GABAergic MECHANISMS OF OPIATE REINFORCEMENT

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Abstract — The neurobiological mechanisms of opiate-induced reinforcement are still not completely understood. Over the past two decades, the vast majority of studies have focused on the role of the mesolimbic dopamine (DA) system. However, current studies strongly suggest that opiate actions on γ-aminobutyric acid (GABA)-ergic cells in both the ventral tegmental area (VTA) and the nucleus accumbens (NAcc) appear to play critical roles. In this review, we focus on the neurochemical substrates of opiate reinforcement and review the role of DA and non-DA substrates, including opioid, GABA, glutamate and serotonin on opiate-reinforced behaviour and the activity of dopaminergic and GABAergic neurons in the VTA and the NAcc.

INTRODUCTION

Opiate abuse remains a major social problem in European and Far Eastern countries and has been making a comeback in the USA over the last several years. Opiates are commonly used clinically to relieve pain and treat chronic diarrhoea. However, repeated administration also produces tolerance, positive reinforcement (reward), physical dependence, and, upon drug cessation, a physical withdrawal syndrome, drug craving and relapse. The precise mechanisms underlying opiates’ addictive effects are still incompletely understood although it has been known for almost three decades that their central nervous system (CNS) effects are mediated by activating opiate μ, δ or κ receptors. With the aid of selective receptor ligands and the subsequent cloning of opiate receptors, it is clear that the majority of morphine’s CNS actions, or that of its predrug, heroin, are mediated by μ-receptors.

In a classical theory of addiction, Wicker and colleagues hypothesized that, since withdrawal is a physical expression of distress, featuring hyperalgesia, gastrointestinal cramps, joint and muscle aches, etc., opiate addiction results, at least in part, from the need to reduce such distress. Accordingly, craving would be a negatively reinforced behaviour related to avoidance of withdrawal distress (see review by Di Chiara and North, 1992). However, this hypothesis has been challenged on both experimental and clinical grounds, as the degree of physical dependence does not predict the intensity of subsequent craving, nor does detoxification and recovery from physical dependence prevent recidivism. Moreover, the motivational (affective) properties of withdrawal are independent of intensity and pattern of the physical symptoms of withdrawal (see reviews by Di Chiara and North, 1992; Schulteis and Koob, 1996).

As such, an alternative hypothesis states that the mesocorticolimbic (MCL) dopamine (DA) system plays a critical role in mediating the positive reinforcing effects of a variety of abused drugs including cocaine, amphetamine, nicotine and opiates (Koob, 1992; Di Chiara, 1995; Wise, 1996). This anatomical pathway originates from the ventral tegmental area (VTA) in the midbrain and projects to several forebrain regions including the nucleus accumbens (NAcc) and medial prefrontal cortex (mPFC) (Kalivas, 1993; White, 1996). However, it is still unclear precisely how abused drugs activate this system and how this system mediates reinforcement. To this end, three major animal models, i.e. drug self-administration (SA), intracranial self-stimulation (ICSS) and conditioned place preference (CPP), have been established and widely used to identify the neuroanatomical and neurochemical mechanisms of drug reinforcement. Several review articles have described various aspects of opiate reward and addiction, including the use of experimental animal models, opiate receptor pharmacology, neural reward circuits, memory, conditioned environmental associations, long-term neuroadaptations and individual genetic vulnerability (Martin, 1983; Schulteis and Koob, 1996; Nestler and Aghajanian, 1997; Bardo, 1998; Shippenberg and Elmer, 1998; Williams et al., 2001). In this review, we focus on the neurochemical substrates of opiate reinforcement and review the role of DA and non-DA substrates, including opioid, GABA, glutamate and serotonin on opiate-reinforced behaviour and the activity of dopaminergic and GABAergic neurons in the VTA and the NAcc.

OPIATE μ AND κ RECEPTOR-MEDIATED POSITIVE AND NEGATIVE REINFORCEMENT

Accumulating experimental evidence indicates that selective activation of μ (and perhaps δ) or κ receptors produces opposite behavioural and physiological effects. For example, acute administration of μ-agonists causes euphoria and feelings of well-being and liking in human subjects, while κ agonists produce dysphoria and psychotomimetic effects (Kumor et al., 1986; Pfeiffer et al., 1986). These observations are further confirmed in experimental animals. For example, opiate μ agonists are self-administered systemically or locally into the VTA or NAcc (Bozarth and Wise, 1981; Devine and Wise, 1984; Goeders et al., 1984). Injections of morphine or other μ agonists into the VTA or NAcc induce a CPP (Phillips and LePiane, 1980; Olds, 1982; Van der Kooy et al., 1982; Mucha and Herz, 1986; Bals-Kubik et al., 1993). In contrast, κ agonists generally lack reinforcing effects (see review by Dykstra et al., 1997) or produce conditioned place aversion (Tang and...
DA-DEPENDENT MECHANISMS OF OPIATE REINFORCEMENT

A large body of experimental evidence supports the DA hypothesis of opiate reinforcement. For example, chemical lesions of VTA DA neurons or DA terminals in the NAcc following 6-hydroxydopamine (6-OHDA) administration not only inhibits the acquisition and maintenance of heroin or morphine SA (Spiryaki et al., 1983; Smith et al., 1985), but also suppresses opiate-induced conditioned preference or aversion (Spiryaki et al., 1983; Shippenberg et al., 1993). Similarly, blockade of D1 receptors with SCH23390 or SCH39166, or D3 receptors with 7-hydroxy-di-n-propylamino tetralin, attenuates the acquisition of morphine-induced CPP behaviour (Leone and Di Chiara, 1987; Shippenberg and Herz, 1988; Daly and Waddington, 1993; Shippenberg et al., 1993; Acquas and Di Chiara, 1994). Further, when an electrode is implanted into either the VTA or the medial forebrain bundle of the hypothalamus, a fibre tract containing ascending DA fibres, animals easily learn to respond on a lever to receive a train of electrical stimulation, thus activating the mesolimbic DA circuit, and increasing NAcc DA release. Morphine or heroin can both decrease the threshold for eliciting ICSS and shift the frequency or current–response function to the left (Esposito and Kornetsky, 1978; Van Wolfswinkel and Van Ree, 1985; Hubner and Kornetsky, 1992). Taken together, these data suggest that the mesolimbic DA system may be the substrate upon which opiates act to produce their reinforcing effects (Bozarth and Wise, 1987).

Considerable evidence suggests that both the positive (rewarding) and negative (aversive) reinforcement of opiate μ and κ receptor agonists are mediated by the mesolimbic DA system (Koob and Nestler, 1997; Pan, 1998; Shippenberg and Elmer, 1998). Electrophysiological studies have demonstrated that systemic or iontophoretic administration of morphine excites DA cells in the VTA and the substantia nigra (Gysling and Wang, 1983; Matthews and German, 1984), whereas U50,488H (κ agonist) inhibits DA cells (Walker et al., 1987). Microdialysis studies have also consistently demonstrated that intracerebroventricular or VTA microinjections of μ agonists cause a significant increase in extracellular DA (Mulder et al., 1984; Di Chiara and Imperato, 1988; Narita et al., 1992; Spanagel et al., 1992; Devine et al., 1993; Xi et al., 1998), while κ agonists significantly decrease extracellular NAcc DA (Di Chiara and Imperato, 1988; Narita et al., 1992; Devine et al., 1993; Ronken et al., 1993; Spanagel et al., 1994; Xi et al., 1998). This ‘push-pull’ or reciprocal modulation of the mesolimbic DA system by μ and κ receptors may, in part, underlie the neurochemical mechanisms of opiate reinforcement.

To further verify the linkage between opiate-reinforced SA behaviour and NAcc DA release, we (Xi et al., 1998) used in vivo fast-cyclic voltammetry (FCV) to monitor the fluctuations in extracellular DA concentration during heroin SA in the rat. These experiments demonstrated that heroin SA behaviour caused a dose-dependent, naltrexone-reversible increase in DA efflux in the NAcc that was inversely proportional to the number of heroin injections. Additionally, activation of κ receptors by U50,488H or Dynorphin A significantly decreased basal DA release and antagonized the SA-stimulated DA release. These data are taken as direct evidence in support of the DA hypothesis of opiate reinforcement.

The mechanisms underlying these opposite cellular and behavioural actions have been attributed to the distinct cellular locations of μ and κ receptors within the mesolimbic DA system, i.e. μ receptors are predominantly located on GABAergic cells in the VTA and NAcc, while κ receptors are mainly located on dopaminergic terminals in the NAcc (Dilts and Kalivas, 1989; Johnson and North, 1992; Svingos et al., 1999). Thus, μ receptor activation in the VTA selectively hyperpolarizes GABAergic interneurons, thereby disinhibiting VTA DA neurons and increasing DA release in the NAcc and the mPFC (Di Chiara and Imperato, 1988; Johnson and North, 1992). In contrast, activation of κ receptors produces a decrease in DA release in the NAcc (Di Chiara and Imperato, 1988; Xi et al., 1998). A similar mechanism may also be involved in the opposite actions of μ and κ agonists on analgesia, and learning and memory-related long-term potentiation in the hippocampus (see review by Pan, 1998).

GABA-MEDIATED DISINHIBITION OF VTA DA NEURONS

Since opiate receptor activation generally inhibits individual neurons, opiate-induced DA release was initially hypothesized to be mediated by a disinhibitory mechanism, i.e. opiates inhibit VTA GABAergic interneurons to decrease GABA release, which subsequently disinhibits VTA DA neurons, leading to an increase in NAcc DA release (Kelley et al., 1980). Several lines of experimental evidence support this hypothesis. For example, systemic or microiontophoretically applied morphine into the VTA increases the firing rate of DA neurons and inhibits the firing rate of inhibitory GABAergic interneurons (Kelley et al., 1980; Gysling and Wang, 1983; Mathews and German, 1984; Johnson and North, 1992). Similarly, microdialysis and electrochemical studies demonstrated an increased NAcc DA release following heroin administration (Rada et al., 1991; Spanagel et al., 1992; Kiyatkin et al., 1993; Xi et al., 1998). Further, anatomical evidence suggests that opiate μ receptors in the VTA are located predominantly on GABAergic interneurons (Mansour et al., 1988; Dilts and Kalivas, 1989), and systemic administration of morphine inhibits GABA release in the midbrain (Renno et al., 1992) and substantia nigra (Starr, 1985). Finally, to determine the causal relationship between GABA and DA or heroin SA behaviour, γ-vinyl GABA (GVG) was either systemically or locally administered into the VTA, NAcc or VP, which significantly elevated extracellular GABA levels by irreversibly inhibiting GABA transaminase (Xi and Stein, 2000). Figure 1 shows a typical in vivo electrochemical
recording demonstrating that intravenous heroin (0.06 mg/kg) injection significantly increases DA-dependent electrochemical signals in the NAcc, which is completely blocked or reversed following GVG microinjection into the VTA. GVG alone significantly lowered the basal levels of DA-dependent signals, an effect that lasted for >1 h of the recording period. Consistent with the reduction in the DA-dependent signals, local administration of GVG into the VTA significantly blocked heroin SA behaviour (Fig. 3A). Similarly, systemic or regional administration of GVG intracerebroventricularly or into the VP, but not NAcc, dose-dependently antagonized heroin reinforcement in rats (Xi and Stein, 2000).

GABA<sub>B</sub> RECEPTOR INVOLVEMENT IN HEROIN-INDUCED DA RELEASE AND REINFORCEMENT

To further determine which receptor subtype(s) underlies this GABAergic disinhibitory mechanism, we observed the effects of GABA<sub>A</sub> and GABA<sub>B</sub> agonists or antagonists on heroin-induced DA release and SA behaviour (Xi and Stein, 1998, 1999). Figure 2 shows another original in vivo electrochemical recording demonstrating heroin increased DA signal (Fig. 2Aa), an effect that was blocked or reversed following blockade of VTA GABA<sub>B</sub> receptors by 2-OH-saclofen (Fig. 2Ab, Ac). Since 2-OH-saclofen alone significantly elevated extracellular DA (Fig. 2Ab), it is suggested that VTA DA neurons are tonically inhibited by endogenous GABA, which is consistent with a microdialysis study by Smolders et al. (1995). Similarly, activation of VTA GABA<sub>B</sub> receptors by baclofen blocks both heroin SA behaviour and NAcc DA release (Xi and Stein, 1998, 1999) and morphine-induced CPP (Tsuji et al., 1996). To evaluate the potential preclinical implications of baclofen in the treatment of opiate addiction, baclofen, when systemically co-administered with heroin, significantly blocked the heroin-induced increase in DA signals (Fig. 2Ba, Bc) and heroin SA behaviour (Fig. 3C). In contrast, co-administration of the GABA<sub>A</sub> agonist muscimol with heroin failed to alter SA behaviour (data not shown). Paradoxically, muscimol, when administered either intravenously or locally into the VTA, increased DA release in the NAcc by activating GABA<sub>A</sub> receptors, an effect that was also prevented by blocking VTA GABA<sub>B</sub> receptors (Xi and Stein, 1998). These results suggest that VTA GABA<sub>B</sub> receptors are predominantly located on GABAergic interneurons or afferents, and the excitatory effect of GABA<sub>A</sub> agonists is similarly mediated by a disinhibitory mechanism. However, two other groups have shown that GABA<sub>A</sub> antagonists can be self-administered into the VTA in mice by a DA-dependent mechanism (David et al., 1997; Ikemoto et al., 1997a,b), suggesting that GABA<sub>A</sub> receptors located on VTA DA neurons also play a role in modulating NAcc DA release and drug reinforcement. Taken together, these data support the hypothesis that VTA GABA<sub>B</sub> receptors may play an essential role in mediating opiate reinforcement.

OPIATE RECEPTOR-MEDIATED INHIBITION OF VTA DA NEURONS

In contrast to the current predominant hypothesis that opiate μ receptors are only distributed on secondary GABAergic
interneurons, but not on primary DA cells (Johnson and North, 1992), our experimental studies demonstrated that some opiate receptors are also located on VTA DA neurons, which may produce direct inhibition of DA release in the NAcc. For example, Figs 1 and 2 show a consistent decrease in DA-dependent signals in the NAcc by heroin following pre-activation of VTA GABA receptors indirectly by GVG or blockade of GABA$_B$ receptors by 2-OH-saclofen. In addition, we also observed a small positive peak that overlaid on a significantly decreased DA signal in ~30–40% of the rats tested. Further, a similar decrease in DA-dependent electro-chemical signals during heroin self-administration behaviour was observed previously by Kiyatkin et al. (1993). Since both the increase and the decrease in the DA signal after heroin SA can be blocked by the opiate antagonist naloxone (Xi et al., 1998; Z. X. Xi and E. A. Stein, unpublished work), these data together support the hypothesis that VTA DA neurons are modulated by both a direct opiate receptor-mediated inhibition and an indirect GABA-mediated disinhibition. Thus, the final amount of DA released after heroin administration will depend on the net effect of these opposing actions.

NON-DA MECHANISMS OF OPIATE REINFORCEMENT IN THE NAcc

Several lines of evidence suggest that non-DA mechanisms also play an important role in mediating opiate reinforcement. First, as discussed above, heroin or other opiate agonists are both self-administered directly into the NAcc and can produce a CPP when passively administered into the NAcc. Although opiates may produce an increase in NAcc DA indirectly by inhibiting NAcc GABAergic cells that subsequently decrease GABA release in both the VTA and the VP, it is possible that this initial inhibition of NAcc GABAergic cells directly contributes to opiate reinforcement. Second, results from DA receptor antagonist administration and chemical lesion studies are not always consistent with the DA hypothesis discussed above. For example, Pettit et al. (1984) demonstrated that 6-OHDA injections into the NAcc selectively attenuated cocaine, but not heroin SA in rats trained to self administer cocaine and heroin on alternate days. These DA lesions also failed to modify the conditioned aversion produced by systemic or intra-NAcc administration of the µ antagonists naloxone or Cys-Tyr-Om-Pen-amide (Shippenberg and
in the NAcc are increased (Wise, 1994; Shippenberg and Bals-Kubik, 1995). Third, DA levels 
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pimozide, sulpiride and spiroperidol, or the mixed antagonists haloperidol and a-flupenthixol, also fail to alter heroin SA
in the VTA or activation of GABA B receptors (Smith and Davis, 1973; Ettenberg et al., 1993). In support of this hypothesis, systemic or local administration of morphine, heroin or DA into the NAcc 
inhibits NAcc neuronal activity (De France et al., 1985; Hakan and Henriksen, 1989; Chang et al., 1997; Lee et al., 1999).

DA receptor activation in the NAcc inhibits GABA release in the VTA and the VP (Swerdlow et al., 1990; Bourdelais and Kalivas, 1992; Cameron and Williams, 1994). This NAcc GABAergic neuronal inhibition can be antagonized by elevating endogenous GABA concentration in the VTA or the VP with GABA transaminase inhibitors or GABA uptake inhibitors, which can dose-dependently reduce heroin-reinforced SA behaviour (Xi and Stein, 2000). In addition, chemical lesions of VP neurons with ibotenic acid block both heroin and cocaine SA behaviour (Hubner and Koob, 1990), whereas intra-pallidal administration of opiates also modulates GABA release within the VP (Napier and Mitrovic, 1999). Taken together, the medium spiny GABAergic projecting neurons in the NAcc may act functionally as a final common target to both DA and non-DA neurotransmitters or neuromodulators. DA may play the critical role in mediating opiate reinforcement, because a functional positive feedback pathway exists to facilitate NA펜 DA release, i.e. opiate/DA-mediated inhibition of NA pensarcelles GABAergic neurons decreases GABA release in the VTA, which subsequently disinhibits VTA DA neurons to facilitate DA release in the NAcc.

Bals-Kubik, 1995). Similarly, systemic or intra-NAcc administration of the D1 antagonist SCH23390, the D2 antagonists pimozide, sulpiride and spiroperidol, or the mixed antagonists haloperidol and a-flupenthixol, also fail to alter heroin SA (Smith and Davis, 1973; Ettenberg et al., 1982; van Ree and Ramsey, 1987; Shippenberg and Herz, 1988; Gerrits et al., 1994; Shippenberg and Bals-Kubik, 1995). Third, DA levels in the NAcc are increased (Wise et al., 1995; Xi et al., 1998), decreased (Kiyatkin et al., 1993; Xi et al., 1998; Xi and Stein, 1999) or unchanged (Hemby et al., 1995) during heroin SA, as assessed by in vivo voltammetry or microdialysis. Taken together, these data suggest that in addition to DA, other non-DA components may also be involved in the maintenance of opiate reinforcement (Hakan and Henriksen, 1989; Xi and Stein, 2000).

Since the majority of neurons in the NAcc are GABAergic, which predominantly project to the VTA and the VP (Walaas and Fonnum, 1981; Groenewegen and Russchen, 1984; Chang and Kitai, 1985; Kalivas et al., 1993), it was hypothesized that opiate receptor-mediated inhibition of the medium spiny GABAergic neurons may directly mediate opiate reinforcement (Xi and Stein, 2000) and locomotor behaviours (Mogensen and Nielson, 1983). In support of this hypothesis, systemic or local administration of morphine, heroin or DA into the NAcc inhibits NAペン neuronal activity (De France et al., 1985; Hakan and Henriksen, 1989; Chang et al., 1997; Lee et al., 1999).

DA receptor activation in the NAcc inhibits GABA release in the VTA and the VP (Swerdlow et al., 1990; Bourdelais and Kalivas, 1992; Cameron and Williams, 1994). This NAcc GABAergic neuronal inhibition can be antagonized by elevating endogenous GABA concentration in the VTA or the VP with GABA transaminase inhibitors or GABA uptake inhibitors, which can dose-dependently reduce heroin-reinforced SA behaviour (Xi and Stein, 2000). In addition, chemical lesions of VP neurons with ibotenic acid block both heroin and cocaine SA behaviour (Hubner and Koob, 1990), whereas intra-pallidal administration of opiates also modulates GABA release within the VP (Napier and Mitrovic, 1999). Taken together, the medium spiny GABAergic projecting neurons in the NAcc may act functionally as a final common target to both DA and non-DA neurotransmitters or neuromodulators. DA may play the critical role in mediating opiate reinforcement, because a functional positive feedback pathway exists to facilitate NAペン DA release, i.e. opiate/DA-mediated inhibition of NAペン GABAergic neurons decreases GABA release in the VTA, which subsequently disinhibits VTA DA neurons to facilitate DA release in the NAcc.

GLUTAMATE MODULATION OF VTA DA NEURONS

In addition to receiving a tonic GABAergic modulation, VTA DA neurons also receive excitatory glutamatergic inputs arising from the mPFC, the pedunculopontine region and the subthalamic nucleus (Kalivas, 1993; White, 1996). One role of this glutamatergic innervation is to mediate a switch from pacemaker-like firing in VTA DA cells to a burst-firing pattern (Gariano and Groves, 1988; Johnson et al., 1992). Pharmacological stimulation of ionotropic or metabotropic glutamate receptors in the VTA elicits an increase in exploratory motor behaviour and promotes DA release in the NAcc and the PFC (Kalivas et al., 1989; Suaud-Chagny et al., 1992; Taber and Fibiger, 1995; Swanson and Kalivas, 2000). Since blockade of presynaptic glutamate release by riluzole prevents the development of morphine-induced CPP (Tzschenkte and Schmidt, 1998), and over-expression of the GluR1 subunit of a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors in the VTA by viral-mediated gene transfer increases morphine’s stimulant and rewarding properties (Carlezon et al., 1997, 2000), glutamate transmission in the VTA also appears to importantly participate in opiate reinforcement.

In support of this hypothesis, systemic administration of the N-methyl-d-aspartate (NMDA) receptor antagonist MK-801...
not only blocks morphine-induced CPP and behavioural sensitization (Jezioski et al., 1994; Tschenke and Schmidt, 1995), but also prevents the acquisition of intravenous morphine SA in mice (Semenova et al., 1999). Direct administration of NMDA and AMPA antagonists into the VTA similarly attenuates heroin reinforcement in rats (Xi and Stein, 2002). Moreover, the opioid NMDA antagonists dextromethorphan and acamprosate have been shown clinically to reduce withdrawal symptoms, drug craving and relapse to heroin, cocaine and ethanol (Herman and O’Brien, 1997; Spanagel and Ziegglansberger, 1997), while acamprosate also prevents, in morphine-dependent rats, the acquisition of naloxone-induced place aversion (Kratzer and Schmidt, 1998). However, David et al. (1998) reported that both NMDA and AMPA receptor antagonists can be self-administered into the VTA in mice, suggesting that glutamatergic inputs onto VTA GABAergic neurons may tonically inhibit VTA DA neurons in some species or under certain conditions.

GLUTAMATE MODULATION OF NAcc GABAergic NEURONS

In contrast to its actions within the VTA, neither NMDA nor non-NMDA antagonists injected into the NAcc alter morphine-induced CPP (Layer et al., 1993) or heroin SA behaviour (Pulvirenti et al., 1992). The exact role of glutamatergic afferents into the NAcc in mediating opiate reinforcement is far from clear. Both NMDA and non-NMDA receptors are located postsynaptically on GABAergic projection neurons, but not on presynaptic DA terminals (Sesack and Pickel, 1992; Doherty and Gratton, 1997). Further, glutamatergic projections from mPFC into the NAcc form excitatory synapses with medium spiny GABAergic neurons (Sesack and Pickel, 1992). Thus, activation of NMDA/AMPA receptors should excite NAcc GABAergic cells, thereby increasing GABA release in the VTA and the VP and inhibiting VTA DA neurons. However, it remains unclear whether opiate administration elevates extracellular glutamate in the VTA and the NAcc. An increase in VTA glutamate would facilitate opiate reinforcement by activating VTA DA cells via NMDA/AMPA receptors, whereas an increase in NAcc glutamate would reduce opiate reinforcement by activating NAcc GABAergic neurons. In support of such mechanisms, several phenecyclidine-like NMDA receptor antagonists have been reported to be self-administered into the NAcc (Carlezon and Wise, 1996), an effect that may be mediated by blocking glutamate effects onto NAcc GABAergic neurons. In the light of this hypothesis, it is perhaps not surprising that blockade of either NMDA or AMPA receptors had no effect on opiate reinforcement. In the light of this hypothesis, it is perhaps not surprising that blockade of either NMDA or AMPA receptors had no effect on opiate reinforcement (Layer et al., 1993; Pulvirenti et al., 1992) since NAcc GABAergic cells may already have been maximally inhibited by both exogenous opiates and endogenous DA. This hypothesis also accounts for the ineffectiveness of baclofen, GVG and nipecot acid (a GABA uptake inhibitor) in the NAcc on heroin SA behaviour, although these drugs all dose-dependently reduce heroin reinforcement when systemic or locally administered into the VTA or the VP (Xi and Stein, 1999, 2000). Clearly, more studies are required to elucidate the role of glutamate in both the VTA and the NAcc in mediating opiate reinforcement.

SEROTONIN (5-HT) INVOLVEMENT IN OPIATE REINFORCEMENT

Several studies suggest an involvement of 5-HT in mediating opiate reinforcement. For example, the acute administration of morphine significantly enhances 5-HT turnover in the rat diencephalon (Grauer et al., 1992), and increases 5-HT release in both the NAcc and the dorsal raphe nucleus, the latter of which projects to the NAcc (Broderick, 1985; Tao and Auerbach, 1994). Direct infusion of morphine into the dorsal raphe nucleus, but not into the NAcc, increases 5-HT release in the NAcc, suggesting an effect mediated by a disinhibitory mechanism (Tao and Auerbach, 1994). However, the exact role of 5-HT and its mechanisms in mediating opiate reinforcement remains unclear. For example, systemic administration of the 5-HT uptake inhibitor dextfenfluramine reduces heroin SA, an effect that can be completely blocked by the 5-HT 1 receptor antagonist metergoline, and partially blocked by the 5-HT 2 receptor antagonist ritanserin, suggesting primary mediation by 5-HT 1 receptors (Higgins et al., 1993, 1994; Wang et al., 1995). Similarly, 5,7-dihydroxytryptamine (5,7-DHT) lesions of NAcc 5-HT innervation significantly increases morphine SA behaviour and drug intake (Smith et al., 1987) but prevents morphine-induced CPP (Spyraki et al., 1988). In contrast, the 5-HT 1B agonist 2-methylserotonin increases DA release in the NAcc and mPFC (Jiang et al., 1990; Chen et al., 1992), while the 5-HT 3 antagonists ondansetron, MDL 7222 or ICS 205930 inhibit morphine-induced DA release, locomotion and CPP (Nomikos and Spyraki, 1988; Carboni et al., 1989; Imperato and Angelucci, 1989; Higgins et al., 1992; Pei et al., 1993). These data suggest that activation of 5-HT 1 receptors may facilitate opiate reinforcement. Since experiments also show that blockade of 5-HT 1 receptors by BRL 46470A neither prevents nor reverses morphine-induced VTA DA neuronal firing (Gifford and Wang, 1994) nor modifies psychostimulant-induced alterations in NAcc DA release (Grant, 1995), it is suggested that both pre- and postsynaptic 5-HT 1 receptors may modulate opiate reinforcement (Van Bockstaele et al., 1996). Taken together, 5-HT 1 and 5-HT 3 receptors appear to modulate opiate reinforcement in a reciprocal fashion, with the final effect of serotonin depending upon the net balance of these actions on NAcc GABAergic cells.

INTRACELLULAR G PROTEIN INVOLVEMENT IN OPIATE REINFORCEMENT

Recent studies have begun to examine the cellular transduction mechanisms that underline effects common to drugs acting at G-protein coupled membrane receptors (including opiates, DA, GABA B, 5-HT and metabotropic glutamate receptors). The role of second messenger systems in the acquisition or maintenance of opiate SA has been investigated by directly inactivating G alpha proteins in the VTA or NAcc with pertussis toxin (Self and Stein, 1993; Self et al., 1994; Nestler and Aghajanian, 1997). The antagonist effects of pertussis toxin were not unique with respect to heroin, as intra-NAcc pertussis toxin also antagonized cocaine SA reinforce (Self et al., 1994), suggesting that intracellular G alpha proteins may act as a common signal pathway-mediating reinforcement of psychostimulants and opiates.
GENERAL CONCLUSIONS AND COMMENTS

Tremendous progress in understanding the neurochemical mechanisms of opiate reinforcement have been made during the past few decades. It now seems clear that a complex neurochemical circuit mediates opiate reinforcement in which VTA DA neurons and NAcc GABAergic neurons play a critical role (Fig. 4). In the NAcc, DA and non-DA substrates, including opioid peptides, GABA, glutamate and 5-HT, mediate opiate reinforcement by differentially modulating NAcc GABAergic projection neurons. Based upon this simplified circuit, the main neurochemical mechanisms responsible for mediating opiate reinforcement are currently understood to include the following. (1) Activation of μ or κ receptors, which are differentially distributed on GABAergic cells in the VTA and NAcc and DA terminals in the NAcc, respectively, produces rewarding and aversive effects by increasing or decreasing, respectively, NAcc DA release. (2) Activation of VTA opiate μ receptors decreases GABA release from VTA GABAergic interneurons or afferents that subsequently disinhibit VTA DA neurons and increase NAcc DA release predominantly via GABA<sub>B</sub> receptors. (3) Inhibition of medium spiny GABAergic neurons in the NAcc by DA and opiates can synergistically facilitate opiate reinforcement. (4) An increase in glutamatergic afferents into the VTA may facilitate opiate reinforcement by activating VTA DA neurons, while an increased glutamatergic activity in the NAcc may decrease opiate action by activating NAcc GABAergic cells. Similarly, the reinforcing effect of ionotropic glutamate antagonists in the NAcc may be mediated by blocking NMDA/AMPA receptors, thereby decreasing excitability of the NAcc medium spiny GABAergic neurons. (5) An increase in NAcc 5-HT by opiates also seems to modulate opiate reinforcement by activation of 5-HT<sub>1</sub> and/or 5-HT<sub>2</sub> receptors.

In view of the above neurochemical framework, a more complete understanding of the mechanisms responsible for mediating the reinforcing properties of opiates will follow from studies designed to clarify the relative role of each non-DA component, the interactions between DA and non-DA components in specific MCL components including the VTA, NAcc and VP and the roles of glutamate and serotonin.

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Fig. 4. Simplified neuroanatomical and neurochemical schematic circuit of opiate reinforcement. Arrows indicate the direction of the projection. DRn, dorsal raphe nucleus; NAcc, nucleus accumbens; mPFC, medial prefrontal cortex; VP, ventral pallidum; VTA, ventral tegmental area.


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