Review

Mechanisms underlying δ - and μ -opioid receptor agonist-induced increases in extracellular dopamine level in the nucleus accumbens of freely moving rats

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Abstract: The nucleus accumbens is a terminal area of the mesolimbic dopaminergic system that arises in the ventral tegmental area. Opioids are thought to enhance dopaminergic activity in the nucleus accumbens by activating δ - and μ -opioid receptors in the ventral tegmental area. However, δ - and μ -opioid receptor agonists increase extracellular levels of accumbal dopamine when infused directly into the nucleus accumbens of rats. Therefore, the roles of δ - and μ -opioid receptors in regulation of accumbal dopaminergic neural activity have been analyzed by using δ - and μ -opioid receptor ligands. This review describes the mechanisms underlying the stimulatory effects on accumbal dopamine efflux, which are induced by local administration of δ - and μ -opioid receptor agonists into the nucleus accumbens of freely moving rats. The focus of this article is neurochemical studies that use *in vivo* microdialysis techniques. Taken together, the *in vivo* neurochemical evidence from these studies indicates that δ - and μ -opioid receptor agonists increase accumbal dopamine efflux by activating naloxone-sensitive opioid receptors, and by mechanisms independent of naloxone-sensitive opioid receptors, in the nucleus accumbens.

Keywords: δ -opioid receptor; μ -opioid receptor; dopamine; nucleus accumbens; microdialysis; rats.

Introduction

Highly potent analgesics in clinical use include opioid receptor agonists. The nucleus accumbens is a terminal area of the mesolimbic dopaminergic system that arises in the ventral tegmental area (VTA), and opioids are thought to enhance dopaminergic neural activity in the nucleus accumbens by activating δ - and μ -opioid receptors in the VTA (for reviews, see 1 and 2). When infused directly into the nucleus accumbens, δ - and μ -opioid receptor agonists increase accumbal extracellular dopamine (DA) levels in freely moving rats (3-5). Therefore, the roles of δ - and μ -opioid receptors in regulating accumbal dopaminergic neural activity have been analyzed by using δ - and μ -opioid receptor ligands.

This review describes the mechanisms responsible in freely moving rats for the stimulatory effects on accumbal DA efflux, which is induced by local administration of δ - and μ -opioid receptor agonists into the nucleus accumbens. In particular, we will focus on findings from neurochemical studies that used *in vivo* microdialysis techniques. These studies provide *in vivo* neurochemical evidence that δ - and μ -opioid receptor agonists increase accumbal DA efflux by activating naloxone-sensitive opioid receptors, and by mechanisms independent of naloxone-sensitive opioid receptors, in the nucleus accumbens.

Normally, the effects of opioids—including δ - and μ -opioid receptor agonists—are reduced by pretreat-

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ment with the non-selective opioid receptor antagonist naloxone (6,7). However, caution is necessary when using increasing doses of naloxone to investigate the roles of opioid receptors, because high doses of this compound can produce non-specific effects (8).

Below, the indicated doses of intracerebrally administered compounds are expressed as total amount (mol) over the duration of infusion.

δ -opioid receptor agonists

The nucleus accumbens contains δ -opioid receptors (9-11). These receptors are encoded by a single gene (12) and there are two known pharmacologically distinct subtypes, namely, δ_1 - and δ_2 -opioid receptors (13).

Intra-accumbal infusion of the δ_1 -opioid receptor agonist DPDPE through a microdialysis probe enhanced the extracellular level of accumbal DA in a dose-related manner (5 and 50 nmol) (4,5). Intra-accumbal administration of the δ_2 -opioid receptor agonist DSLET (5 and 50 nmol) (5) also increased the extracellular level of accumbal DA. Activation of accumbal δ_1 - and δ_2 -opioid receptors mediates these effects of DPDPE and DSLET. Specifically, they were inhibited by co-administration of the δ_1 -opioid receptor antagonist BNTX and the δ_2 -opioid receptor antagonist naltriben, respectively. The non-selective opioid receptor antagonist naloxone inhibited the effects of DPDPE and DSLET. Activation of accumbal μ -opioid receptors increased accumbal DA efflux (see the section *μ -opioid receptor agonists*, below). In contrast to the δ_2 -opioid receptor agonist DSLET (50 nmol)-induced accumbal DA increase, which was not affected by the μ -opioid receptor antagonist CTOP or the δ_1 -opioid receptor antagonist BNTX, the accumbal DA increase induced by the δ_1 -opioid receptor agonist DPDPE (50 nmol) was reduced by the μ -opioid receptor antagonist CTOP and the δ_2 -opioid receptor antagonist naltriben (5). These findings suggest the presence of interactions between δ - and μ -opioid receptors in promoting DA release in the nucleus accumbens (5).

Intra-accumbal infusion of the δ_2 -opioid receptor agonist deltorphin II (50 nmol) induced an increase in accumbal DA efflux that was not influenced by the δ_2 -opioid receptor antagonist naltriben or the non-selective opioid receptor antagonist naloxone (14). This deltorphin II (50 nmol)-induced increase in accumbal DA efflux was inhibited by coadministration of the voltage-dependent sodium ion channel inhibitor tetrodotoxin. Therefore, local administration of deltorphin II (50 nmol) into the nucleus accumbens increases accumbal neural release of DA without stimulating naloxone-sensitive receptors in the nucleus accumbens. We recently found

that intra-accumbal infusion of a lower dose of deltorphin II (25 nmol) induced an increase in accumbal DA efflux that was inhibited by the δ_2 -opioid receptor antagonist naltriben (unpublished data). A low (25 nmol), but not a high (50 nmol), deltorphin II dose administered into the accumbens increases DA efflux by stimulating δ_2 -opioid receptors. These findings indicate that there are dose-related quantitative, as well as qualitative, differences in the effects of deltorphin II after intra-accumbens infusion.

The δ -opioid receptor agonists include a compound that increases accumbal DA efflux through naloxone-insensitive and tetrodotoxin-insensitive mechanisms when infused directly into the nucleus accumbens. ($\pm$)-TAN-67 is a non-peptidergic, centrally acting compound that can interact with δ -opioid receptors (15,16). Despite its strong agonistic activity *in vitro* (15,16), ($\pm$)-TAN-67 has weak or no antinociceptive action *in vivo* (17,18). Interestingly, the (+) enantiomer of TAN-67 induces hyperalgesia (19), while the (–) enantiomer produces profound antinociceptive effects in mice. These effects are mediated through δ_1 -opioid receptor stimulation (18,19). Intra-accumbal infusion of (–)-TAN-67 or (+)-TAN-67 (25 and 50 nmol) induces a transient, dose-dependent increase in accumbal extracellular DA level. However, naloxone (1 mg/kg i.p.) failed to alter the transient increase in accumbal DA level produced by infusion of either TAN-67 enantiomer. The (–)-TAN-67- and (+)-TAN-67-induced increases in accumbal DA level were not affected by subsequent perfusion of tetrodotoxin into the nucleus accumbens. Furthermore, increases in accumbal DA level produced by infusion of (–)-TAN-67 or (+)-TAN-67 were not altered by a Ca^{2+} -freeringer solution (4). Thus, both TAN-67 enantiomers enhance release of DA from dopaminergic nerve terminals in the nucleus accumbens in a manner that is independent of neural activity, and activation of δ -opioid receptors has no role in these events. Local infusion of (–)-TAN-67 (50 nmol) may generate a burst of free radicals, which triggers glutamate release, thus activating NMDA receptors and, ultimately, enhancing DA release from dopaminergic nerve terminals in the nucleus accumbens. This likely occurs because (–)-TAN-67-induced DA efflux was significantly reduced by the NMDA receptor antagonists ifenprodil (20 mg/kg i.p.) and MK-801 (0.5 mg/kg i.p.), respectively, and because the effects of (–)-TAN-67 on DA efflux were also inhibited by the free radical scavenger N-2-MPG (100 mg/kg i.p.) (4).

μ -opioid receptor agonists

The nucleus accumbens contains μ -opioid receptors (20) and the μ -opioid receptor agonist fentanyl dose-

dependently increases accumbal DA levels when given intravenously or via a microdialysis probe placed into the nucleus accumbens (3). The effect of fentanyl (5 nmol) administered into the nucleus accumbens was blocked by systemic administration of the non-selective opioid receptor antagonist naloxone and by intra-accumbal administration of the μ -opioid receptor antagonist CTOP. Thus, the increase in accumbal release of DA caused by intra-accumbens administration of fentanyl (5 nmol) appears to be due to simultaneous activation of μ -opioid receptors and δ_2 -opioid receptors or to activation of μ -opioid receptors that interact with δ_2 -opioid receptors in a complex manner (3). This hypothesis is supported by the observations that, in addition to the μ -opioid receptor antagonist CTOP, the non-selective δ -opioid receptor antagonist naltrindole and the δ_2 -opioid receptor antagonist naltriben readily inhibit fentanyl-induced accumbal DA efflux, while the δ_1 -opioid receptor antagonist BNTX does not (3).

Another μ -opioid receptor agonist, DAMGO (5 and 50 nmol), also increased accumbal DA efflux when directly infused into the nucleus accumbens (3,5), and intra-accumbal infusion of naloxone reduced DAMGO-induced DA efflux. Intra-accumbal infusion of a high dose (50 nmol) of DAMGO induces a biphasic effect, i.e., an early-onset increase lasting for 75 min followed by a late-onset gradual and prolonged increase (5). It has been hypothesized that stimulation of μ -opioid receptors activates δ_1 -opioid receptors, which in turn activate δ_2 -opioid receptors, thereby causing early onset of an increase in extracellular DA. An alternative hypothesis is that stimulation of another group of μ -opioid receptors activates a second group of δ_1 -opioid receptors that are not coupled to δ_2 -opioid receptors and mediate a late-onset increase in extracellular DA (5). This effect occurs because the μ -opioid receptor antagonist CTOP primarily reduces the second, late-onset, component of the DAMGO-induced increase in accumbal DA levels (5). Furthermore, the δ_1 -opioid receptor antagonist BNTX reduces the first, early-onset, component but abolishes the second component of these effects of DAMGO, while the δ_2 -opioid receptor antagonist naltriben reduces only the first component. Thus, stimulation of δ_1 - or δ_2 -opioid receptors may inhibit μ -opioid receptors and thus mediate the late-onset increase in extracellular DA, whereas stimulation of δ_1 - but not δ_2 -opioid receptors may activate μ -opioid receptors involved in the early increase in extracellular DA (5). Because of the lack of detailed information on the synaptic location of opioid receptor subtypes in accumbal neural circuitry, it is unclear how activation of one opioid receptor subtype might lead to

activation or inhibition of another subtype (5).

μ -opioid receptors at the level of the VTA are important in the ability of μ -opioid receptor agonists to increase release of accumbal DA (for reviews, see 1 and 2). Yoshida et al. showed that intra-VTA administration of fentanyl (7.5 nmol) or DAMGO (50 nmol) enhanced accumbal DA efflux, but that the effects elicited by intra-accumbens administration of fentanyl (5 nmol) and DAMGO (50 nmol) were considerably greater than those elicited by administration into the VTA (3). The authors concluded that these regional differences were not simply the result of potency differences between μ -opioid receptor agonists in the VTA and nucleus accumbens. Differential expression of opioid receptor subtypes involved in the effects of a given μ -opioid receptor agonist in the VTA and nucleus accumbens may underlie these differences. However, other factors, such as differences in the sizes of these two structures, the numbers of DA transporters, and the diffusion of the drug through tissue, may contribute to regional differences in *in vivo* probe recovery (3).

Endomorphin-1 (EM-1) is a putative endogenous agonist for μ -opioid receptors (21). Intra-accumbal infusion of EM-1 (5, 25, and 50 nmol) induces a transient increase in the level of accumbal DA (22-24). Systemic administration of the non-specific opioid receptor antagonist naloxone (1 mg/kg, i.p.) inhibited this EM-1 (25 and 50 nmol)-induced increase in accumbal DA. Intra-accumbal perfusion of the μ -opioid receptor antagonist CTOP also abolished the EM-1 (25 and 50 nmol)-induced increase in accumbal DA. Therefore, EM-1-induced accumbal DA efflux could be mediated by stimulation of μ -opioid receptors. μ -opioid receptors are further subdivided into μ_1 - and μ_2 -opioid receptors on the basis of their sensitivity to naloxonazine, a selective antagonist at μ_1 -opioid receptors (25,26). Activation of accumbal μ_1 -opioid receptors is involved in the increase in accumbal DA efflux induced by EM-1 (50 nmol), as naloxonazine inhibited the increase in accumbal DA efflux induced by local administration of EM-1 into the nucleus accumbens (22,27). Because co-administration of the GABA_A receptor agonist muscimol or the GABA_B receptor antagonist saclofen enhanced EM-1 (25 nmol)-induced accumbal DA efflux, a decrease in accumbal GABAergic activity may mediate the increase in DA efflux induced by μ_1 -opioid receptor stimulation (23,24).

Endomorphin-2 (EM-2) is another putative endogenous ligand for μ -opioid receptors (21). Intra-accumbal infusion of EM-2 (5, 25, and 50 nmol) also produces a dose-dependent increase in the level of accumbal DA (22-24). However, this EM-2-induced accumbal DA efflux cannot be mediated by naloxone-sensitive opioid

Table 1 δ - and μ -opioid receptor agonists that increase accumbal extracellular dopamine levels when infused locally into the nucleus accumbens of freely moving rats

	Opioid receptors involved	Tetrodotoxin sensitivity
δ -opioid receptor agonist		
DPDPE	δ_1 , δ_2 and μ	(+)
DSLET	δ_2	(+)
Deltorphan II (50 nmol)	(-)	(+)
Deltorphan II (25 nmol)	δ_2	(+)
(-)- and (+)-TAN-67	(-)	(-)
μ -opioid receptor agonist		
Fentanyl	μ and δ_2	(+)
DAMGO	μ , δ_1 and δ_2	(+)
Endomorphin-1	μ_1	(+)
Endomorphin-2	(-)	(+)

δ - and μ -opioid receptors in the nucleus accumbens that appear to be involved in increasing accumbal dopamine levels are listed: (-) indicates that naloxone-sensitive opioid receptors appear to have no role. The tetrodotoxin sensitivity of each increase in accumbal dopamine efflux induced by δ - or μ -opioid receptor agonist treatments is also summarized: (+) indicates an increase in accumbal extracellular dopamine levels that was inhibited by tetrodotoxin and (-) indicates an increase in accumbal extracellular dopamine levels that was not inhibited by tetrodotoxin.

receptors, as systemic administration of the non-specific opioid receptor antagonist naloxone (1 mg/kg, i.p.) and intra-accumbal perfusion of the μ -opioid receptor antagonist CTOP both failed to alter the EM-2 (50 nmol)-induced increase in accumbal DA (22). Thus, intra-accumbal infusion of EM-2 and EM-1 increases accumbal DA efflux by very different mechanisms. The effects of EM-2 are not mediated via naloxone-sensitive opioid receptors; however, the effects of EM-1 are mediated via μ -opioid receptors in the nucleus accumbens. As mentioned above, in the section *δ -opioid receptor agonists*, naloxone (1 mg/kg i.p.) also failed to alter accumbal DA efflux induced by intra-accumbal infusion of the enantiomers of TAN-67, a δ -opioid receptor agonist. However, the mechanisms of action of locally infused EM-2 and TAN-67 on accumbal dopaminergic neurons appear to differ, as EM-2-induced but not TAN-67-induced DA efflux was inhibited by the voltage-dependent sodium ion inhibitor tetrodotoxin. Increases in accumbal DA level induced by intra-accumbal infusion of EM-2 and EM-1 were fully dependent on neural firing. The mechanisms of action of EM-2 (25 nmol) applied locally into the nucleus accumbens are not known, but accumbal GABA receptor subtypes do not appear to be involved, because GABA_A receptor ligands (muscimol and bicuculline) and GABA_B receptor ligands (baclofen and saclofen) had no effect on EM-2 (25 nmol)-induced accumbal DA efflux (23,24). The finding that EM-2-induced effects are not always mediated via opioid receptors is consistent with an earlier study reporting that the inhibitory action of EM-2 on tachykinergic contrac-

tions of isolated guinea pig bronchus was not sensitive to selective antagonists of μ -, κ -, or δ -opioid receptors or to non-specific opioid receptor antagonists such as naloxone (28). The endomorphins bind non-opioid binding sites in tissues lacking μ -opioid receptors, such as rat cerebellum or in the brain of transgenic mice lacking μ -opioid receptors (for review, see 29).

Table 1 shows the putative involvement of δ - and μ -opioid receptors in increased DA efflux in the nucleus accumbens of rats after intra-accumbal infusion of δ - or μ -opioid receptor agonists. This table also shows the tetrodotoxin sensitivity of increases in accumbal DA efflux induced by δ - or μ -opioid receptor agonist treatments. In summary, evidence from *in vivo* microdialysis experiments indicates that δ - and μ -opioid receptor agonists infused into the nucleus accumbens increase DA efflux in freely moving rats by stimulating δ - and μ -opioid receptors, as well as by mechanisms independent of δ - and μ -opioid receptors, in the nucleus accumbens.

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Conflict of interest

The authors have no conflict of interest to declare.

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