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Review Article

Role of GIRK Channels in Addictive Substance Effects

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Abstract G-protein-activated inwardly rectifying potassium (GIRK) channels are widely expressed in the central nervous system, including brain regions related to reward, and play an important role in mediating the signal transduction pathways of various addictive substances. Studies of GIRK knockout mice have suggested the involvement of GIRK channels in the mechanisms that underlie the effects of addictive substances. Human studies have shown that differences in the genetic sequence of one of the four GIRK channel subunits, GIRK2, are associated with analgesic requirements in patients who undergo major open abdominal surgery. Animal and human studies also showed the possible therapeutic effects of GIRK channel inhibitors in the treatment of methamphetamine dependence and alcoholism. These findings suggest that GIRK channels may be a key molecular target in the reward system for the treatment of addiction.

Keywords GIRK channel; reward system; addictive substance; therapeutic target

1. Introduction

Addictive substances comprise various natural and synthetic substances, including amphetamines, cocaine, opioids, cannabinoids, hallucinogens, alcohol, hypnotics/anxiolytics, inhalants, nicotine, and caffeine. Addictive substances produce immediate good feelings (euphoria) or relieve distress and are more likely to be taken repeatedly. Their reinforcing effects lead to substance abuse and dependence. Although addictive substances have diverse molecular targets in the brain, they commonly increase extracellular dopamine levels in the mesocorticolimbic system. The substances cause adaptations at the neurocircuitry, cellular, and molecular levels in the mesocorticolimbic system and other brain regions that control reward and motivation. G-protein-activated inwardly rectifying potassium (GIRK) channels are widely expressed in the central nervous system, including brain regions related to reward, and play an important role in regulating neuronal excitability and synaptic transmission. Animal models have shown that GIRK channel dysfunction changes some of the responses to several substances and their rewarding effects. This review focuses on GIRK channels in the effects of addictive substances and as a promising target in the treatment of substance dependence.

2. Neural circuits and cellular and molecular mechanisms in the reward system

The reward system includes a collection of brain structures that are activated by the perception and expectation of satisfying craving, and the reward system produces pleasurable sensations in individuals. Neural circuits in the reward system play an important role in the onset and aggravation of substance dependence, which is associated with compulsive addictive behavior, tolerance, and withdrawal. Understanding the biological mechanisms of the reward system may have important social and clinical implications in the treatment of substance dependence.

The major neuronal pathways of the reward system include components of the mesocorticolimbic dopamine system that originates in the ventral tegmental area (VTA) of the midbrain and projects to the limbic forebrain, especially the nucleus accumbens (NAc) [20, 24, 56, 64, 100]. The NAc serves as a dopamine-gated mediator of information that passes from the limbic system to the cortex [13]. Although addictive substances act at distinct targets in different brain regions, addictive substances commonly increase extracellular dopamine levels in the NAc [82]. By contrast, during the withdrawal syndrome associated with opioids, cannabinoids, ethanol, psychostimulants, and nicotine, dopamine levels decrease in the NAc. Thus, the VTA-NAc pathway is thought to play a crucial role in the rewarding effects of addictive substances. Interestingly, the mesolimbic dopamine system is similar between humans and other animals [8]. Furthermore, several brain regions that interact with the VTA-NAc pathway have been shown

to be important for reward neurocircuitry. These regions include the amygdala, hippocampus, prefrontal cortex, locus coeruleus (LC), and raphe nucleus. The relationships with the VTA-NAc pathway include glutamatergic projections from the prefrontal cortex to the NAc and VTA, projections from γ-aminobutyric acid (GABA) neurons in the NAc to prefrontal cortex, the modulation of GABA actions by opioid interneurons in the VTA, opioid actions on norepinephrine neurons in the LC that project to the VTA and frontal cortex, and serotonergic projections from the raphe nucleus to the NAc and VTA [12,72]. The chronic use of addictive substances changes dopamine transmission in the VTA-NAc pathway and other neurotransmitters in brain regions related to reward [71,90,99].

Neurocircuitry alterations induced by chronically used addictive substances are based on molecular and cellular mechanisms that underlie their rewarding effects. The release of dopamine in the NAc stimulates various populations of medium spiny neurons, whose response to the neurotransmitter depends on the types of dopamine receptors they express. Dopamine stimulation induced by various addictive substances activates dopamine receptors (D_1–D_5 subtypes). D_1 and D_1-like receptors (i.e., D_5 receptors) activate heterotrimeric Gs proteins, which activate adenylyl cyclase and lead to the synthesis of the second messenger cyclic adenosine monophosphate (cAMP) [7]. D_2 and D_2-like receptors (i.e., D_3 and D_4 receptors) activate heterotrimeric $G_{i/o}$ proteins, which inhibit adenylyl cyclase activity and lead to a reduction of cAMP levels [2,95]. The level of intracellular cAMP regulates the activity of cAMP-dependent protein kinase A (PKA). PKA can phosphorylate metabolic enzymes, other protein kinases, ion channels, and transcription factors and affect the functions of cytoplasmic, membrane, and nuclear proteins. Protein kinase cascades induce transcription factors, such as cAMP response element binding protein (CREB) and Fos, leading to changes in gene expression. Moreover, G-protein $\beta\gamma$ subunits released from Gα proteins can modulate the functions of Ca^{2+}, Na^+, and K^+ channels, leading to alterations in neuronal excitability [16]. Therefore, G-protein-signaling pathways affect cytoplasmic events and neuronal excitability. Indeed, a considerable evidence indicates that several addictive substances commonly induce cAMP, CREB, and ΔFosB in neurons in the NAc, reflecting adaptations characterized by upregulation of the cAMP pathway [6,65,73,74,94].

$G_{i/o}$ proteins may play a critical role in the effects of addictive substances. Addictive substances, including amphetamines, cocaine, opioids, cannabinoids, hallucinogens, phencyclidine (PCP), alcohol, hypnotics/anxiolytics, inhalants, nicotine, and caffeine, act on diverse target molecules in the brain, such as monoamine transporters, μ-opioid receptors, cannabinoid CB_1 receptors, serotonin 5-hydroxytrypatmine-2A (5-HT_{2A}) receptors, N-methyl-D-aspartate (NMDA) receptors, γ-aminobutyric acid-A ($GABA_A$) receptors, GIRK channels, nicotinic acetylcholine receptors, and adenosine receptors. Among addictive substances, opioids, cannabinoids, and γ-hydroxybutyrate (GHB) directly activate opioid receptors, cannabinoid CB_1 receptor, and $GABA_B$ receptors, respectively (Figure 1). Hallucinogens, such as lysergic acid diethylamide (LSD), mescaline, and psilocybin, act as partial agonists at serotonin 5-HT_{2A} receptors [1], and caffeine acts as an antagonist at adenosine A_1 and A_{2A} receptors [35]. Amphetamines and cocaine indirectly activate dopamine, norepinephrine, and serotonin receptors by increasing the extracellular levels of dopamine, norepinephrine, and serotonin [93]. Target receptors for the actions of these substances include various $G_{i/o}$ protein-coupled receptors (PCRs). $G_{i/o}$ protein-coupled signaling pathways may play a crucial role in the pharmacological effects of addictive substances. Therefore, a better understanding of reward mechanisms may provide novel insights into the treatment of substance abuse and dependence.

3. Physiological regulation of GIRK channels and relationships with actions of addictive substances

GIRK channels are members of a major subfamily of inwardly rectifying K^+ (Kir) channels (Kir1-Kir7) 25. Four GIRK channel subunits (GIRK1 [Kir3.1], GIRK2 [Kir3.2], GIRK3 [Kir3.3], and GIRK4 [Kir3.4]) have been identified in mammals [49,50,54]. The GIRK1, GIRK2, and GIRK3 subunits are expressed in various brain regions, such as the olfactory bulb, cerebral cortex, amygdala, hippocampus, thalamus, VTA, LC, dorsal raphe nucleus, and cerebellum [37,38], suggesting the involvement of GIRK channels in the rewarding effects of addictive substances. By contrast, GIRK4 subunits are expressed mainly in the heart [49] and in only a few regions of the brain, such as deep cortical pyramidal neurons, the endopiriform nucleus and claustrum of the insular cortex, the globus pallidus, the ventromedial hypothalamic nucleus, parafascicular and paraventricular thalamic nuclei, and a few brainstem nuclei [37,69,97]. In the brain, GIRK1 and GIRK2 subunits can assemble with other GIRK subunits to form functional heterotetramers, whereas GIRK2 can form functional homotetramers [101]. As shown in Figure 1, GIRK channels are gated by the direct action of G-protein $\beta\gamma$ subunits [15,25,32,51,57,84,98] released from $G\alpha_{i/o}$ proteins in response to the activation of various $G_{i/o}$ PCRs, such as α_2 adrenergic, $GABA_B$, 5-HT_{1A}, galanin, somatostatin [76], D_2, D_3, and D_4 [96], μ-, δ-, and κ-opioid [29], nociceptin/orphanin FQ [28], cannabinoid CB_1 [63], neuropeptide Y_1 [11], adenosine A_1, and metabotropic glutamate (mGluR2, -3, -4, -6, and -7) [87] receptors. Recent spectroscopic studies have suggested the existence of a macromolecular complex that

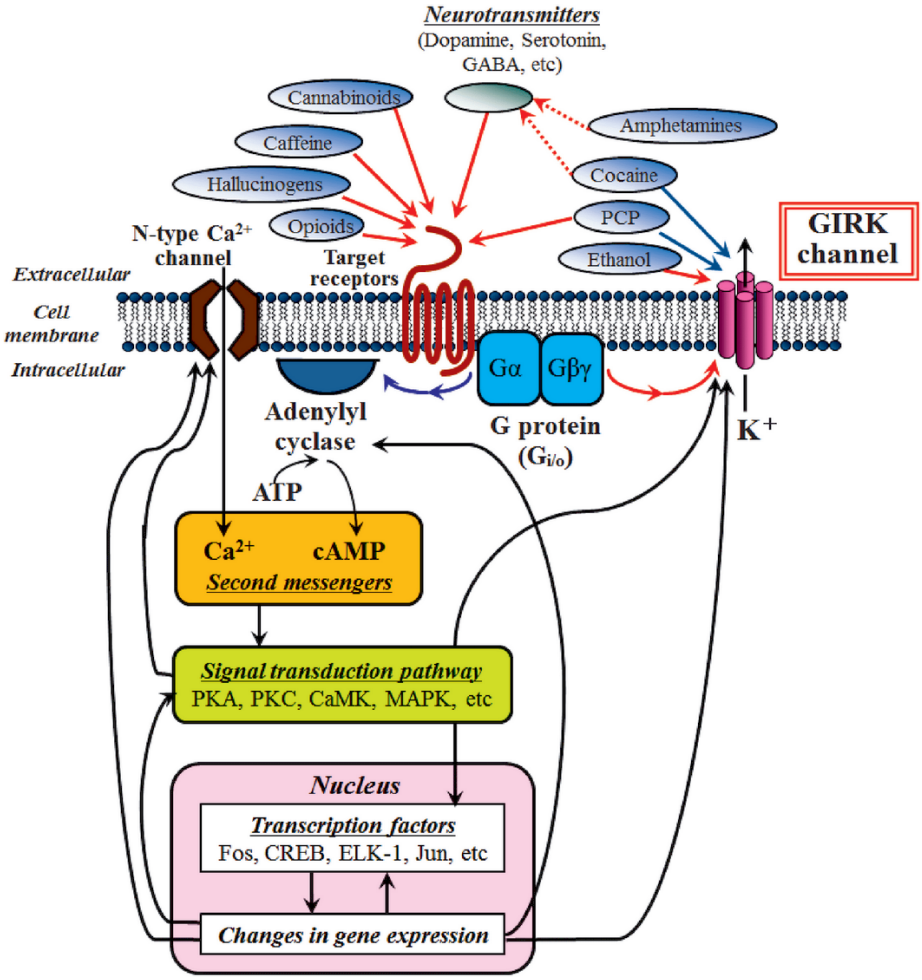

Figure 1: Schematic signal transduction pathways of addictive substances. Ethanol has been found to instantaneously open GIRK channels, whereas cocaine and PCP inhibit GIRK channels. Opioids, cannabinoids, and PCP bind to opioid receptors, cannabinoid receptors, and NMDA-type glutamate receptors, respectively. Amphetamine and cocaine have an indirect effect on receptors, increasing the synaptic levels of neurotransmitters. Receptors as targets for the actions of addictive substances include various $G_{i/o}$ PCRs, including μ-, κ-, and δ opioid receptors, among others. GIRK channels are gated by the direct action of G-protein $\beta\gamma$ subunits released from $G\alpha_{i/o}$ proteins in response to the activation of various $G_{i/o}$ PCRs. These receptors modulate the levels of second messengers like cAMP and Ca^{2+}, which in turn regulate the activity of protein kinase transducers. Such protein kinases affect the function of proteins located in the nucleus. Protein kinase transduction pathways also affect the activity of transcription factors. Changes in the activity of transcription factors may result in long-term functional changes, including changes in the gene expression of proteins involved in signal transduction and/or neurotransmission, resulting in altered neuronal responses. PCP, phencyclidine; $G_{i/o}$ PCRs, $G_{i/o}$-protein-coupled receptors; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKC, protein kinase C; CaMK, calmodulin-dependent protein kinase; MAPK, mitogen-activated protein kinase; CREB, cAMP-response element binding protein. Red arrow: activation. Blue arrow: inhibition.

contains GIRK channels, $G_{i/o}$ PCRs, G-proteins, and regulatory proteins (i.e., a regulator of G-protein signaling (RGS) protein and sorting nexin 27 [SNX27]) [58]. $G\alpha$ proteins have also been shown to be important regulators of the specificity of signaling from G-protein-coupled receptors (GPCRs) to GIRK and basal GIRK channel activity [14, 23, 33, 53, 59, 81, 85, 86]. Furthermore, the channels are modulated by PKA, protein kinase C (PKC), phospholipase C (PLC), and tyrosine kinase (Trk) in G-protein signaling pathways [21]. GIRK channel opening hyperpolarizes the cell membrane and decreases neuronal excitability. Altogether, GIRK channels may act as an important convergence effector in neurons, and the modulation of GIRK channels may affect various brain functions.

Table 1: Roles of GIRK channel genes in animals models.

Subunit	Modification	Result	Reference
GIRK1	Knockout	Decreased antinociceptive effects of morphine	Marker et al. [62]
		A lesser extent of lever press behavior under an operant task using food	Pravetoni et al. [83]
GIRK2	Missense mutation in channel pore	Constitutively active GIRK channels insensitive to G-proteins and ethanol	Kofuji et al. [46] Slesinger et al. [88] Kobayashi et al. [38]
		Reduced antinociceptive effects of ethanol and opioids	Kobayashi et al. [38] Ikeda et al. [30]
	Knockout	Reduced antinociceptive effects of opioids, ethanol, nicotine, cannabinoids, $GABA_B$ receptor agonist, and α_2 adrenergic receptor agonist	Marker et al. [61] Marker et al. [62] Blednov et al. [9]
		Reduced antinociceptive effects of morphine and clonidine in male mice	Mitrovic et al. [66]
		Reduced aversion to saccharin and ethanol-induced conditioned place preference	Hill et al. [26]
		Decreased self-administration of cocaine	Morgan et al. [68]
	Trisomy of *KCNJ6* gene	Increased responses to the availability of sucrose in the sucrose preference test	Cooper et al. [16]
GIRK3	Knockout	Reduced antinociceptive effects of morphine	Marker et al. [61]
		Reduced withdrawal from ethanol and hypnotics (pentobarbital and zolpidem)	Kozell et al. [47]
GIRK2/3	Knockout	Reduced antinociceptive effects of morphine	Marker et al. [61]
		Decreased self-administration of cocaine	Morgan et al. [68]
		Attenuation of morphine withdrawal syndrome and decreased potency but preserved maximal efficacy of the antinociceptive effects of morphine	Cruz et al. [19]

Among addictive substances, ethanol directly activates (i.e., opens) GIRK channels at pharmacologically relevant concentrations [5,39,55], whereas cocaine and PCP directly inhibit GIRK channels at severe toxic concentrations [40, 41] (Figure 1). Addictive substances dynamically increase the levels of neurotransmitters, activate various GPCRs, activate G-protein-mediated signaling, and alter gene expression [47] (Figure 1). Accumulating evidence suggests that GIRK channels are modulated by cellular changes that are induced by several addictive substances. Methamphetamine and cocaine induce the expression of SNX27 [36], which regulates the surface expression of GIRK2 and GIRK3 subunits. GIRK channels composed of GIRK2 and GIRK3 in dopamine neurons in the VTA and heterologous expression systems are less sensitive to $G\beta\gamma$ subunits. The coupling efficiency of GABAB receptors to GIRK channels was found to be lower in dopamine neurons in the VTA than in GABA neurons in the VTA that express GIRK1, -2, and -3 mRNA [34]. Furthermore, cocaine administration reduced $GABA_B$ receptor-dependent GIRK currents in dopamine neurons in the VTA, which was prevented by pretreatment with the D_2-like receptor antagonist sulpiride [4]. The reduction correlated with a reduction of GIRK2-containing channels on the plasma membrane [4]. In GABA neurons in the VTA, a single administration of methamphetamine or cocaine depressed $GABA_B$ receptor-mediated GIRK currents [79]. After methamphetamine administration, phosphatase-dependent reductions of GIRK2 channels and $GABA_B$ receptors were observed on the plasma membrane, with a concomitant increase in intracellular GIRK2 channels and $GABA_B$ receptors. The phosphatase-dependent reduction of GIRK2 channels and $GABA_B$ receptors in plasma membrane correlated with the depression of $GABA_B$ receptor-mediated GIRK currents [79]. Furthermore, expressions of several genes: GIRK1-3, μ-opioid receptors, nociceptin/orphanin FQ receptors, NMDA receptor channel GluN2D subunit, Kir2.2 and Kir2.3, decreased even after 3 weeks following chronic administration of methamphetamine in a cDNA array system [102]. Additionally, repetitive administration of morphine and GHB increased the coupling efficiency of $GABA_B$ receptors to GIRK channels through the downregulation of RGS2 in VTA dopamine neurons [52]. Therefore, GIRK channels can be both directly and indirectly involved in the complex effects of addictive substances. GIRK channels may play an important role in the rewarding effects and neuroadaptations induced by addictive substances.

4. Involvement of the GIRK channel genes in addictive substance effects

4.1. Studies in animal models

Studies that have used mutant mice to investigate GIRK channel genes have implicated GIRK channels in the alterations of in vivo responses to addictive substances (Table 1). We demonstrated that abnormal GIRK channels are involved in the reduction of the antinociceptive effects of ethanol in *weaver* mutant mice, which have a missense mutation in the pore-forming region of the *GIRK2* gene [30,31], leading to a

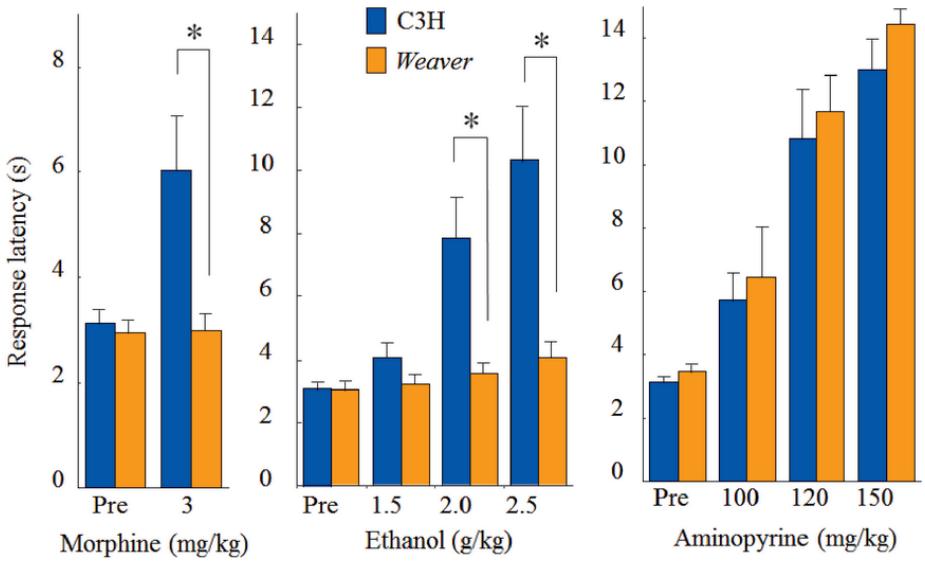

Figure 2: Reduced antinociceptive effects of morphine and ethanol in *weaver* mutant mice. Control: nonsteroidal anti-inflammatory drug aminopyrine, which dose-dependently exerts similar antinociceptive effects in C3H and *weaver* mice. $^{*}P < .05$, significant difference (repeated-measures ANOVA). (Kobayashi et al. [39]; Ikeda et al. [30]).

loss of K^+ selectivity with Na^+ influx and insensitivity to G-proteins and ethanol [39,46,88] (Figure 2). Although some of the physiological and behavior responses to ethanol, including hypnotic effects, hyperactivity, hypothermia, and bradycardia, were similar in wildtype and *weaver* mice [39], *weaver* mice exhibited various impairments, including neuronal cell death in the cerebellar cortex, substantia nigra, and pontine nuclei and abnormal channel properties [31,78, 80]. To further assess the in vivo roles of GIRK channels, GIRK-deficient mice without such anatomical anomalies have been generated. GIRK2 knockout mice also exhibited a marked reduction of ethanol-induced antinociceptive effects [9]. The activation of GIRK channels by ethanol is linked to analgesic effects. Moreover, GIRK2 knockout mice were less sensitive than wildtype mice to some of the acute effects of ethanol, including anxiolysis, habituated locomotor stimulation, and acute handling-induced convulsions [26]. Additionally, GIRK2 knockout mice exhibited a reduction of ethanol-induced conditioned taste aversion and conditioned place preference, suggesting that GIRK channels are involved in the rewarding and aversive motivational effects of ethanol [26]. Interestingly, GIRK3 knockout mice also exhibited a reduction of handling-induced convulsions after the administration of ethanol, pentobarbital, or zolpidem, indicating less severe withdrawal from ethanol and other hypnotics [48].

GIRK channels are also involved in the antinociceptive effects of various analgesics and pain thresholds (i.e., nociceptive sensitivity). *Weaver* mutant mice [30] (Figure 2) exhibited a reduction of the antinociceptive effects of opioids (i.e., morphine and the κ-opioid receptor agonist U50488), and GIRK2 knockout mice showed a reduction of the antinociceptive effects of the opioid receptor agonist morphine, M_2 muscarinic agonist oxotremorine, nicotinic acetylcholine receptor agonist nicotine, $GABA_B$ receptor agonist baclofen, α_2 adrenergic agonist clonidine, and cannabinoid agonist WIN 55,212-2 [9,62, 66]. GIRK2/GIRK3 double-knockout mice and GIRK3 knockout mice also showed a reduction of the antinociceptive effect of morphine [19,61]. The antinociceptive potency of morphine was decreased in GIRK2 knockout and GIRK2/GIRK3 double-knockout mice, but the maximal efficacy of the high dose was preserved [19,66]. By contrast, the antinociceptive effects of ketamine and nonsteroidal anti-inflammatory drug (NSAID) aminopyrine, which do not affect GIRK channels, remained intact in GIRK2 knockout mice and *weaver* mice, respectively [9,39] (Figure 2). These findings indicate that the coupling of various $G_{i/o}$ PCRs to GIRK channels contributes to the antinociceptive effects of many classes of analgesics. Additionally, the effects of nicotine appear to be mediated by the indirect activation of GIRK channels. Moreover, GIRK2 and GIRK3 knockout mice displayed low thermal hyperalgesia in the hot plate test [9,61], and GIRK2/GIRK3 double-knockout mice displayed hyperalgesia in the hot plate test and tail flick test [19]. In wildtype mice, females were more sensitive than males to the noxious heat stimulus in the tail flick test [66], and the antinociceptive effects of morphine were more potent in males than in females, but the maximal efficacy was similar in the dose-response curves [19]. The sex differences were abolished in GIRK2 knockout mice and GIRK2/GIRK3 double-knockout

mice [19]. Additionally, in GIRK2 knockout mice, the antinociceptive effects of ethanol, oxotremorine, baclofen, clonidine, and WIN 55,212-2 were markedly reduced or eliminated in males and reduced in females [9]. Therefore, GIRK channels may play a crucial role in pain perception and the antinociceptive effects of several classes of analgesic drugs and contribute to sex differences in nociception.

GIRK2 and GIRK3 knockout mice exhibited decreased cocaine self-administration [68]. GIRK2/GIRK3 double-knockout exhibited a strong attenuation of morphine withdrawal signs, without an increase in LC neuron firing rate in brain slices (i.e., the loss of an electrophysiological hallmark of opioid withdrawal) [19].

Pravetoni and Wickman [83] reported that although GIRK1 knockout mice, GIRK2 knockout mice, and GIRK3 knockout mice were able to learn an operant task using food as the reinforcing agent, within-session progressive-ratio responding revealed an increase in lever press behavior in GIRK2 knockout mice and, to a lesser extent, in GIRK1 knockout mice. Moreover, Cooper et al. [17] reported that mice with trisomy of the *KCNJ6* gene that encodes the GIRK2 channel subunit exhibited deficits in hippocampal-dependent learning and memory in the fear-conditioning paradigm and altered responses to the availability of sucrose in the sucrose preference test, which may be related to the altered function of reward mechanisms.

GIRK-deficient mice are very useful for evaluating the behavioral, electrophysiological, and cellular functions of GIRK channels. GIRK channels are hypothesized to play an important role in the pharmacological effects of various addictive substances, including ethanol, opioids, cannabinoids, hypnotics, GHB, GABAB receptor agonists, nicotine, and cocaine. However, in addition to a simple loss of GIRK channel signaling, GIRK knockout mice have been suggested to present secondary neuroadaptations, such as D_1 receptor-dependent basal hyperactivity in GIRK2 knockout mice [3] and elevated glutamatergic neurotransmission in dopamine neurons in the VTA and medium spiny neurons in the NAc in GIRK1 and GIRK2 knockout mice, with elevated synaptic densities of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptors [3]. More selective genetic and pharmacological approaches will clarify the functional roles of GIRK channels.

4.2. Studies in humans

Opioids, such as morphine, fentanyl, and buprenorphine, are widely used to relieve severe pain. The analgesic dosages that are required to achieve satisfactory pain control are well known to vary widely among individual subjects [27]. Although individual differences in the sensitivity to analgesics are thought to be attributable to both genetic and environmental factors, the relative influence of each of these various factors remains largely unknown [18]. We previously showed that differences in the genetic sequence of GIRK2 subunit were associated with analgesic requirements in patients who underwent major open abdominal surgery [75] (Figure 3). A total of nine single-nucleotide polymorphisms (SNPs) were identified in the entire exon, 5′ flanking region, and exon-intron boundary region of the *KCNJ6* gene that encodes GIRK2. G-1250A and A1032G were selected for the association study by considering the linkage disequilibrium structure, allele frequencies of the SNPs, and expected impact on gene function. Carriers of the A/A genotype in the A1032G SNP or -1250G/1032A haplotype required rescue analgesics more often than other genotypes and haplotypes and tended to require higher doses of rescue analgesics converted to equivalent oral morphine doses for all opioids and NSAIDs used during the first 24 h postoperative period, especially in females. Additionally, *KCNJ6* gene expression levels in the 1032A/A subjects were significantly decreased compared with the 1032A/G and 1032G/G subjects in a real-time quantitative polymerase chain reaction (PCR) analysis that used human anterior cingulate cortex tissues, suggesting a decrease in GIRK2 subunit expression and a decrease in GIRK channels. Altogether, the 1032A/A subjects felt more pain and required more analgesics, likely because of lower *KCNJ6* gene expression levels and consequently lower pain thresholds or insufficient analgesic effects. These findings provide valuable information for determining the analgesic doses required to achieve satisfactory pain control.

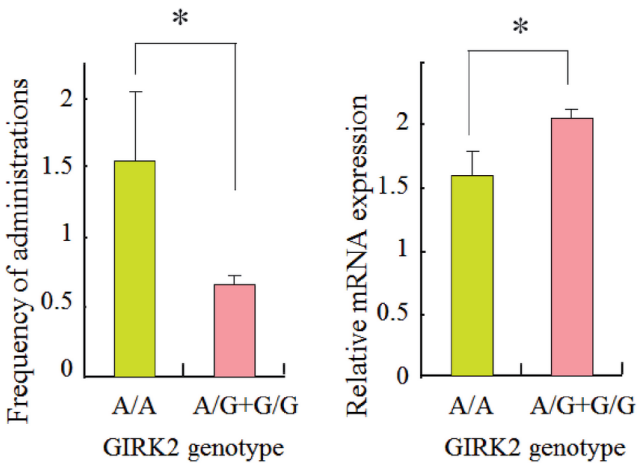

Figure 3: Associations between GIRK2 polymorphism and analgesic requirements and relative mRNA expression. The A/A genotype showed significantly more frequent analgesic administration during the 24 h postoperative period (left) and lower *KCNJ6* gene expression levels than the A/A genotype and combined A/G and G/G genotypes (A/G+G/G) in the A1032G SNP in the real-time quantitative PCR analysis that used human brain tissues (right). $^*P < .05$, significant difference (Student's t-test). (Nishizawa et al. [75]).

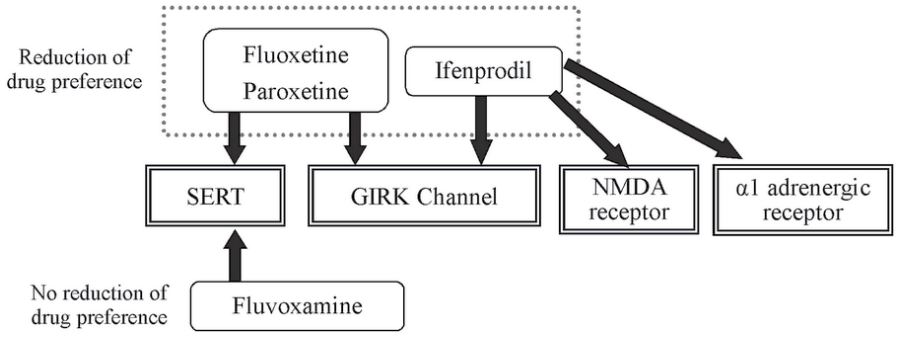

Figure 4: Schematic illustration of the effects of candidate medications on drug preference and their target molecules. Methamphetamine-induced conditioned place preference in mice was reduced by fluoxetine and paroxetine but not fluvoxamine, although these medications similarly block the serotonin transporter (SERT). Ifenprodil was shown to suppress the rewarding effects of morphine [60]. Solid arrows: inhibitory effects.

Further studies of *KCNJ6* gene polymorphisms may clarify individual differences in pain control.

5. Therapeutic effects of GIRK channel inhibition on substance dependence

5.1. Animal studies

The selective serotonin reuptake inhibitor (SSRI) antidepressants fluoxetine and paroxetine but not fluvoxamine inhibited GIRK channels in the *Xenopus* oocyte expression system [42,43,45] (Figure 4). Pretreatment with fluoxetine or paroxetine decreased methamphetamine-induced conditioned place preference in mice, whereas pretreatment with fluvoxamine did not affect it [91,92] (Figure 4). The results suggest that fluoxetine and paroxetine may be useful for treating methamphetamine dependence. Furthermore, ifenprodil, which is a well-known antagonist of the α_1 adrenergic receptor and GluN2B subunit-containing NMDA receptor, also inhibited GIRK channels [44] and attenuated methamphetamine-induced conditioned place preference [67]. Common molecules may be involved in these suppressive effects on methamphetamine-induced conditioned place preference. Psychostimulants, such as cocaine and amphetamines, increase the extracellular levels of dopamine, norepinephrine, and serotonin, and their subjective effects are similar [93]. GIRK knockout mice exhibited a reduction of cocaine self-administration [68]. The inhibition of GIRK channels may be an effective treatment strategy for psychostimulant addiction. Moreover, ifenprodil also inhibited GIRK currents induced by opioid receptor activation [44]. Ifenprodil was shown to suppress morphine-induced conditioned place preference [60]. Fluoxetine, paroxetine, and ifenprodil all inhibited ethanol-induced GIRK currents [42,44,45]. GIRK2 knockout mice were less sensitive than wildtype mice to some of the acute effects of ethanol, including anxiolysis and handling-induced convulsions [10]. GIRK3 knockout mice exhibited a reduction of handling-induced convulsions after ethanol administration [48]. Fluoxetine and ifenprodil reduced the anxiolytic effects of ethanol [22] and ethanol withdrawal signs, including convulsions [70], respectively. Altogether, agents that act as GIRK channel inhibitors might suppress the effects of addictive substances.

5.2. Studies in humans

Ethanol directly activates GIRK channels. GIRK knockout mouse studies have suggested that GIRK channels are involved in some ethanol-related behaviors [26], providing support for the therapeutic potential of GIRK channel inhibition for treating individuals with alcoholism. Retrospective studies have demonstrated the possible pharmacotherapeutic effects of inhibiting GIRK channels in individuals with alcoholism. We showed that GIRK channel inhibition with paroxetine, sertraline, ifenprodil, and chlorpromazine improved the lack of negative expectancy for alcohol drinking (i.e., a component of relapse risk) in outpatients with alcohol dependence [77]. Because environmental factors during the study and the time of initiation of drug treatment cannot be well controlled in outpatients, we secondly examined the influence of GIRK channel inhibition on relapse risk in Japanese alcohol-dependent inpatients while controlling environmental factors and the drug treatment schedule [89]. The results of the second study in inpatients suggested that GIRK channel inhibition improved the positive expectancy for alcohol, a component of relapse risk (Figure 5). Although the first study in outpatients [77] reported that GIRK channel inhibition improved the lack of negative expectancy for alcohol, both previous studies in outpatients and inpatients showed that GIRK channel inhibition may be useful for improving the maladaptive expectancy of the effect of alcohol. Altogether, the results from animal and human studies suggest that GIRK channel inhibition may be an effective treatment strategy for substance dependence.

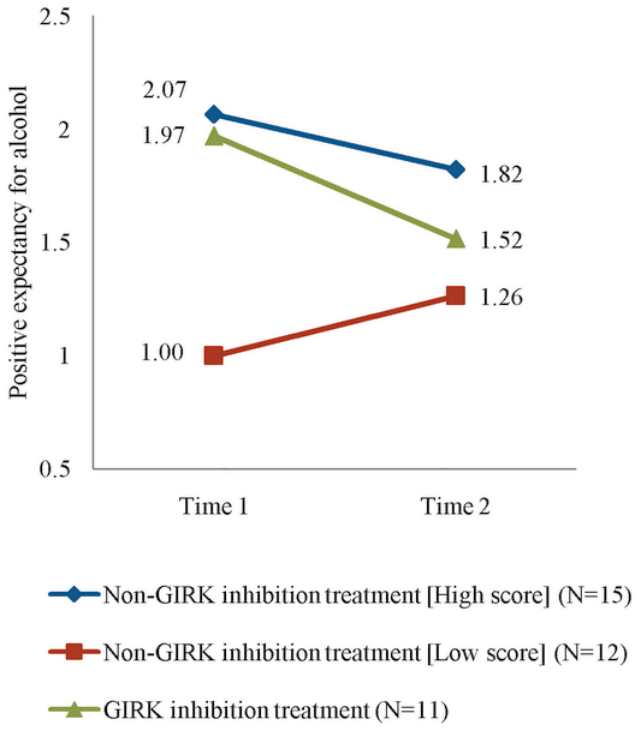

Figure 5: Effects of GIRK channel inhibitors on alcohol relapse risk. GIRK channel inhibitors (e.g., paroxetine, sertraline, and chlorpromazine) reduce the positive expectancy for alcohol, a component of relapse risk, in inpatients with alcohol dependence. Because of the difference between groups in positive expectancy score on the Alcohol Relapse Risk Scale at Time 1, the non-GIRK inhibition treatment group was divided by a median of positive expectancy score at Time 1 into a high-score group and low-score group, and a comparison was made between these groups and the GIRK inhibition treatment group in positive expectancy score. Time 1: 2 weeks after hospitalization. Time 2: 45–60 days after Time 1. (Sugaya et al. [89]).

Nonetheless, some problems in these previous studies in humans have been noted. First, the patients in these studies took a wide range of drugs with GIRK channel inhibition properties, including anxiolytics, antidepressants, and antipsychotics. Second, these previous studies had retrospective designs. Therefore, a prospective randomized controlled study may be useful for investigating the effects of GIRK channel inhibitors on relapse prevention in patients with substance dependence.

6. Conclusion

The available treatments for substance addiction are currently insufficient. A better understanding of the neurobiology of substance addiction may lead to the discovery of new therapeutic strategies. GIRK channels are involved in the rewarding and neuroadaptive effects induced by addictive substances. Studies on GIRK mutant and knockout mice have suggested the involvement of GIRK channels in the mechanisms that underlie the effects of addictive substances. Animal and human studies have demonstrated the possible therapeutic effects of GIRK channel inhibitors for the treatment of methamphetamine dependence and alcoholism, respectively. Selective GIRK channel inhibitors may be useful for treating substance dependence. The chemical structures of pharmacological agents that inhibit GIRK channels may provide insights into the development of novel drugs for the treatment of substance dependence.

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