eccitatori della morfina nel comportamento si sviluppano in tre differenti stadi. Durante la prima settimana la morfina induce attivazione comportamentale che non è mediata da recettori oppioidi specifici. Nella seconda settimana la morfina induce punte EEG in maniera dose-dipendente, ma a questa età le punte non possono essere bloccate da antagoantisti oppioidi. Le punte EEG specificamente indotte da oppioidi e altri effetti eccitatori specifici da oppioidi cominciano a divenire preponderanti durante la terza settimana di vita.

Parole chiave: ratti neonati, sviluppo, analgesia, oppioidi, effetti convulsivanti.

Introduction

The importance of the last two decades for our understanding of opiate action can hardly be overstated. The discovery of specific opiate receptors [1-3], the existence of endogenous opiates [4], and the presence of multiple opiate receptors (for review, see [5]) have enabled us to learn more about opiate action than in the preceding thousands of years that opiates were known to the human species.

The present review focuses on three behavioral effects produced by opiates that have been extensively investigated in adult, but barely so in immature animals: analgesia, epileptiform and anti-convulsant action. We hope to show that the study of these behavioral effects in immature animals not only is worthwhile for its own sake, but also that the sequential development of physiological systems helps understanding mature systems.

Does the neonate perceive pain?

Traditionally, the human neonate was considered to be incapable of perceiving pain (for review see [6-8]). An early study which tested limb withdrawal in response to a pinprick asserted that infants could not perceive or localize pain in the first postnatal year [9]. In addition, infants were not considered to experience significant discomfort during a procedure such as circumcision [10]. Moreover, neonates were thought not to be able to remember painful experiences [10, 11]. It was suggested
that neonates were insensitive to pain and therefore they did not suffer during birth [12]. As a result, physicians rarely prescribed postoperative analgesics for children and nurses tended to administer less than was prescribed [7].

This view has changed over the last decades [6, 8]. It was found that ascending nociceptive pathways such as the spino-thalamic tract, are present even before birth [13]. The maturation of nociceptive pathways is at least sufficient to produce physiological stress in neonates. Thus, procedures such as circumcision [14], heel lancing [15, 16], or tracheal intubation [17] produce an increase in blood pressure, heart rate and palmar sweating, all signs of stress in neonates. Hormonal studies in human neonates have also shown that surgical procedures, which were performed under minimal anesthesia, produced intense stress, as measured by an increased release of corticosteroids [18].

Evidence for early maturation of nociceptive mechanisms was also generated by behavioral research in animals. Thus, flexor withdrawal reflexes activated by mechanical and thermal nociceptive stimuli appear not only to be present at, or immediately after birth, but appear to be hypersensitive when compared to adults [19-22] in that these reflexes were exaggerated and of long duration. We (Falcon, Guendelman, and Frenk, unpublished observations) studied the development of the tail-flick withdrawal reflex to thermal stimuli. We found, that in pups up till 9 days of age the tail-flick reflex could be activated at temperatures as low as 410, lower than the adult nociceptive threshold (450). Tail-flick latencies paralleled this development, with animals up till 9 days of age responding at shorter latencies to all effective stimuli.

In summary, it is clear that nociceptive stimuli produce nociceptive responses in neonates, and, although there is no conclusive evidence that neonates do feel pain (pain is a subjective emotional experience, the above described research does indicate that nociceptive processes are at least sufficiently developed to enable nociceptive reflexes and physiological and psychological stress in infants.

Opiate analgesia in neonates

The observations that nociceptive stimuli produce a wide array of pain related responses in the newborn provide both the rationale and the methods to answer the questions pertaining to the analgesic potential of morphine and its sites of action.

The development of endogenous opiates and their receptors

The anatomical distribution and levels of the three groups of endogenous opioids (endorphins, enkephalins and dynorphins) and their different receptors (μ, δ and κ) have been assessed both pre- and postnatally in rats. Opioid peptides are present in fetal rats [23, 24] but at much lower levels than adult rats and after birth mature levels are reached only at three weeks (for review see [25]). Ligand binding studies showed that opioid receptors, too, are present in fetal rats [26, 27] and continue to increase in quantity postnatally, until the third postnatal week [28]. However, each group of opioid peptides and every receptor sub-type has its own pattern of development. Endorphins appear before enkephalins, and enkephalins prior to dynorphins during gestation [23, 29]. Mu and kappa receptors are present at birth, whereas delta receptors can only be detected from the second postnatal week onwards [30-34].

Analgesia following systemic injections of morphine

During development of the neonate, neurons proliferate together with their receptors. This results in an increase in opiate receptor binding during the first two postnatal weeks in rat pups [35, 36]. Consequently, the effects of opiate drugs on the nociceptive threshold do not remain constant over the first postnatal weeks [37]. Morphine induced analgesia has been observed already in 1-day-old pups [38], but it was reported [35-37] that morphine’s potency in producing inhibition of the nociceptive tail-flick reflex increases rapidly during the first two weeks after birth. Morphine’s analgesic effective dose (ED50) was found to be 40-fold greater in two-day-old, than in 14-day-old rats. This difference in analgesic sensitivities between 2 and 14-day-old rats was attributed to proliferation of endogenous opioids and their receptors [36].

Supra-spinal substrates of opiate analgesia

In adult rats, antinociception elicited by systemic injection of opiates is mediated by spinal [39, 40] and supraspinal [41] mechanisms. Although systemic injection of opiates also produces analgesia in rat pups from the first postnatal week onwards [19, 35-38], these experiments did not elucidate whether both spinal and supraspinal sites are involved in antinociception in newborn rats. We [42] therefore investigated the development of supraspinal antinociception.

Supraspinal inhibition of spinal nociceptive reflexes results from activation of fibers contained in the DLF [43]. These descending fibers can be activated by systemic injection of opiates [44] and also by electrical stimulation of, [45] and microinjection of opiates [41] or other compounds such as glutamate [46, 47], bicuculline and picrotoxin [48] into, the PAG. Activation of the DLF causes inhibition of the response of spinal dorsal horn neurons to nociceptive stimuli [49]. The neurotransmitters that are released into the spinal cord from DLF terminals, and mediate antinociception, are apparently norepinephrine and serotonin [50, 51].
Several studies suggest that the DLF is present or functional from the first postnatal day onwards. Anatomical studies showed that neuronal projections from the brainstem to the spinal cord are present from the first postnatal day onwards [52-54]. In addition, pharmacological studies found that monoaminergic agonists and antagonists affect nociception as early as 3 days after birth [55, 56]. Thus, it appears that descending inhibition of nociceptive reflexes is functional from 3 days after birth.

In contrast to this anatomical, physiological and behavioral evidence, there are studies which indicate that functional maturation of the DLF occurs only at 3 weeks postnatally. First, stimulation of the DLF partially inhibited dorsal horn neurons only 10–12 days after birth in rat pups [52]. Second, lower levels of forepaw shock induced analgesia were observed in 10-day-old as compared to 28-day-old pups [57]. Third, lesioning of descending fibers in rat pups did not decrease analgesia produced by systemic morphine in 14-day-old pups [36], as opposed to adult rats [58]. Finally, a study of descending serotonergic axons showed that serotonergic immunoreactivity was sparse at 14 days after birth in the rat spinal cord, and that adult levels are only reached 21 days after birth [59].

In order to provide evidence for early involvement of supraspinal mechanisms in nociception, we activated the descending pathway directly by means of electrical stimulation of the PAG at postnatal days 7, 14 and 21. It was found that PAG stimulation inhibits the nociceptive tail-flick reflex only at 3 weeks after birth. Thus, despite the anatomical presence of the DLF at birth [53, 54], it becomes functional in antinociception only three weeks postnatally. Hence, systemically administered morphine induces antinociception which is only mediated by opiate receptors in the spinal cord until 3 weeks postnatally [36, 60, 61].

Mechanisms of SPA in 3-week-old and adult rats

In adult animals analgesia elicited from the PAG is mediated by both opiate and non-opiate receptors (for review see [62]). It has been suggested that the substrate which mediates analgesia produced by specific opiate receptors is located in the ventral PAG, whereas non-naloxone reversible analgesia involves the dorsal PAG [63].

Evidence exists which suggests that these two systems of antinociception develop differentially. A study of forepaw shock induced analgesia, which is also mediated by the DLF [64] found that this analgesia was reversed by naloxone at 28 days postnatal, but not at 5-7 months after birth [57]. Thus, antinociception mediated by specific opiate receptors, and therefore by the ventral PAG, may develop prior to non-naloxone reversible antinociception from the dorsal PAG.

The results of our study [42] showed that the ventral PAG does indeed mature earlier than the dorsal PAG. Current intensities needed to produce SPA were higher in 3-week-old pups from the dorsal PAG, when compared to the ventral PAG. Since this difference between the current intensities needed to produce SPA from either site disappears in adult rats, this indicates that the ventral PAG matures prior to the dorsal.

Although we found, like others did in adult animals, that naloxone blocks SPA from the ventral PAG in pups, we failed to replicate this finding in adult animals. The finding that naloxone reversibility of SPA in the ventral PAG is a transient age-related phenomenon, could possibly explain the contradictory findings of others. Thus, some investigators report that naloxone reverses SPA from the ventral PAG in adult rats [63], whereas others could not replicate this effect [65]. Based on the present findings, it is possible that age of the animals is the crucial factor for opiate mediation of descending inhibition of nociceptive reflexes. Since the researchers of naloxone reversibility of SPA do not report the age of their subjects [63, 65], a conclusive statement cannot be made at this time.

Do opiates produce tolerance in infant rats?

Tolerance occurs if an increased amount of drug over time is required to produce the same degree of effect [66]. Tolerance to morphine's effects has been shown to occur after chronic [67] and even after a single [68] administration of the drug to adult rats. Tolerance in adult animals has been suggested to be mediated by two mechanisms: psychological [69, 70] and physiological [71, 72]. Evidence pertaining to psychological variables was observed when development of tolerance was found to be facilitated as a result of repeated exposure to the testing procedure and environment [69, 73, 74]. It was suggested that learned tolerance occurs according to mechanisms of classical (Pavlovian) conditioning. Tolerance, or the conditioned response (CR), has been shown to develop more rapidly if there is a specific cue or conditioned stimulus (CS), such as the environment, associated with the administration of morphine, the unconditioned stimulus (UCS) [70, 75]. On the other hand, repeated drug administration in different environments causes a slower development of tolerance since there is no association between environmental cues and drug administration [76].

As for physiological mechanisms, there is ample evidence that opiate tolerance is mediated by cellular processes. Thus, cross tolerance occurs among opioid actions at the same receptors [77] but there is little cross tolerance between opiates that activate different receptor subtypes [78-80]. Moreover, pretreatment with β-
funulateamine, a specific and irreversible μ opioid receptor antagonist, prevented development of tolerance to morphine’s analgesic effects [81, 82].

Tolerance to morphine’s analgesic effects has not been observed in pups within the first two postnatal weeks [38], but was observed thereafter [19]. The lack of evidence for tolerance in newborn rats may be due to absence of one, or both, of the mechanisms which mediate tolerance in adult animals. On the other hand, it is possible that rat pups may be unable to form associations and therefore not become tolerant to opiates [38]. This assumption is unlikely, since studies have shown that neonatal rats can learn associations involving an odor cue [83-85]. In addition, 3-day-old pups can learn to administer intracranial self-stimulation [86]. Both findings indicate that rat pups can learn. On the other hand, learning may not be a necessary prerequisite for tolerance acquisition. A decrease in morphine’s potency was demonstrated in 3-day-old [87] and 5-week-old [88] rat pups born to mothers that received morphine during pregnancy. Tolerance in these pups cannot be due to learning since it was observed during the first postnatal analgesia test session. Thus, an impairment in, or absence of, learning cannot be the reason for lack of tolerance in pups that received morphine in the first two postnatal weeks.

We [89] hypothesized that the failure to observe tolerance in these young animals was due to three developmental processes which counteract the development of tolerance. First, during the first postnatal weeks opiate receptors proliferate continuously which results in increased morphine potency. Second, receptors that develop after the first morphine injection are less exposed to the drug, and therefore less subject to tolerance, than receptors that were already present on day 5. Finally, tolerance in newborn rats is mediated by spinal cord mechanisms only, since the descending pathways, which mediate SPA from the PAG [90] become functional only three weeks after birth. It has been suggested, that simultaneous pre- exposure of spinal and supraspinal sites to morphine [44, 91] results in stronger tolerance than pre-exposure of each separate site to morphine [92].

The results of our study show that tolerance does indeed exist prior to 2 weeks of age but that it can only be demonstrated on a full dose- response curve. Thus, when pups aged 5-8 days are pretreated with a relatively high (20 mg/kg) dose of morphine, a rightward shift of this curve is observed on day 9. The effect was small since it occurred only at doses (0.5-4 mg/kg) that were far lower than the pretreatment dose. In addition to replicating others [30] and demonstrating that tolerance cannot be observed with the dose used for pretreatment, we found weak, but significant tolerance at the lower doses (0.5-4 mg/kg).

In adult animals, exposure to a single [93] or several [39] injections of morphine produces hypersensitivity to opiate antagonists. It was suggested that this increase in potency of antagonist drugs is a measurement of the onset of tolerance [94]. We (Van Praag and Frenk, unpublished observations) found that naloxone was more effective in reversing morphine analgesia in morphine pretreated pups than in saline pretreated animals. Together with the rightward shift of the dose-response, supersensitivity to naloxone on day 9 constitutes proof that tolerance occurs within the first two postnatal weeks.

**Pro- and anticonvulsant effects of morphine in infant rats**

In adult animals opiates exert proconvulsant action in at least three ways (For review, see [95]). First, opiates produce epileptiform EEG activity with myoclonic twitches and wet dog shakes, but without generalized convulsions, when administered intracerebroventricularly (i.c.v.) or within discrete brain structures. Second, systemic injections of opiates may produce generalized convulsions. Third, opiates may enhance epileptic activity produced by other chemical agents or manipulations. In addition, in certain conditions morphine may also show an anticonvulsant effect. In the following sections studies with relevance to the ontogeny of these effects will be summarized.

**Opiate nonconvulsive epileptiform activity in the neonate**

Intracerebroventricular (i.c.v.) administration of morphine and endogenous opioids cause non-convulsive electrographic seizures [96, 97]. Similar epileptiform changes have been reported following intracerebral (i.c.) administration of morphine or enkephalins into the hippocampus [98], thalamus [99] and caudate nucleus [100]. These effects were reversed by naloxone [96]. Recent studies in rat pups support the above conclusions. It was found that leucine-enkephalin first produced spikes in two day old, whereas β-endorphin only did so later [101]. I.c.v. morphine and morphiceptin produced electrographic spiking from five days of age and seizure-like activity from 7 days old, while delta agonists, D-Ala2-D-Leu5-enkephalin (DADL) and D-Tyr-D-Ser-Gly-Phe-Leu-Thr (DSLET), produced spiking from day 12 which progressed to seizures over the following 2 weeks. All epileptiform activity was naloxone reversible [101, 102].

**Convulsant and general excitatory effects of opiates in neonates**

High doses of systemic morphine may produce electrographic spikes, electrographic seizures and overt convulsions in adult rats [103] and several other species such as the rabbit [104], dog [105], and man [106]. These
convulsions were neither subject to tolerance nor reversible by specific opiate antagonists, hence are not mediated by specific opiate receptors [103]. It is likely that seizures produced by systemically administered morphine are mediated by the GABAergic and glycnergic system [95], since morphine is an antagonist to both GABA and glycine [107, 108].

The convulsant effect of systemic morphine in human and animal neonates was investigated in early studies. It was reported that morphine may elicit convulsions in human infants [109]. The few animal studies that exist all reported that morphine produces convulsions in neonates, and that young animals are far more sensitive to morphine than adults [110, 111]. Thus, the average lethal systemic dose of morphine was found to be lower in young than in adult rabbits and the minimal convulsive dose nearly coincided with the average fatal dose in the first two postnatal months [110]. It was also found that, in rabbits, the average lethal dose of morphine increased and reached a peak in the second month of life, indicating a decrease in sensitivity to morphine during subsequent development [110]. Similar results were reported for newborn rats, that were found to be ten times more susceptible to the lethal effects of morphine than adults, with convulsions being the purported cause of death. In addition, these researchers reported a steady decrease in susceptibility over the first three postnatal weeks [112].

These studies [110-112] have technical and methodological limitations. They did not make use of EEG recordings and made very few behavioral observations. Only a distinction between tonic and clonic seizures was made [111]. A clear description of the behaviors observed, was lacking in these studies. Moreover, convulsions were reported to occur already in the first postnatal week [111, 112], a finding which stands in marked contrast with modern studies using proconvulsants other than morphine, that failed to report convulsions within the first week of life.

The early studies which report convulsions following morphine in newborn animals [111, 112] are at odds with current data. The recent studies, using proconvulsant compounds such as pilocarpine [113] and kainic acid [114], did use EEG recordings and made detailed behavioral observations in rat pups and reported that neither well-defined electrographic seizures, nor tonic-clonic convulsions occurred during the first postnatal week [113-115]. At the end of the second postnatal week, however, a peak susceptibility to the proconvulsant effects of manipulations such as kindling [116] and compounds such as kainic acid [114, 117], pilocarpine [113], and aminophylline [118] is reported, which declines to adult values in the following weeks.

In order to investigate whether the discrepancy between the present and past studies results from incomplete techniques or special, and yet unknown, convulsant properties of morphine, we [119] performed a series of studies in which systemic morphine was administered from postnatal day 1 and its effects were monitored by EEG recordings and behavioral observations.

Our studies showed that morphine's excitatory effects develop in 3 different stages of maturation. In the first stage (days 1-3), excitation is apparent only in behavior and not in the EEG. For example, morphine (100 mg/kg) produces an increase in locomotion in 1- and 3-day-old rats, but only a few spikes which were not dose-related. In the second stage (days 12-24), morphine produces spikes in a dose-related manner. Spike frequency increases from day 12 to day 24. In the third stage (day 24 - adult) opiate specific excitatory effects become more pronounced, whereas non-specific excitation becomes less apparent. For example, naltrexone reversible spikes were observed only at a dose of 50 mg/kg on day 12, whereas on day 24 spikes induced by 300 mg/kg of morphine were transiently reversed by opiate antagonists, as was observed in adult rats [103].

Although morphine failed to produce convulsions in any of our animals, this was clearly not because of immaturity, at least not on and after day 12, since other researchers reported that convulsant compounds such as kainic acid [114, 117], aminophylline [118] and pilocarpine [113] produce seizures and convulsions in rats at two weeks after birth. However, even the highest dose administered (300 mg/kg) which produced convulsions in adult rats [103], did not produce convulsions in either 12- or 24-day-old rats. It is likely, that differences in techniques used in the adult [103] and our pup study account for the different findings. In the present study electrodes were glued to the skull, whereas in animals skull screws were used [103]. Damage to the blood-brain-barrier caused by these screws lowers the seizure threshold [120]. Indeed, overt convulsions in animals that were not implanted with screws were reported to be rare [121, 122].

Opiate specific and non-specific excitatory effects of morphine in combination with PTZ

Opiates can potentiate convulsions induced by manipulations such as kindling [123] or by administration of compounds such as pentyleneetetrazol (PTZ) [124, 125] or picrotoxin [124]. The ability of morphine to exacerbate convulsions induced by other manipulations is reversed by naltrexone and hence mediated by specific opiate receptors [125].

Since there exists no information pertaining to these effects in the developing organism, we (Van Praag and Frenk, in preparation) investigated the effects of morphine on PTZ induced convulsions in rat pups. We could produce PTZ convulsions in 12-day-old as well as 24-day-old rats. In both age groups these seizures were potentiated by pretreatment with morphine (50 mg/kg). Although studies utilizing other proconvulsants, such as
kainic acid [114, 117], aminophylline [118] and pilocarpine [119] reported peak susceptibility to seizures at 2 weeks after birth. PTZ alone as well as in combination with morphine did not produce a greater number of seizures in 12-day-old as compared to 24-day-old pups.

However, 24-day-old rats differed from 12-day-old animals in that morphine (50 mg/kg) pretreatment induced potentiation of PTZ seizures, as reported for adult rats [122, 125, 126], which was blocked by administration of naltrexone in the first, but not in the latter. Even when the dose of morphine was lowered to 12.5 mg/kg we did not find evidence for involvement of opiate receptors in 12-day-old rats, since at this dose the proconvulsant effect of morphine on PTZ-induced seizures was no longer detectable. Thus, opiate-specific receptors mediate morphine's proconvulsant effect on PTZ-induced seizures on day 24, but not on day 12.

Although specific opiate receptors do not mediate morphine's proconvulsant effect on PTZ-induced seizures on day 12, these receptors are involved in morphine-induced electrographic spikes [119]. Electrographic spikes induced by morphine alone were reversed by opiate antagonists following 50 mg/kg of morphine but were not affected at higher doses of morphine (100 and 300 mg/kg). In 24-day-old animals, on the other hand, spikes were reversed by naltrexone following 300 mg/kg of morphine. Thus, the involvement of specific opiate receptors in the excitatory effect of morphine by itself, becomes more pronounced upon maturation.

The increased involvement of opiate receptors in the excitatory effect of morphine results from two developments. First, the number of opiate receptors continues to increase until 3 weeks after birth [28] hence, there are less receptor sites available for opiate specific action on day 12 than on day 24. Second, the blood-brain barrier develops only on day 15 postnatally [127]. This was demonstrated by showing that morphine's analgesic potency increased from birth to day 15, but decreased from day 15 to day 30, whereas phenoperidine, on the other hand, which passes from the brain to the blood more rapidly than morphine, had the same analgesic potency from day 15 to day 30 [37]. Taken together, these points suggest that (a) 50 mg/kg dose of morphine in 12-day-old rats may be a higher effective dose than on day 24 and (b) exceeds the number of available opiate receptor sites. Hence, it is likely that morphine induces excitatory effects and has a proconvulsant effect on PTZ-induced seizures primarily by antagonism of GABA/glycine receptors on day 12 [107, 108], whereas these effects are mainly mediated by specific opiate receptors on day 24.

The opiate receptor subtype that mediates morphine's excitatory and proconvulsant effects

Opiate epileptiform activity, which was reversible by opiate antagonists was first reported following intracerebral administration of opiates [96-98, 109]. These electrographic seizures have been suggested to be mediated by δ receptors for the following reasons. Since (a) the i.c.v. dose of the μ receptor agonist morphine [128] necessary to produce electrographic seizures and spikes is twice as high as the amount that produces analgesia [97], (b) the δ receptor agonist leu-enkephalin produced electrographic seizures and spikes at doses that are lower than those needed to produce analgesia [96, 129], and (c) relatively high doses of naltrexone are needed to reverse the epileptogenic effects of morphine as well as endogenous opioids [96], it was suggested that this epileptiform activity is mediated by δ receptors.

A second opiate specific proconvulsant effect of morphine occurs when morphine is administered in combination with other compounds such as PTZ [125]. The proconvulsant effect of morphine administered with other compounds is induced by low doses of morphine [95]. In addition, this effect is reversed by low doses of opiate antagonists [125, 126]. Therefore, μ receptors were suggested to mediate morphine's proconvulsant effect on convulsions produced by other compounds or manipulations [95].

Studies in adult animals have repeatedly reported that high doses of systemic morphine produce electrographic spikes [103, 122], and these spikes are, at least transiently, reversed by naltrexone. Therefore, these electrographic spikes are mediated by specific opiate receptors. Since systemic injections of morphine reach all parts of the CNS, including the periventricular sites, it could be hypothesized that the electrographic spikes induced by systemic morphine are elicited by the same receptor population as those mediating epileptiform activity induced by i.c.v. injected morphine [96]. On the other hand, electrographic spikes are elicited by very low (10 mg/kg) of morphine [130]. This suggests that these spikes are produced by activation of the same receptor subtype that mediates the proconvulsant effect of morphine on PTZ-induced seizures. No evidence exist pertaining to the opiate receptor subtype that mediates these electrographic spikes.

The results of studies in the immature animal [119] Van Praag and Frenk, in preparation) provide evidence for the contention that morphine's naltrexone reversible spikes are mediated by μ receptor, but not delta receptors. Thus, opiate specific electrographic spikes produced by morphine occur already on day 12, prior to development of δ receptors. Mu receptors, on the other hand, are present [31, 33] and functional [36, 37, 131] from birth. Therefore, μ receptors are both necessary and sufficient to mediate this opiate specific effect.

Morphine's proconvulsant effect on PTZ-induced seizures, on the other hand, is not mediated by specific opiate receptors on day 12, but is reversed by naltrexone on day 24. Therefore, the receptor subtype which mediates morphine's proconvulsant effect may well be the δ receptor, since this receptor subtype is first detected at
weeks after birth [31, 33]. Thus, the receptor subtype which mediates morphine’s proconvulsant effect on PTZ induced seizures in adults is identical to the one that has been suggested to mediate epileptiform activity induced by intracerebral administration of opiates [99].

**Anticonvulsant effects of morphine**

It has been often observed that the convulsant potency of morphine by itself is not as great as when this compound is administered together with opiate antagonists. Administration of nalorphine following injection of morphine or the baine in rats and mice hastened the onset of seizures [124]. Similar findings were reported in the rabbit and cat [132]. Furthermore, following pretreatment with methadone, injection of naloxone potentiated the appearance of epileptiform activity in anesthetized monkeys [133]. In rats, administration of naltrexone decreased latency to onset and increased the frequency of convulsions. However, it was also reported that increasing the dose of naltrexone did not result in further potentiation of convulsions [103]. Thus, the proconvulsant activity depends on the dose of morphine administered, and is unmasked by injection of naltrexone. This implies that, simultaneous with its non-specific convulsant activity, morphine also has an anticonvulsant effect, which is mediated by specific opiate receptors. Inhibition of this anticonvulsant effect by naltrexone therefore triggers the convulsant activity of morphine [103].

Morphine’s anticonvulsant effect is also observed upon administration in combination with other proconvulsants. Electrophographic seizures induced by i.e.v. morphine, leu-enkephalin or -endorphin were blocked by pretreatment with systemic morphine [134]. In addition, morphine delayed the onset of pentyleneetetrazol (PTZ) [122, 125, 126] and flurothyl [121] induced seizures. These anticonvulsant effects are reported to be mediated by μ receptors since they are easily reversed by naloxone [95, 134]. Other researchers, however, using agonists and antagonists which bind preferentially to the receptor subtypes, proposed that delta receptors mediate anticonvulsant effects of opiates [135, 136].

Our recent studies in the immature pups (Van Praag and Frenk, in preparation) provided evidence supporting the view that δ receptors are involved in morphine’s anticonvulsant effects. We found that morphine increased the latency both to the first PCJ and to the first PTZ convolution in 24-day-old rats. This effect was blocked by naltrexone and therefore mediated by specific opiate receptors. On the other hand, no anticonvulsant effect of morphine on PTZ seizures was observed in 12-day-old pups. Since δ receptors are first detected at day 12 and after, as opposed to μ receptors which are present at birth [31, 33], our findings indicate that δ receptors are necessary for morphine’s anticonvulsant effect, as was suggested previously by Tortella et al. [135, 136].

Although the presence of δ receptors appears to be necessary for morphine’s anticonvulsant effect, it may not be sufficient. It was reported that etorphine, a specific μ agonist, as well as the δ agonist DADL, raised the threshold to flurothyl seizures [137]. The anticonvulsant effect of DADL on these seizures was partially blocked by the specific mu antagonist beta-flunaltrexamine [138] as well as by the δ antagonist ICI 154,129 [137]. These investigators therefore, suggested that μ and δ receptors interact to produce an anticonvulsant effect of opiates and this hypothesis is not contradicted by evidence obtained in the developing animal (Van Praag and Frenk, in preparation).

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**REFERENCES**


