ABSTRACT—In mammalian cells, protein de novo synthesis is mainly regulated at the stage of gene transcription by RNA polymerase II in the nucleus. Transcription factors are proteins that bind to the specific nucleotide sequences at promoter or enhancer regions on target genes to control the transcription of mRNA from genomic DNA. In this article, we have outlined the signal responsiveness of different transcription factors to particular drugs in the brain. Nuclear transcription factors rapidly respond to a variety of extracellular signals carried by neurotransmitters, hormones and autacoids as a third messenger in frequent situations. Translated proteins are responsible for a number of physiological and pathological events for a long period in the brain. We have also discussed possible involvement of transcription factors in molecular mechanisms underlying development of tolerance and dependence to drugs following acute and chronic administration.

Keywords: Transcription factor, Neurotransmitter, Hormone, Autacoid, Drug dependence

1. Introduction

There are at least two major signal cascade pathways for cells to change their functions following reception of stimuli from the outside. One is rapid signal transduction accompanied by changes in cellular movement, secretion, metabolism, etc., while the other is slow signal transduction that leads to cellular development, differentiation, cell death, etc. (1). The former is often responsible for quick and transient appearance of an influence on cellular functions through posttranslational modifications of a number of different functional proteins, whereas the latter is involved in delayed and long-lasting appearance of functional alterations through changes in gene expression and protein de novo synthesis in particular situations. This review will mainly deal with the latter signaling pathway with a focus on consolidation and/or amplification of altered responsiveness to particular drugs in the brain.

Extracellular signals carried by hydrophilic first messengers are usually transformed into the second messengers, such as cyclic nucleotides, lipophilic molecules and ions, at the level of membrane receptors, followed by distinct signaling cascades in the cytoplasm and subsequent alterations of a variety of cellular functions. These intracellular signals could be also propagated to the third messengers, which are often referred to as IEGs, in the nucleus for consolidation of functional alterations for a relatively long period (2). By contrast, most of all hydrophobic extracellular first messengers are thought to easily penetrate cytoplasmic membranes to the cytoplasm and gain access to their receptors located on genomic DNA in the nucleus. Finally, both hydrophilic and hydrophobic first messengers could lead to long-term alterations of a number of cellular...
functions through modulation of de novo synthesis of particular target proteins following specific interactions with their membrane and nuclear receptors, respectively (Fig. 1). There processes could be at least in part involved in mechanisms underlying amplification and long-term consolidation of transient extracellular signals such as neuronal plasticity and drug dependence in the brain. In this article, we will outline the expression of particular TFs in response to different extracellular first messengers including neurotransmitters, hormones and autacoids in the brain.

2. TFs

TFs are nuclear proteins that regulate the activity of RNA polymerase II responsible for the transcription of genomic DNA to the corresponding mRNA through recognition of particular core nucleotide sequences at the promoter or enhancer regions on inducible target genes. They have in principle abilities to bind to the core nucleotides for specific recognition and to interact with RNA polymerase II for modulation of the activity to transcribe DNA to mRNA in the nucleus. TFs would bind to specific base sequences in the noncoding region of DNA via their DNA binding domain through a hydrogen bond, an ionic bond and/or hydrophobic interactions. The specific base sequences, also referred to as consensus sequences, are often located at the promoter or enhancer region of genes. The recognition is attributed to a unique DNA binding motif with α-helices or β-sheets for binding to the major or minor groove of DNA.

On the basis of these unique protein motifs at the DNA binding domain, TFs are categorized into several groups with zinc finger, b-Zip, HTH and HLH motifs to date (3). For instance, AP1 is a homo- and heterodimer between Jun and Fos family member proteins with b-Zip motif and able to specifically recognize the core base sequence TGACTCA on inducible target genes for transcriptional modulation. By contrast, CREB is a protein with b-Zip
motif and able to modulate transcription of target genes through phosphorylation on serine 133 following specific recognition of the core element TGACGTCA. From another point of view, therefore, TFs are divided into 3 major categories (4, 5): 1) ITFs expressed as IEGs in response to a particular stimulus (Fos, Jun, Krox, etc.); 2) CTFs constitutively expressed but regulated by posttranslational modifications (CREB, ATF, SRF/TCF, etc.); 3) ligand-activated TFs (glucocorticoid receptor, etc.) (Table 1).

By interacting with RNA polymerase II, TFs are able to affect gene transcription in a positive or negative way. TFs would activate the activity of RNA polymerase II via their transactivation domain to facilitate the synthesis of mRNA from DNA. For specific gene transcription, however, RNA polymerase II alone is insufficient, but a group of both general and regulatory TFs are required. General TFs comprise at least 20 proteins that form multiple preinitiation complexes at the promoter region enriched with the base sequence TATA, named the TATA box, or its equivalent regulatory sequence. The general TF complex TFII D is composed of TBP and more than 8 TBP-associated factors with an ability to bind to the TATA box, which leads to binding of RNA polymerase II to the complex for the initiation of gene transcription. The transcription dependent on the promoter is called basal transcription, while a regulatory territory usually exists upstream of the promoter region to affect such control (cis-element) in a form of an enhancer or silencer for determination of a basal transcription level. The promoter region is essential for the initiation of transcription, whereas the enhancer modifies the activity of the promoter but is not effective alone. Regulatory transcription factors bind to the promoter or enhancer on the genomic DNA close or distant to the TATA box, followed by activation or repression of gene transcription by RNA polymerase II in the nucleus (Fig. 2).

Accordingly, gene expression is controlled at two territories, promoter and cis-element, by two groups of protein factors, general and regulatory TFs. Cells could raise or lower the level of the basal transcription through modulation of the expression of different TFs in response to changes in physiological environments and stimuli from the extracellular world.

### Table 1. Subclassification of typical transcription factors

| TFs                | Peculiar protein motifs | Consensus sequences |
|--------------------|------------------------|---------------------|
| Inducible TFs      | Fos, Jun               | Leucine zipper      | TGACTCA           |
|                    | Krox                   | Zinc finger         | GCGGGGGCG         |
| Constitutive TFs   | CREB, ATF              | Leucine zipper      | TGACGTCA          |
|                    | SRF/TCF                |                      | CC(AT)_{6}GG      |
| Ligand-activated TFs | GR                    | Zinc finger         | GTTACANNTGTCT    |

3. Neurotransmitters

In the mammalian CNS, extracellular neurotransmitters induce two major distinct intracellular responses at synapses, such as excitatory and inhibitory postsynaptic potentials. In excitatory neurons, a neurotransmitter evokes action potentials on postsynaptic cells following the release from nerve endings, while an inhibitory neurotransmitter prevents the evocation of action potentials in postsynaptic cells. The difference between excitatory and inhibitory responses is attributed to characteristic profiles of membrane receptors to which excitatory and inhibitory neurotransmitters specifically bind. Of a number of central neurotransmitters identified to date, both glutamate and GABA are the major excitatory and inhibitory neurotransmitters, respectively.

3.1. Glutamate

Glutamate is the dominant excitatory neurotransmitter responsible for a variety of physiological and pathological events in the mammalian brain. GluRs play a critical role in the neuronal survival and refinement of neuronal connections during brain development, as well as in the synaptic...
plasticity underlying learning and memory. In contrast, over-activation of GluRs often leads to neurodegeneration. Some of these long-lasting phenomena would require coordinated programs of changes in gene transcription and subsequent de novo synthesis of particular functional proteins (4).

GluRs are nowadays categorized into 2 major subclasses, such as iGluR and mGluR, on the basis of intracellular signal transduction systems. iGluRs are classified into NMDA, AMPA and KA subtypes according to the sensitivity to excitation by the respective exogenous agonists. NMDARs are composed of a protein oligomeric complex between the NR1 subunit and one of the NR2 (A - D) subunit members, whereas the NR1 subunit is essential for expression of a functional ion channel permeable to Ca\(^{2+}\) ions. AMPA receptors consist of a protein oligomer between different subunit members including GluR1, GluR2, GluR3 and GluR4, while KA receptors are constructed through a combination of GluR5, GluR6 and GluR7 in addition to both KA1 and KA2 subunits that are supposed to participate in expression of KA receptors with high affinity. Of the mGluR subtypes, in contrast, group I mGluR is linked to phospholipase C to stimulate the formation of 1,3-diacylglycerol and IP\(_3\) from PIP\(_2\) in the cytoplasmic membrane. Both group II and group III mGluR subtypes are coupled to adenylate cyclase to inhibit the formation of cyclic AMP from ATP in the cytoplasm. The group I mGluR subtype is formed from either the mGluR1 or mGluR5 subunit, while both mGluR2 and mGluR3 subunits participate in construction of the group II mGluR subtype. Four different subunits of the group III mGluR subtype have been cloned to date.

A variety of ITFs are induced by activation of the NMDAR. In vitro activation of NMDAR leads to transcriptional activation of c-fos, fosB, c-jun, junB, zif/268 and nur/77 in hippocampal neurons (6) and increases c-fos, c-jun and zif/268 mRNA levels with rapid and transient kinetics in cerebral cortical neurons (7) and c-fos, c-jun, junB and zif/268 mRNA induction in both cortical and striatal neurons (8), respectively. The early phase of the induction by glutamate of c-fos, c-jun, junB and zif/268 mRNA is preferentially regulated through the stimulation of NMDAR in cerebellar neurons (9). Brief exposure to NMDA leads to a transient increase in AP1 DNA binding in immature cultured rat hippocampal neurons in a manner sensitive to both nifedipine and dantrolene in addition to MK-801 (10). In vivo administration of NMDA leads to expression of c-Fos protein and potentiation of AP1 DNA binding in murine brain (11), while the potentiation of AP1 DNA binding is highly selective for the hippocampus among the discrete brain structures (12). In murine hippocampus, the systemic administration of NMDA results in preferential potentiation of AP1 DNA binding in the dentate granule cells, but not in the CA1 and CA3 pyramidal layers (13). In addition to the nuclear extracts described above, NMDA is also effective in potentiating AP1 DNA binding constitutively detected in cytosolic fractions of murine hippocampus (14). An intracerebroventricular injection of NMDA significantly potentiates binding of probes for AP1 and CREB in nuclear extracts of murine whole brain (15). Moreover, NMDA induces more rapid degradation of inducible c-Fos protein than constitutive Fra-2 protein in nuclear, but not cytosolic, fractions of murine hippocampus (16).

Activation of AMPA or KA receptors is less frequently effective in inducing expression of ITFs than NMDARs. Antagonists at these non-NMDARs do not usually inhibit ITF expression induced by a numbers of pharmacological and physiological stimuli. In vitro, however, AMPA receptor stimulation is shown to induce expression of c-fos, c-jun, junB and krox-24 mRNA in cultured cortical, hippocampal and striatal neurons (5). The systemic administration of KA leads to marked and sustained potentiation of AP1 DNA binding in the hippocampus (11, 17), in addition to expression of c-Fos, FosB, Fra-2 and c-Jun proteins (18), and to drastic enhancement of AP1 DNA binding in both mitochondrial and nuclear fractions of murine cerebral cortex and hippocampus through expression of c-Fos, FosB and JunB proteins (19). This potentiation could involve Ca\(^{2+}\) entry through particular subclasses of AMPA and KA channels permeable to this cation, while both iGluR subtypes would induce membrane depolarization with subsequent opening of NMDAR channels required for expression of the AP1 protein complex.

Activation of mGluR in vivo by unilateral striatal injection of the mGluR-selective agonist 1S,3R-ACPD causes c-Fos expression in the striatopallidal neurons (20). Intrastriatal infusion of the selective group I mGluR agonist, DHPG, upregulates phosphorylation of CREB and Elk-1 in the striatum (21). In vitro addition of 1S,3R-ACPD increases c-fos, c-jun and zif/268 mRNA levels in cultured cerebral neurons (7). Moreover, the increases in mRNA levels for c-fos, c-jun, junB and NGFI-A by the addition of quisqualate are antagonized by the mGluR antagonist AP3 in cultured cortical and striatal neurons (8). However, it remains to be determined if all or only one of the second messenger pathways and calcium influx activated by these receptors are capable of causing ITF expression (Fig. 3).

3.2. GABA

In the mammalian CNS, GABAergic neurons exist in routes projected from the hypothalamus to the cerebral cortex, from the cerebellum cortex to the vestibulum nucleus lateralis medulla oblongata, and from the substantia nigra to the thalamus/colliculus superior, in addition to various other places of the brain as interneurons. GABA receptors
have been classified into at least two categories such as GABA_A and GABA_B receptors. The former is a hetero-
gorous multimeric ligand-gated Cl^-/G_2d channel, while the latter is a metabotropic receptor subtype coupled to G proteins with seven transmembrane domains.

The administration of pentylenetetrazol or picrotoxin, the inhibitor of GABA_A receptor Cl^- channel, leads to
convulsive seizures, followed by expression of c-fos, c-jun and Fra and subsequent increase in nuclear AP1 DNA binding (22).

Baclofen and 3-hydroxybutyric acid could activate the
release of Ca^{2+} from intracellular Ca^{2+} stores via GABA_B receptors coupled with G proteins in membranes and the
increased intracellular Ca^{2+} plays an important role in
increasing both CREB and AP1 DNA binding in cultured
cerebellar granule neurons (23).

4. Hormones

Unlike synaptically released compounds, hormones can enter the CNS and simultaneously affect the functions of a number of neurons and/or glia to coordinate gene expression. Hormones could acutely induce ITF expressions, as well as modulation of their expressions caused by other acute stimuli (5).

4.1. Glucocorticoid hormones

Due to its lipophilic nature, GC readily enters the brain and stays at those cells that contain GC receptors. Both GC receptor immunoreactivity and GC receptor mRNA is widespread in neurons and glia throughout the brain. GC receptor belongs to the superfamily of nuclear receptors that act as transcription factors in the mechanism underlying the regulation of gene expression in the nucleus (24).

GC receptor is a constitutive protein attached to a member of the HSP family with a molecular weight of 90 kDa (HSP90) in the cytoplasm. Activation of GC receptor by a GC agonist leads to dissociation of HSP90 from the complex and subsequent import of the GC receptor bound by an agonist from the cytoplasm to the nucleus. In the nucleus, GC receptor is able to recognize particular nucleotide sequences referred to as a GRE (GTTACANNNTGTTCT) located at the upstream (or downstream) on double stranded target genes and thereby to modulate the activity of RNA polymerase II as a member of transcription factors with zinc finger motifs through homodimerization. GC receptor is a protein complex consisting of DNA binding and trans-
scription domains (1) (Fig. 4).

The DNA binding domain comprises two zinc fingers with four cysteine residues placed in a way that they tetrahedrally coordinate zinc and thereby form a loop. The domain is sufficient to activate transcription itself.
Transcriptional domains are identified in the C-terminal and N-terminal regions of the receptor. These domains are an important determinant of the final transcriptional efficacy. The transactivational domains also play a role in the interactions with other TFs. The best-documented example so far concerns interactions between GC receptor and the AP1 complex. GC receptor enhances transcription when AP1 is composed of c-Jun homodimers, but represses transcription in the presence of c-Jun/c-Fos heterodimers (24). GC receptor inhibits binding of the c-Jun/c-Jun homodimer to TRE in certain situations (25–27). In addition, GC receptor is shown to exacerbate DNA binding activities of c-Fos (28, 29) and CREB (30) through a direct protein-protein interaction in vitro. Moreover, in vitro dexamethasone, which is a potent synthetic GC analogue, suppresses the basal expression of c-jun, but not c-fos, and inhibits CRF-induced c-fos expression and AP1 activity in corticotrophic pituitary AtT 20 cells (31). However, in vivo footprinting disagrees with the direct protein-protein interaction between AP1 complex and GC receptor (32). Transcriptional modulation by AP1 also seems to be under negative and positive control by GC receptor through as-yet unidentified mediators (33). In vivo administration of the GC receptor agonist TA significantly inhibits AP1 DNA binding in the adrenal and pituitary but not in the brain, with concomitant facilitation of translocation of immunoreactive GC receptor from the cytoplasm into the nucleus in all central and peripheral excitable tissues examined (34). Heterogeneity of ligand binding domain is also proposed in cytosolic fractions of brain and liver from rats with intact adrenals using [3H]TA as a radioligand (35, 36). These findings give rise to the possible in vivo crosstalk between transcription factors with different protein motifs in murine peripheral but not central excitable tissues.

4.2. Sex steroid hormones

Estrogen exerts a pivotal role in the female sexual behaviors through the action of ER not only in the peripheral tissues but also in the CNS. Circulating estrogen dissociates from the estrogen binding protein to pass through the cell membrane to the cytoplasm and enters the nucleus to form the estrogen-ER complex. The estrogen-ER complex responds to the estrogen-responsive element on the target genes to activate the synthesis of proteins and peptides. It is widely accepted that ER is an inevitable protein for the regulation of transcription of the specific DNA in the nucleus. ER is localized in the nucleus of neurons and glia, but not in the cytoplasm, in the CNS including the hypothalamus that is the highest center of the autonomic nervous system.
system related to the sexual and maternal behaviors of female animals. Using immunocytochemistry and in situ hybridization for ER and ER mRNA, it became known that ER is mainly located in the hypothalamus of the female brain to regulate the female sexual cycle and behaviors (37).

As seen with corticosteroids, sex steroids affect stimulation-induced ITF expression. Gonadectomy is shown to reduce expressions of c-fos, c-jun and krox-24, but not junB, induced by placement of male rats in a novel environment in the CA1, but not other hippocampal regions, and these expressions are potentiated in castrated rats given dihydrotestosterone. In female rats, estrogen and progesterone have complex inhibitory and stimulatory effects on the expression of c-fos mRNA and c-Fos protein in central estrogen-responsive areas such as the lateral septum and medial preoptic area, which is mediated by vagion-cervical stimulation (5).

4.3. Growth hormones

Growth hormones instigate expression of c-fos in the arcuate and the peri- and paraventricular nuclei of the hypothalamus in hypophysectomized rats. GHRP-6, but not GHRH, elicits expression of c-Fos protein in the rat hypothalamic arcuate nucleus, hypothalamic paraventricular nucleus, dorsomedial hypothalamus, lateral hypothalamus, and nucleus of the tractus solitarius (5, 38).

5. Autacoids

Autacoids are synthesized under different physiological and pathological conditions in peripheral organs and mainly secreted around the formation part. They produce strong alterations of a number of physiological functions in various organs in spite being present in only a small quantity and are also called local hormones. There are histamine, serotonin, Ang, prostaglandin, and so on as typical autacoids. Among these, the function of Ang II is relatively well investigated in terms of the relation to TFS in the brain. Ang II exerts a number of actions in the brain and appears to be an important factor in central cardiovascular and osmotic regulations. These actions include increases in blood pressure, modulation of sympathetic nerve activity, and inductions of urinary sodium excretion, kaliuresis, and drinking responses (39).

Ang analogues exert their actions via binding to specific receptors. Two of the four known Ang II receptor subtypes have been cloned: type 1 (AT₁) and type 2 (AT₂). Both subtypes belong to the family of seven-transmembrane domain receptors that functionally couple to G proteins. Protein phosphorylation triggered by the AT₁ receptor is important for cell growth, in which tyrosine kinase, serine/threonine kinase, and protein kinase C are all involved. AT₁-receptor activation induces hydrolysis of PIP₂ by phospholipase C and creates IP₃, which releases calcium from cytosolic calcium pools. Cytosolic calcium can also be elevated by activation of voltage-gated calcium channels via a linkage between the AT₁ receptor and G protein. The AT₂ receptor has a conserved motif in its third intracellular loop, which is unique among G protein coupled seven transmembrane receptors known to date. However, increasing evidence suggests that the AT₂ receptor is coupled to G protein as well. Activation of AT₂ receptor is shown to activate protein tyrosine phosphatase, followed by repression of calcium channel and subsequent inactivation of mitogen-activated protein kinases (39, 40) (Fig. 5).

The effects of Ang II have been examined on the expression of transcription factors in the rat brain in a wide range of studies, following both peripheral and central administrations of the peptide. Ang II would probably reach the brain tissues via an intravenous (i.v.) bolus injection or infusions to stimulate the nuclei of the lamina terminals such as SFO and OVLT, both lacking the blood-brain barrier due to a special fenestrated epithelium. Consequently, peripheral administration of Ang II induces strong expression of c-Fos, c-Jun, or Fos/Jun-like immunoreactivity in the SFO and OVLT. Other regions of strong c-Fos and c-Jun expression include MnPO, SON and PVN (39, 41).

An injection of Ang II into the lateral ventricle results in the expression of a number of ITFs (c-Fos, FosB, c-Jun, JunB, and Krox-24) in several rat brain regions. The principal regions of expression are the SFO, MnPO, PVN, SON and OVLT. The expression is dose-dependent, with a threshold dose between 1 and 10 ng Ang II, the agonists at the AT₁ subtype, and is specific with respect to the pattern of various ITF expressed in each region. Injections of higher doses of Ang II (250 ng) or infusions of the peptide over a period of time (1 ng/min for 90 min) result in expression of c-Fos protein in other regions such as the bed nucleus of the stria terminals and the central amygdaloid nucleus. The ITF expression is also temporally differentiat-
ed, with some ITF such as c-Fos and Krox-24 showing fast onset and decline of expression within 4 h after stimulation, while other ITFs show delayed onset of expression (FosB) or persistent expression for up to 8 to 24 h (c-Jun and JunB). These findings suggest that periventricular AT₁ receptor stimulation may lead to successive regulation of transcription of different target genes in a manner selective for each gene in the nuclei (39).

6. Drug tolerance and dependence

Transcription factors are believed to play a crucial role in the development of tolerance and dependence through mechanisms similar to those operating in other instances of neural plasticity such as learning and the development of chronic pain. Here we review the responsiveness of TFs
to acute and chronic administration, as well as withdrawal, of drugs inducing dependence, particularly cocaine, morphine and ethanol (5).

6.1. Cocaine

Cocaine blocks the DA reuptake transporters, thereby increasing the availability of this catecholamine to synaptic DA receptors within the brain. Increased DA levels subsequently lead to a cascade of intracellular events, including activation of cAMP-dependent protein kinase, phosphorylation of CREB and induction of AP1 complex in striatal synapses. For instance, acute administration of cocaine elicits rapid and transient increases in the expression of c-fos, junB and NGFI-A mRNAs, and Fra members in rat striatal and cerebellar neurons (42, 43). Moreover, acute cocaine increases AP1 DNA binding in several CNS regions, including the striatum and cerebellum. In the striatum, cocaine induces AP1 complexes containing c-Fos, FosB, JunB and JunD, whereas in the cerebellum, only c-Fos and JunD are involved (43). Using the in situ hybridization technique, cocaine is shown to elicit a robust but transient increase in arc mRNA levels in the rat striatum (44).

Chronic cocaine administration results in long-lasting behavioral alterations in rodents. Such alterations are accompanied by changes in signal transduction mechanisms that regulate gene transactivity. For example, chronic cocaine treatment leads to the desensitization of c-fos and NGFI-A mRNAs in the rat forebrain, caudate putamen and nucleus accumbens (42). Chronic cocaine administration abolishes the inducibility of c-fos, c-Fos, c-jun and krox-24 in the nucleus accumbens and striatum. This does not occur nonspecifically throughout the brain since the inducibility of c-fos is enhanced in the cerebellum, probably because the actions of cocaine occur via NMDA and D1 receptors in the striatum, but not via other receptors in the cerebellum (43, 45). Chronic cocaine additionally produces the expression of novel 35- and 37-kDa Fras (‘chronic’ Fras), which are especially stable isoforms of ΔFosB, in specific brain regions (46, 47). These chronic Fras show electrophoretic patterns different from those induced by acute cocaine, with properties to dimerize mostly with JunB and JunD rather than c-Jun. The increase is dependent upon D1 receptors and blocked by D1 antagonists that themselves do not alter chronic Fra levels in some situations. By contrast, D2 antagonists increase chronic Fra levels, without affecting the increase caused by cocaine. Chronic Fras are also expressed in response to repeated treatment with dopamine uptake inhibitors. Finally, chronic cocaine can alter the amount and phosphorylation of CREB in several central

Fig. 5. Modulation of gene transcription via angiotensin receptors. Activation of AT1 receptor induces an increase in cytosolic free calcium concentration through the permeation across IP3-gated and voltage-gated channels, followed by activation of phosphorylation cascade and subsequent facilitation of transcription of particular genes. By contrast, activation of AT2 receptor leads to reduction in cytosolic free calcium concentration, followed by repression of phosphorylation cascade and subsequent suppression of gene transcription.
nuclei, without affecting CREB mRNA levels in the hippocampus (5) (Table 2).

### 6.2. Morphine

Morphine is thought to produce reinforcement phenomena via stimulation of $\mu$-, $\kappa$-, and $\delta$-opioid receptors in reward-relevant brain systems. Receptor binding studies show that morphine has the highest affinity for $\mu$ among these subclases, with progressively lower affinity for $\kappa$ and $\delta$ subtypes. These opioid receptors are all coupled to G proteins with seven transmembrane domains, and have stimulatory effects on neurotransmission in several brain regions (42).

Morphine acts at $\mu$-opioid receptors to indirectly facilitate the release of dopamine in striatal synapses. Such a membrane event triggers transcriptional activation of the prototypic IEGs, c-fos and junB, in caudate and accumbal cell bodies (48). Acute or chronic morphine administration suppresses the induction of c-fos mRNA, whereas an abrupt and significant induction of the IEG is noted in the locus ceruleus, amygdala, neocortex, hypothalamus and brain stem after withdrawal (49, 50). Single or repeated administration of morphine and naltrexone only slightly alters c-Fos expression in the guinea-pig brain, but in morphine-dependent animals, naltrexone induces strong c-Fos expression in numerous CNS nuclei. Both acute and chronic administrations of morphine decrease c-Fos levels in the rat locus ceruleus, while in dependent animals, opioid antagonists cause a marked induction of c-jun, c-fos, krox-24, but not junD, as well as their corresponding proteins in the locus ceruleus, cerebral cortex, hippocampus, thalamus, cerebellum, brain stem and spinal cord (5).

Repeated morphine administration also increases the levels of chronic Fras, almost exclusively in the striatum and nucleus accumbens, and withdrawal from morphine initiates expression of ‘acute’ Fras including c-Fos, FosB, Fra-1, Fra-2 and A-FosB within 6 h in the striatum and nucleus accumbens. Chronic Fras are present at this time, and their levels increase in these and other regions 72 h after withdrawal (46).

Chronic morphine administration decreases CREB levels in the nucleus accumbens, but increases AP1 and CREB DNA binding, as well as phosphorylation of CREB. In contradistinction, chronic morphine treatment is also shown to increase the levels of CREB, but decreases its phosphorylation in the locus ceruleus (5). In another report, acute morphine treatment decreases rates of CREB phosphorylation in the rat locus ceruleus, which is not seen after chronic morphine exposure. When opiate withdrawal is precipitated by naltrexone, however, PKA-dependent phosphorylation of CREB is abruptly increased (51). Chronic morphine treatment decreases CREB phosphorylation in accumbal neurons, suggesting that two morphine-sensitive brain structures display contrasting features of adaptation, regulation and expression of phosphorylated CREB (52). The importance of phosphorylated CREB for the induction of c-fos and NGFI-A mRNA after morphine treatment is evidenced in striatonigral neurons (42) (Table 2).

### 6.3. Ethanol

Ethanol dependence has been well defined by diagnostic criteria with well-described cellular pathophysiology. For instance, chronic and long-term ethanol use often results in encephalopathies, such as cerebellar degeneration and pontine myelinolysis, that seriously compromise the health of affected individuals. Therefore, a great deal of effort has been devoted to understanding the molecular mechanisms underlying these neurological abnormalities and cellular steps that give rise to tolerance and dependence induced by this simple two-carbon molecule. As seen with most drugs of abuse, acute ethanol treatment increases the activity of certain dopaminergic and serotonergic pathways in the brain. Exposure to ethanol increases endogenous levels of DA and 5-HT in the caudate putamen and nucleus accumbens of rat brain. Acute increases in monoaminergic

| Drugs   | Administration | Genes                                      | TFs                                      |
|---------|----------------|--------------------------------------------|------------------------------------------|
| Cocaine | Acute          | c-fos $\uparrow$, junB $\uparrow$, NGFI-A $\uparrow$, arc $\uparrow$ | c-Fos $\uparrow$, FosB $\uparrow$, JunB $\uparrow$, JunD $\uparrow$, Fras $\uparrow$ |
|         | Chronic        | c-fos $\downarrow$, c-jun $\downarrow$, NGFI-A $\downarrow$, CREB $\rightarrow$ | c-Fos $\downarrow$, ‘Chronic’ Fras $\uparrow$ |
| Morphine| Acute          | c-fos $\uparrow$, junB $\uparrow$          | c-Fos $\downarrow$, CREB $\downarrow$   |
|         | Chronic        | c-fos $\downarrow$                         | c-Fos $\downarrow$, CREB $\uparrow$$\downarrow$$\rightarrow$ |
|         | Withdrawal     | c-fos $\uparrow$                           | ‘Acute’ Fras $\uparrow$, ‘Chronic’ Fras $\uparrow$, CREB $\uparrow$ |
| Ethanol | Acute          | c-fos $\rightarrow$, c-jun $\rightarrow$, NGFI-A $\rightarrow$ | CREB $\uparrow$ |
|         | Chronic        | c-fos $\rightarrow$, c-jun $\rightarrow$, NGFI-A $\rightarrow$ | CREB $\downarrow$ |
|         | Withdrawal     | c-fos $\uparrow$, c-jun $\uparrow$, NGFI-A $\uparrow$ | c-Fos $\uparrow$, FosB $\uparrow$, c-Jun $\uparrow$, JunB $\uparrow$, JunD $\uparrow$ |

$\uparrow$: increase, $\downarrow$: decrease, $\rightarrow$: no change.
content would involve the stimulation of appropriate receptor subtypes (e.g., D_1, D_2, 5-HT_1A, 5-HT_1B and 5-HT_3) that modify resting membrane potential dynamics regulating excitatory or inhibitory synapses. Ethanol also interferes with the function of particular ionotropic receptor complexes by potentiating GABA_A ion (e.g., Cl^-) currents or reducing NMDA-activated ion (e.g., Ca^{2+}) fluxes in different regions and even in different neurons of the rodent brain (42).

The functional consequences of ethanol treatment have also been studied on IEGs in rat brain. In all of these studies, ethanol by itself did not alter total brain c-fos, c-jun or NGFI-B mRNA expression (42). Exposure of rats to ethanol vapor over 21 days does not alter the basal CNS levels of c-fos, c-jun and krox-24, but 8 to 24 h after discontinuation of the exposure, there is a more potent increase in expression of mRNA for all these three transcription factors in the hippocampus than in the cortex. There is a concomitant increase in c-Fos protein in a manner that peaks at 15 h after the discontinuation in the cerebral cortex, olfactory bulb, inferior colliculus and several brain stem nuclei, but not in the hippocampus (5). These increases are prevented by prior administration of MK-801, again indicating a principal role for NMDARs in induction of the ITFs after withdrawal (53). This induction is probably caused by the increased level of NMDARs produced after chronic ethanol intake (54). Acute ethanol intake inhibits AP1 DNA binding enhanced via NMDARs, while in cultured cerebellar neurons, chronic treatment increases AP1 complex induced by AMPA receptors (55). Acute ethanol treatment triggers an increase in CREB content in granule cell layers of the rat cerebellum, with peak levels of phosphorylated CREB 30 min later. In contrast, ethanol consumption for 5 weeks results in a significant decrease of levels of phosphorylated CREB in striatal and cerebellar neurons (56).

In animal models of ethanol withdrawal, there are significant changes in transcriptional mechanisms when ethanol is withheld after chronic use. These animals show transient increases in the expression of c-fos, c-jun and NGFI-A mRNAs in the cerebral cortex, olfactory bulb, hippocampus and granular layers of the cerebellum (57, 58). Withdrawal from chronic ethanol results in a transient increase in AP1 DNA binding composed of FosB, c-Jun, JunB and JunD, with the induction of c-Fos protein in a manner dependent on the brain structures and the time after withdrawal (58). The hyperexcitability in a variety of membrane-dependent events leading to the induction of IEGs seems to be at least in part responsible for mechanisms associated with increased activities of 5-HT and GluR and decreased inhibitory function of GABA_A receptors, all of which are upregulated or downregulated, respectively, during chronic ethanol intake (42) (Table 2).

7. Conclusions

Drug-derived signals could modulate the activity of several different types of TFs, of which the IEGs are the best characterized. Induction of these cellular genes activates the expression of a number of late-response genes, resulting in behavioral manifestations of addiction. There is no doubt that TFs are indeed involved in regulating the first biological amplification step. As cited in this paper, a variety of TFs are induced in response to administration of various drugs in the brain. It appears that TFs may play a critical role in long-lasting consolidation of transient signals through modulation of de novo synthesis of inducible target proteins in the brain. Binding between TFs and nucleotides is also as specific in the cell nucleus, as binding between ligand and receptor on the cell membrane. Search for drugs targeting the interaction between TFs and nucleotides could give us new points of view toward the therapy and treatment of various diseases in the brain.

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