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Alterations in the levels of heterotrimeric G protein subunits induced by psychostimulants, opiates, barbiturates, and ethanol: Implications for drug dependence, tolerance, and withdrawal

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Abstract

Neuronal adaptations have been found to occur in multiple brain regions after chronic intake of abused drugs, and are therefore thought to underlie drug dependence, tolerance, and withdrawal. Pathophysiological changes in drug responsiveness as well as behavioral sequelae of chronic drug exposure are thought to depend largely upon the altered state of heterotrimeric GTP binding protein (G protein)-coupled receptor (GPCR)-G protein interactions. Responsiveness of GPCR-related intracellular signaling systems to drugs of abuse is heterogeneous, depending on the types of intracellular effectors to which the specific G α protein subtypes are coupled and GPCR-G protein coupling efficiency, factors influenced by the class of drug, expression levels of G protein subunits, and drug treatment regimens. To enhance understanding of the molecular mechanisms that underlie the development of pathophysiological states resulting from chronic intake of abused drugs, this review focuses on alterations in the expression levels of G protein subunits induced by various drugs of abuse. Changes in these mechanisms appear to be specific to particular drugs of abuse, and specific conditions of drug treatment.

Keywords

drug of abuse; amphetamine; cocaine; opiate; morphine; barbiturate; ethanol; gene expression; heterotrimeric G protein subunit; intracellular effector

Introduction

Abused drugs, including morphine, ethanol, nicotine, *d*-amphetamine (AMPH), cocaine, Δ^9 -tetrahydrocannabinol, and toluene, all have the common property of releasing dopamine in the nucleus accumbens (NAc) (Di Chiara and Imperato, 1988; Chen et al., 1990; Riegel et al., 2007) and other dopamine terminals area, brain regions involved in the rewarding effects of drugs of abuse. Effects of these drugs on the mesolimbic dopaminergic pathway alter psychological state acutely, and long-term changes in this circuitry are thought to mediate behavioral and psychological changes critical to the development of addiction (Koob and

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Bloom, 1988; Self and Nestler, 1995; Leshner and Koob, 1999; Gardner, 2004). Chronic or repeated activation of dopaminergic systems by chronic drug intake is also thought to influence postsynaptic neuronal adaptations in multiple brain regions, culminating in drug dependence, tolerance, and withdrawal. This is a property shown by all classes of abused drugs, including drugs generally classified as stimulants and depressants of the central nervous system (CNS). Neuronal adaptations include enduring changes in presynaptic and postsynaptic neuronal function, as well as alterations in neuronal structure. Thus, changes in neurotransmission as well as changes in the expression levels of immediate-early genes, neurotransmitter transporters, and heterotrimeric GTP binding protein (G protein)-coupled receptors (GPCRs) have been observed (for reviews, see Robinson and Becker, 1986; Harlan and Garcia, 1998; Wolf et al., 2004; Barr and Markou, 2005; Kitanaka et al., 2008).

Ultimately the pathophysiological sequelae of chronic drug exposure depend largely upon the nature of GPCR-G protein interaction, presynaptic events that produce stimulation of these receptors by neurotransmitters and post-receptor signal transduction. Changes at each of these levels have been observed, but GPCR-G protein interactions appear to mediate pivotal positions in specific aspects of this process. For instance, a reduction in μ opioid receptor-inhibitory G protein coupling efficiency (as measured by GTPase activity) in the locus coeruleus is thought to be associated with the development of morphine tolerance in rats after chronic morphine treatment (Selley et al., 1997). The dysphoric state of cocaine withdrawal is characterized by increased secretion of adrenocorticotrophic hormone and corticosterone as a consequence of the supersensitivity of hypothalamic 5-HT_{2A} receptor function (Levy et al., 1992). Supersensitivity of 5-HT_{2A} receptor function was attributed primarily to the elevation of G protein expression, specifically the $G\alpha_q$ and $G\alpha_{11}$ subunits, to which 5-HT_{2A} receptors are coupled (Carrasco et al., 2003, 2004).

Although chronic changes induced by drugs of abuse may share many common mechanisms, there are a number of differences, particularly with regard to the initial molecular target. In line with this observation and the data discussed above, the responsiveness of GPCR-related intracellular signaling systems to drugs of abuse is heterogeneous, depending on which GPCRs are stimulated (largely influenced by the class of drug involved, but also including downstream targets in the neuronal circuitry), GPCR-G protein coupling efficiency, expression levels of G protein subunits, and drug treatment regimens (dose, route, and number of injections, duration between injections, and the time of experimental assessment relative to drug exposure) (Barr and Markou, 2005; Kitanaka et al., 2008).

G proteins consist of three different subunits, designated $G\alpha$, $G\beta$, and $G\gamma$, which regulate neurotransmitter-induced transmembrane signaling by coordinating the interaction of GPCRs with intracellular effector enzymes (such as protein kinases and phospholipases) and ion channels (Casey and Gilman, 1988; Neer, 1995). Usually, $G\beta$ and $G\gamma$ subunits form a functional monomer. Regarding the alterations of levels of G protein subunits induced by drugs of abuse, most work has focused on $G\alpha$ subunits because these subunits are largest in molecular size, and bind and hydrolyze GTP as a “G protein” to interact with downstream effectors. $G\alpha$, $G\beta$, and $G\gamma$ subunits are encoded by 16, 5, and 12 genes in mammals, respectively, with some alternatively spliced isoforms (Downes and Gautam, 1999). Studies examining the expression levels of subtypes of $G\alpha$ subunits can identify and unravel specific intracellular effectors associated with drug abuse. For instance, $G\alpha$ proteins, $G\alpha_s$, $G\alpha_{olf}$, $G\alpha_o$, $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$, encoded by genes *GNAS1*, *GNAL*, *GNAO*, *GNAI1*, *GNAI2*, and *GNAI3*, respectively, couple neurotransmitter receptors to adenylate cyclase, regulating intracellular levels of cyclic AMP. To enhance understanding of the molecular mechanisms that underlie the development of the pathophysiological state induced by chronic intake of abused drugs, this review focuses on alterations in the expression levels of G protein subunits induced by drugs of abuse. The molecular mechanisms whereby G protein subunits function have been described in previously

published reviews (Casey and Gilman, 1988; Simon et al., 1991; Neer, 1995; Cabrera-Vera et al., 2003; Johnston and Siderovski, 2007).

Drugs of abuse and G α subunits

Of 16 G α subunits, 8 have been investigated in animal models after administration of various drugs of abuse (Table 1).

Psychostimulants

Repeated administration of psychostimulants such as AMPH and cocaine produces a phenomenon called behavioral sensitization in response to intermittent treatment of rodents with the same or related drugs (Robinson and Becker, 1986; Kalivas and Stewart, 1991; Steketee, 2003). The most common method used to evaluate this type of response involves the measurement of the locomotor stimulant effects of a drug, and sensitization refers to increased locomotor responses to drug treatment with repeated treatment. Changes in the G protein-cyclic AMP signal transduction system have been causally implicated in behavioral sensitization (for reviews see Nestler, 1993; Self and Nestler, 1995; Nestler et al., 1996). In particular, up-regulation of the cyclic AMP signal transduction system in the mesolimbic dopamine pathway has been proposed to play a critical role in psychostimulant-induced behavioral sensitization (Self and Nestler, 1995). However, such changes are not by any means uniform. Crawford et al. (2004) observed a down-regulation of striatal and accumbal protein kinase A (PKA) activity in rats behaviorally sensitized to AMPH (2.5 mg/kg i.p. once daily for 6 days followed by a challenge injection, 0.5 mg/kg) and cocaine (15 mg/kg i.p. once daily for 6 days followed by a challenge injection, 7.5 mg/kg). A significant reduction in the expression level of the stimulatory G protein α subunit G α_{olf} (gene symbol GNAL) immunoreactivity may mediate this reduction in accumbal PKA activity after repeated psychostimulant treatment (Table 1). Decreased G α_{olf} expression levels may lower the functional coupling of GPCR-G α_{olf} -adenylate cyclase. Alternatively, increased interaction between G $\beta\gamma$ dimers and other G α □□□□□□ may consequently down-regulate striatal and accumbal PKA activity in a less direct manner through competition for available G $\beta\gamma$ dimers. The overall importance of GNAL expression levels in the regulation of psychostimulant-induced behavioral sensitization remains unclear, although cocaine and AMPH both reduce GNAL expression levels. The critical similarity in the actions of these drugs may be increased extracellular dopamine and consequential stimulation of postsynaptic GPCRs, despite having quite different initial mechanisms. Cocaine elevates extracellular dopamine by blocking the activity of the cocaine-sensitive dopamine transporter (DAT) located in presynaptic plasma membranes, while AMPH acts as a releaser, blocking the vesicular monoamine transporter-2 (VMAT-2) located in vesicular membranes, and the monoamine oxidase (MAO) isozymes (MAO-A and MAO-B) located in mitochondrial outer membranes (Seiden et al., 1993; Sulzer et al., 2005). VMAT-2 is a key factor in transporting cytoplasmic monoamines into synaptic vesicles for storage and release, and MAO isozymes catalyze the oxidative deamination of monoamines. DAT is necessary for AMPH actions, since VMAT-2 and MAO are extracellular proteins and AMPH enters the cell via DAT. Despite these differences in initial mechanism, both cocaine and AMPH elevate extracellular dopamine levels in NAc, and elsewhere, so that repeated psychostimulant-induced dopaminergic neuronal excitation may mediate the reduction in the expression levels of GNAL products in NAc. A significant reduction of the expression levels of GNAL mRNA (but not GNAL protein immunoreactivity) was also observed in the ventromedial caudate putamen (15 mg/kg i.p. three times daily for 1 and 3 days), NAc shell (15 mg/kg i.p. three times daily for 1 and 3 days) and NAc core regions (15 mg/kg i.p. three times daily for 1, 3, and 14 days) of rats after binge cocaine treatment (Perrine et al., 2005). Since the protein level is not changed, the alteration in GNAL expression was suggested not to be a necessary step for the development of cocaine-induced locomotor sensitization (Perrine

et al., 2005). However, this GNAL alteration appears to be specific because the expression of two other major $G\alpha$ subunits ($G\alpha_s$, $G\alpha_o$), also coupled to adenylate cyclase, were unchanged after binge cocaine treatment (Perrine et al., 2005), while the level of another subunit ($G\alpha_{i1}$) was elevated (Table 1) after binge cocaine treatment (Perrine et al., 2005). Because there is no additional experimental data, the molecular mechanisms whereby the alterations in GNAL signal transduction could modulate overall mesolimbic G protein function relevant to behavioral sensitization remain to be determined, but since the changes in the expression of G protein subunits are highly specific it would seem to indicate that this might be highly relevant to the changes in sensitivity to psychostimulants after a sensitizing treatment regimen.

The role of signal transduction systems other than the cyclic AMP signal transduction system have been less well investigated with regard to potential involvement in drug abuse, so the specificity of the sensitization-induced changes discussed above remains to be determined in this regard as well. The $G\alpha$ proteins $G\alpha_q$ and $G\alpha_{11}$, encoded by genes *GNAQ* and *GNA11* respectively, can couple neurotransmitter receptors to phospholipase C (PLC), regulating intracellular levels of calcium and inositol 1,4,5-trisphosphate. Van de Kar and colleagues have investigated alterations in the expression levels of *GNAQ*, *GNA11*, and *GNAZ* gene products after binge cocaine treatments in rats. In the hypothalamic paraventricular nucleus and amygdala (but not in the frontal cortex), $G\alpha_q$ and $G\alpha_{11}$, but not $G\alpha_z$, proteins were significantly increased after binge cocaine treatment (15 mg/kg i.p., twice or three times daily for 2 weeks; or 15 mg/kg i.p., twice daily for 1, 3, 5, and 7 days) (Carrasco et al., 2003, 2004). Elevated $G\alpha_q$ and $G\alpha_{11}$ protein levels should increase PLC activity in the hypothalamus. Such changes are likely to be involved in aspects of responses to chronic exposure to drugs of abuse, aside from drug seeking or acute drug reward, such as withdrawal effects. For instance, it was suggested that the supersensitivity of hypothalamic 5-HT_{2A} receptor function was attributed primarily to elevation of $G\alpha$ protein expression (Carrasco et al., 2003, 2004), although a functional linkage between 5-HT_{2A} receptors and PLC activity in the hypothalamus has not yet been demonstrated. PLC activity has also been found to be affected by drugs of abuse. Repeated administration of *d*-methamphetamine (METH) reduced histamine-induced phosphoinositide hydrolysis in the frontal cortex of mice without changing histamine H₁ receptor mRNA levels (Kitanaka et al., 2003). In this report, expression of $G\alpha$ protein subunits was not determined, but appear likely to be involved; reduced PLC activity may predict decreased levels of free $G\alpha$ subtypes coupled to PLC.

One recent study indicated that dopamine D₂-like receptors can be functionally coupled to $G\alpha_z$ proteins in mice (Leck et al., 2006). Since both AMPH and cocaine can stimulate dopamine D₂-like receptors as indirect dopaminergic agonists, it is possible that $G\alpha_z$ proteins participate in neurochemical and/or behavioral sequelae of psychostimulant treatment. There is some data that addresses this hypothesis, which indicates that the expression levels of *GNAZ* products are not affected by psychostimulants (Carrasco et al., 2003, 2004).

Opiates

Chronic opiate treatments produce both behavioral sensitization and tolerance to different opiate-mediated responses. The levels of μ opioid receptors are generally unchanged after chronic opiate treatments (Sim-Selley et al., 2000; Stafford et al., 2001; Patel et al., 2002), although more selective μ opioid agonists do down-regulate μ opioid receptor levels (Tao et al., 1987; Stafford et al., 2001; Patel et al., 2002). Thus, some other mechanism must account for these differences in opiate responses. Changes in G-protein signaling are likely to be involved, as chronic opiate treatment reduces adenylate cyclase activity in many brain areas (Noble and Cox, 1996; Deng et al., 2001; Sim-Selley et al., 2007), although, as is the case for other compounds, acute down-regulation appears to be greater and more widespread than chronic down-regulation (Sim-Selley et al., 2007). Other evidence suggests that the

development of opiate-induced tolerance and dependence depends largely on the activity of the opioid receptor-G protein (G_s , G_i , and G_o)-adenylate cyclase system (Nestler, 1993; Self and Nestler, 1995; Nestler et al., 1996; Przewlocki, 2004). Basal opioid receptor signaling is mediated primarily by G_i/G_o proteins that inhibit adenylate cyclase, resulting in a decrease in the intracellular levels of cyclic AMP. Chronic treatment with opiates is thought to produce fundamental changes in this signaling pathway, so that after chronic opiate treatment the consequence of opioid receptor signaling is reversed in many brain regions, including the locus coeruleus and NAc (Nestler et al., 1989; Terwilliger et al., 1991), resulting in increased intracellular cyclic AMP levels in response to opiates. This is thought to be one of the neuronal adaptations of fundamental importance for opioid tolerance and dependence (Nestler, 1993; Crain and Shen, 1995; Wang et al., 2005). Such a switch in basic signaling consequences of opioid receptor stimulation may indicate a fundamental switch in G protein subtype expression and coupling to opioid receptors. Expression levels of G protein subunits after chronic administration with morphine (morphine pellet infusion or p.o. route; see Table 1) produce up-regulation of inhibitory $G\alpha$ proteins (e.g. GNAO products) in the locus coeruleus (likely to affect μ opioid receptors) and the claustrum/endopiriform nucleus (likely to affect the κ receptor as discussed below) in the anterior forebrain of rats (Nestler et al., 1989; Parolaro et al., 1993). Nestler and colleagues hypothesized that increased expression of inhibitory $G\alpha$ subunits may reduce the probability of association between $G\beta\gamma$ dimers and stimulatory $G\alpha$ subunits, resulting in increased free stimulatory $G\alpha$ subunits which can activate adenylate cyclase (Nestler et al., 1989). The importance of the noradrenergic locus coeruleus for the development of morphine tolerance and dependence has been well established (Nestler et al., 1989; Selley et al., 1997; McClung et al., 2005). The potential contribution of the claustrum/endopiriform nucleus is still uncertain, although increased expression of opioid receptors was revealed by autoradiography using selective radioactive ligands in the claustrum/endopiriform nucleus (Mansour et al., 1987), suggesting a role of these opioid receptors in physiological functions of the claustrum/endopiriform nucleus.

Changes in other brain regions may be more relevant to other opiate mediated effects. It has recently been shown that changes in opioid GPCR signaling may be critical for several other behavioral consequences of chronic opiate exposure (Wang et al., 2005; Olmstead and Burns, 2005). These authors found that chronic morphine treatment reduced MOR- $G_{i/o}$ coupling, while concomitantly increasing G_s coupling, as well as a direct interaction of $G\beta\gamma$ with adenylate cyclase in the striatum, periaqueductal gray and spinal cord (Wang et al., 2005). This change in function occurred in the absence of any changes in MOR or $G\alpha$ protein levels. Nonetheless, this effect on MOR-G protein coupling could be attenuated by administration of ultra-low dose naloxone, concomitant with a reduction in measures of both opiate tolerance and dependence (Wang et al., 2005). Furthermore, antagonist treatment also prevented the development of both morphine conditioned place preference and withdrawal-induced conditioned place aversion (Olmstead and Burns, 2005).

In the prefrontal cortex, withdrawal from chronic morphine is associated with changes in noradrenergic and dopaminergic neuronal activity that are opposite to those observed after acute administration (Devoto et al., 2002). Acute administration with morphine (30 mg/kg i.p.) increases expression of GNAO gene, but decreases expression of the GNAI1 and GNAI2 genes, while a chronic escalating treatment regimen (10-50 mg/kg i.p., twice daily for 2 weeks) results in increased expression levels of GNAI1 and GNAI2 genes with no change in GNAO levels (Kaewsuk et al., 2001). Although prefrontal adenylate cyclase activity was not assessed in this study, this phenomenon was suggested to be a neuronal adaptation in response to the duration of morphine treatment involved in some aspect of morphine tolerance and dependence, similar to the sort of changes previously observed in the locus coeruleus (Nestler et al., 1989).

Acute administration of morphine releases dopamine in the NAc (Di Chiara and Imperato, 1988), a brain region with a crucial role in drug reward and reinstatement of drug seeking behavior. By contrast, chronic morphine treatment reduces dopaminergic neurotransmission in the NAc as well as in the ventral tegmental area (Acquas et al., 1991; Diana et al., 1995). Therefore, the nature of long-term morphine treatment-induced alterations in G protein subunit gene expression in the NAc is important to clarify in order to understand the behavioral changes that occur in response to chronic morphine treatment. Regarding this point, chronic treatment of rats with heroin (diacetylmorphine; 50 µg/kg/infusion i.v. for 3 weeks) increased expression of GNAI1 mRNA in the NAc shell (Jacobs et al., 2002). Heroin and morphine actions are mediated primarily by μ opioid receptors as indicated by the elimination of analgesic, locomotor stimulant, and rewarding effects in μ opioid receptor knockout mice (Tian et al., 1997; Kitanaka et al., 1998; Sora et al., 2001; Contarino et al., 2002; Hall et al., 2003). Although the NAc is certainly involved in some of these opiate mediated behavioral responses, there is limited information available concerning the association of chronic opiate-induced alterations in gene expression, including GNAI1, with dopaminergic hypoactivity observed in the NAc shell after morphine withdrawal (Spiga et al., 2005).

Butorphanol, a synthetic opioid, produces dependence by a putatively more complex mechanism of drug-receptor interactions than morphine, acting as a partial agonist-antagonist analgesic (Commiskey et al., 2005). One report has suggested that subchronic butorphanol treatment (26 nmol/µl/h i.c.v. for 3-day infusion and 7-h withdrawal after cessation of infusion) decreases expression of both GNAS1 and GNAI2 mRNA in specific brain regions of rats (Kim et al., 2003; Table 1). Since the expression levels of adenylate cyclase did not differ, as assessed by [3 H]-forskolin binding (Kim et al., 2003), neuronal adaptations associated with butorphanol tolerance and/or withdrawal might be attributed to altered ratios of mRNA expression of stimulatory versus inhibitory G α subunits that couple to adenylate cyclase in specific brain regions. Further studies are needed to address whether cortical neuronal adaptations after butorphanol treatment are directly influenced by noradrenergic projections from the locus coeruleus where chronic morphine (and perhaps butorphanol) treatment up-regulates adenylate cyclase activity via μ receptors (Nestler et al., 1989) or to some other mechanism.

In addition to alterations in G protein mechanisms involved in μ opioid receptor signaling, it is also important to investigate the roles of other opioid receptor subtypes in alterations of G protein gene expression. Parolaro and colleagues have addressed this issue in rats following sustained treatment with selective opioid receptor ligands. According to their results, the mixed μ/δ receptor ligand DADLE, but not the selective μ or δ ligands DAMGO and DPDPE, increased expression of the GNAS1 gene in the hypothalamus (Rubino et al., 1994). This observation was supported by another study using morphine which has high μ and δ affinity as well (Parolaro et al., 1993; see Table 1), although, as noted above, most behavioral effects of morphine are μ opioid receptor dependent. In addition, up-regulation of GNAS1 gene expression in the hypothalamus following chronic opioid treatment was reduced after naltrexone-induced withdrawal (Rubino et al., 1995), suggesting a close relationship between the expression levels of G α proteins and opioid-induced dependence, tolerance, and withdrawal. The κ opioid receptor is also likely to affect G protein expression. For instance, in the claustrum and endopiriform nucleus, the κ selective ligand U-50,488H increased expression of the GNAO gene (Rubino et al., 1994), which was also observed after morphine treatment (Parolaro et al., 1993). It is likely that activation of specific opioid receptor subtypes contribute to changes in specific mRNAs for G α proteins in specific brain regions.

Thus, overall, it appears that stimulation of opioid receptor subtypes by either selective or non-selective agonists can alter specific mRNAs for G α proteins in a brain region dependent manner. In some instances functional alterations are observed in the absence of changes at the protein level, in a manner that is not yet understood. In either case, regulation of GPCR-G

protein interactions is closely tied to the behavioral states associated with opiate dependence, tolerance, and withdrawal.

Barbiturates

In addition to other affects occasioned by AMPH withdrawal after chronic AMPH exposure, alterations of γ -aminobutyric acid (GABA) neurotransmission are also observed (for review see Kitanaka et al., 2008). Thus, the GABA receptor-G protein-effector system may play a role in the sequelae of withdrawal from barbiturates. Receptors for GABA, especially the GABA_A receptor subtype, play an important role in the sedative/hypnotic action of barbiturates, and changes in this receptor and its signaling pathways are thought to play a role in barbiturate tolerance, dependence, and withdrawal (Saunders and Ho, 1990). Regarding the effects of barbiturates on levels of G protein subunit expression, Kim and Oh (2002) have shown that chronic treatment of rats with pentobarbital (300 μ g/10 μ l/h, i.c.v. infusion through osmotic minipumps for 1 week) increases expression of GNAS1, GNAI2, and GNAO genes in specific brain regions. There is no experimental evidence that pentobarbital-induced alterations of G α protein mRNAs are directly mediated by specific GABA receptor subtypes, although barbiturates are known to act directly on the GABA_A receptor, so that this is likely to be the initial site of barbiturate action. Nonetheless, changes in the expression of G α proteins are likely to involve other receptors as well. The GABA_A receptor contains an intrinsic chloride channel within its subunit complex, with binding sites for several modulatory agents, including barbiturates and benzodiazepines, and thus the structure is unlike GPCRs. The GABA_A receptor consists of 5 subunits derived from 6 subunit groups, and forms a ligand-gated ion channel within the center of the protein complex (Hevers and Lüddens, 1998). The GABA_B receptor is a GPCR but does not bind barbiturates (Bettler and Tiao, 2006). Thus, although barbiturates may induce alterations in G protein subunit expression, the particular receptors with which these may be acting is uncertain.

Drugs of abuse and G β subunits

Of the 5 G β subunits, 2 have been investigated in animals after administration of various drugs of abuse (Table 1). In particular, a pivotal role of the G β ₁ subunit in the development of psychostimulant abuse has been proposed (Wang et al., 1997), as described below.

Psychostimulants

Single injections of AMPH (7.5 mg/kg i.p.) or cocaine (50 mg/kg i.p.) up-regulate the expression of the GNB1 gene in the striatum and the NAc in rats, as determined by subtracted differential display PCR (Wang et al., 1997). This up-regulation of mRNA expression is accompanied by increased expression of G β ₁ immunoreactivity in the NAc. Furthermore, treatment of rats with antisense oligonucleotides that effectively suppress GNB1 gene expression significantly inhibits psychostimulant-induced behavioral sensitization, suggesting that up-regulation of functional G β ₁ plays a pivotal role in the expression of psychostimulant-induced behavioral sensitization (Wang et al., 1997). This series of observations clearly demonstrates a direct association between changes in G β ₁ function, both at the level of gene expression and protein expression, and psychostimulant-induced behavioral sensitization. Consistent with these findings, up-regulation of the GNB1 gene has also been observed in mice treated with a single dose of METH (1 mg/kg i.p.), consistent with a role of GNB1 expression in behavioral sensitization induced by a range of psychostimulant compounds in both rats and mice (Kitanaka et al., 2003). The exact nature of this role remains to be determined however, as in most of the cases cited above the changes in GNB1 expression were the result of acute administration of psychostimulant compounds. It should be noted that alteration in the expression of the GNB1 gene was solely observed after acute, but not chronic, psychostimulant treatment (Kitanaka et al., 2003), indicating that changes in the levels of these genes were part

of a sequence of alterations and not the ultimate causative factor in behavioral sensitization. Nonetheless, since antisense oligonucleotides for GNB1 prevented the development of behavioral sensitization, this was obviously a necessary step in this sequence of events. Obviously, further studies are needed to address whether the significant association between GNB1 and psychostimulant-induced behavioral sequelae comes from a monogenic cellular event, since G β and G γ subunits are closely associated with each other, acting as a functional monomer, and changes in the G α subunits have also been observed.

Opiates

GNB gene expression and G β immunoreactivity are increased in the locus coeruleus, frontal cortex, and prefrontal cortex after both acute and chronic opiate treatments (Nestler et al., 1989; Kaewsuk et al., 2001). In the NAc, a significant increase in expression of the GNB1 gene was observed after chronic morphine administration using a subcutaneously implanted morphine pellet for 5 days in mice (Grice et al., 2007). Morphine-treated mice demonstrated dorsiflexion of the tail (Straub tail), indicating that morphine acted centrally during treatment. As mentioned previously, the levels of μ opioid receptors are generally unchanged after chronic opiate treatments (Sim-Selley et al., 2000; Stafford et al., 2001; Patel et al., 2002), although adenylate cyclase activity is altered (Noble and Cox, 1996; Deng et al., 2001; Sim-Selley et al., 2007). It would appear then that changes in the expression of GNB1 gene products may play a role in these alterations in adenylate cyclase activity, in a manner similar to that observed in AMPH- and cocaine-treated subjects (Wang et al., 1997), although the precise role of this gene remains to be determined. One obvious difference in the alteration of GNB1 expression between psychostimulant and morphine-treated rodents is the duration of up-regulation, which is longer in morphine-treated subjects than in psychostimulant-treated subjects, but this may be a procedural issue, relating to dose and treatment regimen.

Changes in GNB1 expression in different brain regions may involve signaling in different neuronal populations, mediated by different neurotransmitter receptors, and involved in different aspects of tolerance and sensitization. The changes noted above in the NAc may be involved in behavioral sensitization, as indicated by the psychostimulant studies in which GNB1 was essential for the development of locomotor sensitization (Wang et al., 1997). By contrast, a significant decrease in GNB1 expression levels was observed in the locus coeruleus of mice after withdrawal from chronic morphine treatment (induced by a single injection of naltrexone) (McClung et al., 2005). Reduced GNB1 levels predict a possible increase in free G α subunits which can regulate adenylate cyclase in the locus coeruleus, a brain region implicated in the behavioral manifestations of morphine tolerance and withdrawal.

There have been no reports on the association of opioid receptor ligands other than morphine with GNB gene expression levels. Investigation of a wider range of opioid agonists will help clarify to the nature of some of these changes.

Ethanol

Ethanol has sedative-hypnotic properties similar to barbiturates, and in some respects acts as a CNS depressant, while of course acting through other mechanisms to stimulate behavior and reward mechanisms like all addictive compounds (Wise, 1987). Nonetheless, chronic ethanol intake culminates in dependence and tolerance in a manner similar to other CNS depressants. The molecular targets for ethanol are multiple but include the GABA_A receptor complex (Sundstorm-Poromaa et al., 2002) and G-protein-coupled inwardly rectifying potassium channels (GIRKs) (Lewohl et al., 1999; Kobayashi et al., 1999). The G proteins coupled to GIRKs include pertussis toxin-sensitive G_i and G_o. The activation of GIRKs is triggered by the direct association of released G $\beta\gamma$ dimers with GIRKs (Reuveny et al., 1994). Chronic treatment of rats with ethanol (p.o. route of 12% vol/vol ethanol solution for 15 months)

decreased expression of the GNB1 gene in the hippocampus (Saito et al., 2002), so that chronic ethanol intake may reduce the availability of G $\beta\gamma$ dimers, resulting in reduced GIRK activation. This may result in increased hippocampal neuronal excitability, which may therefore play a role in ethanol dependence.

Drugs of abuse and G γ subunits

Since G β and G γ subunits form a dimer, acting as a functional monomer under normal conditions, and ablation of G β subunits by means of an RNA interference technique destabilized other G protein subunits (Hwang et al., 2005), concomitant alteration in the expression levels of the two subunits may be expected to occur after chronic treatment with abused drugs. However, there is no experimental evidence that addresses this question in the literature. The critical role of the G γ subunit as a G β partner is recognized in the relapse of heroin-seeking behavior in rats (Yao et al., 2005). The influence of G γ subunits *per se* on the development of drug tolerance, dependence, and withdrawal has not been examined and needs to be explored further given the close association of the two subunits.

Molecular mechanisms by which the expression levels of G protein subunits are altered after drug treatment

As discussed above, increasing evidence suggests that (1) drugs of abuse alter the expression levels of G α , G β , and possibly G γ protein subunits at the level of gene expression and that (2) these alterations may affect intracellular signaling pathways in specific brain regions with crucial roles in different aspects of drug dependence, tolerance, and withdrawal. There is also evidence that changes in GPCR-G protein coupling can occur in the absence of overall changes in receptor or G protein expression. It might be interesting to speculate that these changes might reflect differential expression of mRNA variants in different cells or targeted to different parts of the cell, but such speculation is beyond the scope of our present stage of knowledge.

How do drugs of abuse alter the expression levels of G protein genes? These drugs have long been known to alter the expression of immediate-early genes, including the fos family of genes, in specific brain regions (Hope et al., 1994; Doucet et al., 1996; Harlan and Garcia, 1998), resulting in altered expression of numerous genes which are sensitive to these transcription factors. With regard to the potential impact of these mechanisms on GNB1 expression, comparative DNA sequencing analysis revealed that several potential elements involved in putative transcriptional control (AP2, Sp1, CRE, and NF- κ B) were located in the promoter region of the human and mouse GNB1 gene locus (Kitanaka et al., 2002). These elements are thought to be essential for regulation of GNB1 gene expression, including that induced by drugs of abuse in that they are responsible for modulation of gene expression induced by cyclic AMP and/or calcium ion signaling pathways. Acute administration of psychostimulants and opiates affect these pathways, and chronic treatments are known to modulate the cyclic AMP signal transduction system in a variety of ways. Stimulation of these pathways is then likely to mediate increased expression of the GNB1 gene in rodents (Wang et al., 1997; Kaewsuk et al., 2001; Kitanaka et al., 2003; Grice et al., 2007) via these transcription factors. Of the transcription factors which bind to the potential elements of the promoter/enhancer region, cyclic AMP response element binding protein (CREB) has been investigated extensively with regard to its roles in drug addiction (for review see Carlezon et al., 2005). CREB activity influences a variety of neuronal responses in specific brain regions (for example, the NAc and the ventral tegmental area) by binding to the CRE and playing a pivotal role in the development of drug dependence, tolerance, and withdrawal (Cole et al., 1995; Shaw-Lutchman et al., 2002; Olson et al., 2005). In order to understand the significance of alterations of the expression levels of G protein subunits in response to drug treatment and the involvement of G protein subunits in drug abuse in humans, it will be necessary to compare the DNA sequences of

promoter and/or enhancer regions of G protein subunit genes from different species carefully and extensively.

Significance of alterations of G protein expression by drug treatment

Evidence has accumulated to support the hypothesis that neuronal adaptations leading to drug dependence, tolerance, and withdrawal involve alterations of the expression levels of G protein subunits that may affect cellular signaling. Although drugs of abuse alter expression levels of G protein subunits under certain conditions (Table 1), the anatomical distributions of gene responses differ substantially for each drug, suggesting that the expression pattern may be an important factor determining the pathophysiological state after drug treatment. Although there are certainly many mechanisms that drugs of abuse share in common, which are likely to be involved in the primary reinforcing properties of addictive drugs, many of these drugs produce effects that are unique, particularly in terms of tolerance and withdrawal. This pattern of regional changes likely reflects these differences.

This review has attempted to survey the functional roles of G protein subunits in the development of drug abuse, as indicated by alterations in gene expression levels. It is clear that the alterations of G protein gene expression are not same in response to all drugs of abuse, suggesting that multiple cellular signaling pathways are responsible for drug-induced behavioral and neurochemical sequelae, and this may be particularly true for those ways in which the chronic effects of these drugs differ from each other. Although information on G protein gene expression for drugs of abuse is still incomplete, alterations in these mechanisms could account for numerous drug-induced outcomes, an assertion supported by the findings that G protein subunits are candidate genes for human psychiatric disorders (Hudson et al., 1993; Friedman and Wang, 1996; Lee et al., 2004) which share substantial comorbidity with addiction.

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Table 1
Change in the expression levels of G protein subunits after administration of drugs of abuse in rodents

Species	Treatment regimen	Brain region(s)	Subunit type ^d	Molecule	Effect	Reference
<i>Psychostimulant</i>						
AMPH/METH						
Rat	2.5 mg/kg i.p., q.d., 6 dy $\pm$ 0.5, 1 inj	NAc	GVAL	Protein	Decrease	Crawford et al. (2004)
Rat	2.5 mg/kg i.p., q.d., 6 dy $\pm$ 0.5, 1 inj	NAc	GNAS1	Protein	NC	Crawford et al. (2004)
Rat	7.5 mg/kg i.p., 1 inj	Str + NAc, Hipp	GNB1	mRNA	Increase	Wang et al. (1997)
Rat	7.5 mg/kg i.p., 1 inj	Str + NAc	GNB1	Protein	Increase	Wang et al. (1997)
Rat	7.5 mg/kg i.p., 1 inj	PFC, Tha, Hyp	GNB1	mRNA	NC	Wang et al. (1997)
Mouse	1 mg/kg i.p., 1 inj	Str	GNB1	mRNA	Increase	Kitanaka et al. (2003)
Mouse	1 mg/kg i.p., 1 inj	Cx	GNB1	mRNA	NC	Kitanaka et al. (2003)
Mouse	1 mg/kg i.p., b.i.d., 5 dy	7 regions ^b	GNB1	mRNA	NC	Kitanaka et al. (2003)
Cocaine						
Rat	15 mg/kg i.p., b.i.d./i.d., 2 wk	PVN, AMY	GNA11, GNAQ	Protein	Increase	Carrasco et al. (2003)
Rat	15 mg/kg i.p., b.i.d./i.d., 2 wk	FCx	GNA11, GNAQ	Protein	NC	Carrasco et al. (2003)
Rat	15 mg/kg i.p., b.i.d./i.d., 2 wk	PVN, AMY, FCx	GNAZ	Protein	NC	Carrasco et al. (2003)
Rat	15 mg/kg i.p., b.i.d., 1, 3, 5, 7 dy	PVN, AMY	GNA11, GNAQ	Protein	Increase	Carrasco et al. (2004)
Rat	15 mg/kg i.p., b.i.d., 1, 3, 5, 7 dy	FCx	GNA11, GNAQ	Protein	NC	Carrasco et al. (2004)
Rat	15 mg/kg i.p., b.i.d., 1, 3, 5, 7 dy	PVN, AMY, FCx	GNAZ	Protein	NC	Carrasco et al. (2004)
Rat	15 mg/kg i.p., t.i.d., 1, 3, 14 dy	NAc, CPu, FCx	GNA1, GNAS1, GNAO	Protein	NC	Perrine et al. (2005)
Rat	15 mg/kg i.p., t.i.d., 1, 3, 14 dy	NAc, CPu	GNA11	Protein	NC	Perrine et al. (2005)
Rat	15 mg/kg i.p., t.i.d., 1, 3, 14 dy	CPu, NAc	GNAO	mRNA	NC	Perrine et al. (2005)
Rat	15 mg/kg i.p., t.i.d., 1, 3, 14 dy	CPu, NAc, cing Cx	GNAS1	mRNA	NC	Perrine et al. (2005)
Rat	15 mg/kg i.p., t.i.d., 1, 3 dy	vm CPu, NAc shell	GNA1	mRNA	Decrease	Perrine et al. (2005)
Rat	15 mg/kg i.p., t.i.d., 1, 3, 14 dy	NAc core	GNA1	mRNA	Decrease	Perrine et al. (2005)
Rat	15 mg/kg i.p., t.i.d., 3 dy	CPu, NAc, cing Cx	GNA11	mRNA	Increase	Perrine et al. (2005)
Rat	15 mg/kg i.p., q.d., 6 dy $\pm$ 7.5, 1 inj	NAc	GNA1	Protein	Decrease	Crawford et al. (2004)

Abbreviations: AMPH, *d*-amphetamine; AMY, amygdala; b.i.d., twice daily; Ch, cerebellum; CeA, central amygdaloid nucleus; cing Cx, cingulate cortex; CPu, caudate putamen; Cx, cerebral cortex; dy, day(s); DADLE, [D-Ala², D-Leu⁷]-enkephalin; DMGGO, [D-Ala², N-Methyl-Phe⁵]-enkephalin; dy, day(s); DPDPE, [D-Pen², D-Pen⁵]-enkephalin; FCx, frontal cortex; Hipp, hippocampus; Hyp, Hypothalamus; IC, inferior colliculus; i.c.v., intracerebroventricular; inj, injection; i.p., intraperitoneal; L.C., locus coeruleus; METH, *d*-methamphetamine; mo, month(s); n., nucleus; NAc, nucleus accumbens; NAcSh, nucleus accumbens shell; NC, no change; PFC, prefrontal cortex; p.o., orally; PVN, paraventricular nucleus; q.d., once daily; s.c. subcutaneous; SON, supraoptic nucleus; Str, striatum; Tha, thalamus; t.i.d., three times daily; U-50,488H, trans-(1S,2S)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]-benzenacetamide; vm CPu, ventromedial caudate putamen; wk, week(s).

Species	Treatment regimen	Brain region(s)	Subunit type ^d	Molecule	Effect	Reference
Rat	15 mg/kg i.p., q.d., 6 dy $\pm$ 7.5, 1 inj	NAc	GNAS1	Protein	NC	Crawford et al. (2004)
Rat	50 mg/kg i.p., 1 inj	Str + NAc	GNB1	mRNA	Increase	Wang et al. (1997)
Rat	50 mg/kg i.p., 1 inj	PFC, Tha, Hyp, Hipp	GNB1	mRNA	NC	Wang et al. (1997)
<i>Opioid</i>						
Morphine (μ δ)						
Rat	75 mg/pellet s.c., q.d., 5 dy	LC	GNAO	Protein	Increase	Nestler et al. (1989)
Rat	75 mg/pellet s.c., q.d., 5 dy	LC	GNB1	Protein	NC	Nestler et al. (1989)
Rat	75 mg/pellet s.c., q.d., 5 dy	FCx	GNAO, GNB1	Protein	NC	Nestler et al. (1989)
Rat	0.1-0.6 mg/ml p.o., 30 dy	claustrum/endopiriform n.	GNAO	mRNA	Increase	Parolaro et al. (1993)
Rat	0.1-0.6 mg/ml p.o., 30 dy	PVN	GNAS1	mRNA	Increase	Parolaro et al. (1993)
Rat	0.1-0.6 mg/ml p.o., 30 dy	SON, CeA	GNAS1	mRNA	NC	Parolaro et al. (1993)
Rat	0.1-0.6 mg/ml p.o., 30 dy	Hyp	GNAS1	Protein	Increase	Parolaro et al. (1993)
Rat	30 mg/kg i.p., 1 inj	PFC	GNAO	mRNA	Increase	Kaewsuk et al. (2001)
Rat	30 mg/kg i.p., 1 inj	PFC	GNAI1, GNAI2	mRNA	Decrease	Kaewsuk et al. (2001)
Rat	30 mg/kg i.p., 1 inj	PFC	GNB1, GNB2	mRNA	NC	Kaewsuk et al. (2001)
Rat	10-50 mg/kg i.p., b.i.d., 2 wk	PFC	GNAI1, GNAI2, GNB1	mRNA	Increase	Kaewsuk et al. (2001)
Rat	10-50 mg/kg i.p., b.i.d., 2 wk	PFC	GNAO, GNB2	mRNA	NC	Kaewsuk et al. (2001)
Mouse	25 mg pellet x 2 s.c. 6 dy ^c	LC	GNB1	mRNA	Decrease	McClung et al. (2005)
Mouse	25 mg total/pellet s.c., 5 dy	NAc	GNB1	mRNA	Increase	Grice et al. (2007)
Heroin						
Rat	50 μ g/kg/infusion i.v., 3 wk	NAcSh	GNAI1	mRNA	Increase	Jacobs et al. (2002)
Butorphanol (μ δ/κ)						
Rat	26 nmol/ μ l/h i.c.v., 3 dy	FCx, cing Cx, Septum, Tha, central gray, IC, Cb	GNAS1	mRNA	Decrease	Kim et al. (2003)
Rat	26 nmol/ μ l/h i.c.v., 3 dy	FCx, cing Cx	GNAI2	mRNA	Decrease	Kim et al. (2003)
Rat	26 nmol/ μ l/h i.c.v., 3 dy	FCx, cing Cx, Hipp, Cb	GNAO	mRNA	NC	Kim et al. (2003)
DAMGO (μ)						

Abbreviations: AMPH, *d*-amphetamine; AMY, amygdala; b.i.d., twice daily; Cb, cerebellum; CeA, central amygdaloid nucleus; cing Cx, cingulate cortex; CPu, caudate putamen; Cx, cerebral cortex; dy, day(s); DADLE, [D-Ala², D-Leu⁷]-enkephalin; DAMGO, [D-Ala², N-Methyl-Phe⁵, Gly⁶-ol]-enkephalin; dy, day(s); DPDPE, [D-Pen², D-Pen⁵]-enkephalin; FCx, frontal cortex; Hipp, hippocampus; Hyp, Hypothalamus; IC, inferior colliculus; i.c.v., intracerebroventricular; inj, injection; i.p., intraperitoneal; LC, locus coeruleus; ME/TH, *d*-methamphetamine; mo, month(s); n., nucleus; NAc, nucleus accumbens; NAcSh, nucleus accumbens shell; NC, no change; PFC, prefrontal cortex; p.o., orally; PVN, paraventricular nucleus; q.d., once daily; s.c. subcutaneous; SON, supraoptic nucleus; Str, striatum; Tha, thalamus; t.i.d., three times daily; U-50,488H, trans-(1S,2S)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]-benzenacetamide; vm CPu, ventromedial caudate putamen; wk, week(s).

Species	Treatment regimen	Brain region(s)	Subunit type ^d	Molecule	Effect	Reference
Rat	0.5 µg/µl i.c.v., 1 µl/h, 1 wk	14 regions ^d	GNAS1, GNAO	mRNA	NC	Rubino et al. (1994)
DPDPE (δ)						
Rat	3.4 µg/µl i.c.v., 1 µl/h, 1 wk	14 regions ^d	GNAS1, GNAO	mRNA	NC	Rubino et al. (1994)
U-50,488H (κ)						
Rat	4 µg/µl i.c.v., 1 µl/h, 1 wk	14 regions ^d	GNAS1	mRNA	NC	Rubino et al. (1994)
Rat	4 µg/µl i.c.v., 1 µl/h, 1 wk	claustrum/endopiriform n.	GNAO	mRNA	Increase	Rubino et al. (1994)
DADLE (µδ)						
Rat	11.4 µg/µl i.c.v., 1 µl/h, 1 wk	14 regions ^d	GNAO	mRNA	NC	Rubino et al. (1994)
Rat	11.4 µg/µl i.c.v., 1 µl/h, 1 wk	Hyp	GNAS1	mRNA	Increase	Rubino et al. (1994)
Barbiturate						
Pentobarbital						
Rat	300 µg/10 µl/h i.c.v., 1 wk	Septum, central gray, IC	GNAS1	mRNA	Increase	Kim and Oh (2002)
Rat	300 µg/10 µl/h i.c.v., 1 wk	FCx, cing Cx, Hipp, central gray, Cb	GNAI2	mRNA	Increase	Kim and Oh (2002)
Rat	300 µg/10 µl/h i.c.v., 1 wk	FCx, cing Cx	GNAO	mRNA	Increase	Kim and Oh (2002)
Ethanol						
Rat	12% ethanol p.o., 15 mo	Hipp	GNB1	mRNA	Decrease	Saito et al. (2002)

Abbreviations: AMPH, *d*-amphetamine; AMY, amygdala; b.i.d., twice daily; Cb, cerebellum; CeA, central amygdaloid nucleus; cing Cx, cingulate cortex; CPu, caudate putamen; Cx, cerebral cortex; dy, day(s); DADLE, [D-Ala², D-Leu⁵]-enkephalin; DAMGO, [D-Ala², N-Methyl-Phe⁴, Gly⁵-ol]-enkephalin; dy, day(s); DPDPE, [D-Pen², D-Pen⁵]-enkephalin; FCx, frontal cortex; Hipp, hippocampus; Hyp, hypothalamus; IC, inferior colliculus; i.c.v., intracerebroventricular; inj, injection; i.p., intraperitoneal; LC, locus coeruleus; METH, *d*-methamphetamine; mo, month(s); n., nucleus; NAc, nucleus accumbens; NAcSh, nucleus accumbens shell; NC, no change; PFC, prefrontal cortex; p.o., orally; PVN, paraventricular nucleus; q.d., once daily; s.c. subcutaneous; SON, supraoptic nucleus; Str, striatum; Tha, thalamus; t.i.d., three times daily; U-50,488H, trans-(1S,2S)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]-benzenacetamide; vm CPu, ventromedial caudate putamen; wk, week(s).

^aGene symbol: GNAL, Gα_{OLF}; GNAS1, Gα_s; GNAO, Gα_o; GNAI1, Gα_{i1}; GNAI2, Gα_{i2}; GNAQ, Gα_q; GNAZ, Gα_z; GNB1, Gβ₁; GNB2, Gβ₂. For a review of gene nomenclature, see Downes and Gautam (1999).

^bRegions include olfactory bulb, thalamus plus hypothalamus, cerebellum, hippocampus, brain stem, striatum, and cerebral cortex.

^cMice were treated with naltrexone (50 mg/kg s.c.) to induce a state of morphine withdrawal.

^dRegions include cerebral cortex, olfactory cortex, claustrum and endopiriform nucleus, septum, striatum, hippocampus, habenula, thalamus, hypothalamus, amygdala, central gray, mesencephalic nucleus, red nucleus, and substantia nigra plus ventral tegmental area.