Ghrelin/GHS-R1As and Stimulants

Subjects: Substance Abuse

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Psychostimulants include a number of synthetic and natural compounds. The most prevalent and harmful (causing health damage, violence, death, etc.) are traditionally considered to be cocaine, amphetamine, and its derivative methamphetamine. In Europe, nearly 3 million young adults aged 15–35 (2.4% of this age population) are estimated to have used cocaine and about 1.5 million are estimated to have used amphetamines (1.2%) in the past year, and the trend of the stimulant use is increasing. Concerning their pharmacological effects, stimulants directly increase the dopamine concentrations in the NAC, mainly through axonal membrane monoamine-dopamine transporters (MATs) manipulation. Cocaine acts as short-term monoamine reuptake inhibitor, while amphetamines promote reverse transport (efflux) through MATs and also induce synaptic vesicle depletion (the blockage of VMATs), thus causing a massive prolonged accumbens dopamine increase. Psychostimulants show stimulatory and sympathomimetic effects and induce a loss of appetite. Food restriction (FR) increases stimulant (amphetamine, D1 agonist A77636) consumption and signs of reward, and increases amphetamine/cocaine intake and the reinstatement of drug-seeking behavior. Thus, the relationship between ghrelin/GHS-R1As and the pro-addictive effects of stimulants has been explored in a considerable number of preclinical studies and two clinical studies so far.

ghrelin signaling growth hormone secretagogue receptor type A (GHS-R1A)

1. Preclinical Studies

The results of experiments in rodents suggest that central ghrelin signaling plays an important role in the reinforcing effects of stimulants. The administration of stimulants increased peripheral ghrelin availability. The single intraperitoneal administration of either methamphetamine or 3,4-methylendioxy-methamphetamine (MDMA, “ecstasy”) significantly increased total ghrelin blood levels in rats [1][2]. Another study described a positive correlation between total ghrelin serum levels and increased cue-induced cocaine-seeking activity in rats [3]. A recent elaborated investigation showed that the intravenous self-administration (IVSA) and extinction/anticipation of cocaine in rats significantly increased both acyl- and des-acyl ghrelin blood levels, and the cocaine self-administration experience was associated with enhanced responses of acyl- and des-acyl ghrelin to cocaine [4][5]. Thus, the elevation of ghrelin blood levels by cocaine seems to play a critical role in the maintenance of cocaine IVSA and cocaine-seeking motivated by cocaine-conditioned stimuli. The achievement of cocaine IVSA also significantly up-regulated the GHS-R1A mRNA expression in the rat’s VTA [5]. Furthermore, it was found that blockade of peripheral adrenergic ß1 receptors by hydrophilic atenolol potently attenuated the elevation in circulating ghrelin induced by cocaine [5]; atenolol pretreatment also inhibited the IVSA of cocaine [5]. Stomach ghrelin secretion is modulated by the sympathetic nervous system and is increased by ß-sympathomimetics [6].

The observed adrenergic—ghrelinergic interactions require further study. As previously mentioned, the GHS-R1A interacts with the sigma-1 receptors in CNS neurons [7], and cocaine mediates many of its central effects via the sigma-1 receptors through molecular interactions with dopamine and possibly other metabotropic G-protein coupled receptors. Cocaine increases colocalisation of sigma-1R and GHS-R1A at the cell surface (in vitro), and it was indicated that cocaine's effects on the GHS-R1A were mediated by the sigma-1R [7]. Thus these relationships should be further investigated. Pretreatment with external acyl-ghrelin before the administration of cocaine significantly augmented the cocaine-induced locomotor hyperactivity [8]. Furthermore, repeated systemic acyl-ghrelin administration before acute cocaine exposure augmented the cocaine-induced hyperlocomotion [9]. Ghrelin's enhancement of psychostimulants' functions may be caused by its direct action on the mesolimbic dopamine function or may reflect an indirect action of ghrelin on the glucocorticoid pathways [10][11]. It was suggested that NAC-core (rather than NAC-shell) is the neuronal substrate mediating the expression of behavioral activation and drug-induced sensitization [12][13]. It was found that the microinjection of acyl-ghrelin directly into the NAC core significantly sensitized the cocaine- and amphetamine-induced hyperlocomotion, which was abolished by the co-microinjection of GHS-R1A antagonist (D-Lys3-GHRP6), while microinjected ghrelin alone did not change the rats' behavior [14][15]. Furthermore, previous subchronic exposure to amphetamine in rats produced behavioral sensitization to the microinjection (NAC-core) of acyl-ghrelin when administered together with dopamine D1 agonist (SKF81297), indicating the important interaction of ghrelin with D1 receptors in sensitized locomotion [15].

Similarly to food restriction, which increased ghrelin blood levels (see above), acyl-ghrelin administration also increased the reinforcing effects of stimulants. Pretreatment with systemic [16] as well as central acyl-ghrelin (into the VTA and/or NAC) [17][18] together with intraperitoneal cocaine during a place-conditioning procedure increased cocaine-conditioned place preference (CPP) development in rats, which was attenuated when GHS-R1A antagonist (JMV2959) was centrally coadministered with ghrelin [18]. Another study indicated that the GHS-R1A's effects on reward are independent from peripheral acyl-ghrelin binding [19], because the administration of acyl-ghrelin antibodies, which blocked the peripheral acyl-ghrelin access to the CNS, did not influence the development of cocaine-conditioned place preference (CPP) in mice, even though the mice weight gain was significantly blunted. However, both weight gain and cocaine rewarding effects (CPP) were blocked by the direct GHS-R1A antagonist (JMV2959). These results indicate that the central GHS-R1As that regulate weight-gain behavior are physiologically dependent on peripheral acyl-ghrelin activation, whereas hedonic reward due to cocaine administration does not appear to require peripheral acyl-ghrelin [19]. It was further found that ghrelinergic and dopaminergic signaling interact in the neuronal control of motivation with other drug rewards (e.g., ethanol). Low doses of acyl-ghrelin (2.5 nmol intraperitoneally) and also ghrelin microinjection into the VTA augmented cocaine-induced voluntary alcohol consumption in rats; conversely, a high ghrelin dose (10 nmol) suppressed it [20]. This seems in accordance with observations recorded when only high doses of ghrelin (including 10 nmol) elicited anxiogenic responses [21][22].

A number of studies have used the pharmacological or genetic suppression of the GHS-R1A to further support the crucial role of central ghrelin signaling in the stimulant's reinforcement and motivation effects. Initially, it was noted in mice that pretreatment with the systemic GHS-R1A antagonist (JMV2959) attenuated both intraperitoneal cocaine and methamphetamine-induced hyperlocomotion, the expression of drug-induced CPP, and the

accumbens dopamine efflux [23]. Furthermore, sub-chronic GHS-R1A blocking by repeated JMV2959 administration attenuated the ability of acute amphetamine to stimulate locomotion in mice [24], while there was no effect of sub-chronic JMV2959 treatment on locomotor activity per se or on the expression of the GHS-R1A gene in the VTA or the NAC compared with vehicle treatment. In another study, the repeated systemic administration of JMV2959 together with cocaine, as well as genetic ablation of the GHS-R1A, significantly attenuated cocaine-induced locomotor sensitization in rats, which suggests that GHS-R1A activity is required for the induction of locomotor sensitization to cocaine [25]. Furthermore, pretreatment with JMV2959 significantly reduced the expression of methamphetamine-conditioned place preference (CPP) in rats, and the simultaneous repeated administration of JMV2959 with methamphetamine during conditioning significantly reduced methamphetamine-CPP development [26], which indicates, that ghrelin antagonism reduced the methamphetamine reward as well as the signs of craving/desire for methamphetamine.

The first intravenous self-administration (IVSA) study found that the blockading of the GHS-R1A by JMV2959 significantly reduced the spontaneous maintenance methamphetamine intake and reduced or prevented the tendency to relapse, as tested in rats on the 12th day of the abstinence period [26]. A recent innovative and comprehensive study noted that JMV2959 pretreatment dose-dependently inhibited cocaine-IVSA, cocaine-seeking, and reinstatement of cocaine-seeking triggered by cocaine in rats [5]. Furthermore, JMV2959 pretreatment also inhibited the brain stimulation reward (BSR) and cocaine-potentiated BSR maintained by optogenetic stimulation of the VTA dopamine neurons in DAT-Cre mice [5].

A recent microdialysis study in rats explored in detail the mesoaccumbal ghrelin—dopamine interactions participating in the stimulants' reinforcing effects [27]. Previously, it was suggested that not only the dopamine release in the NAC shell, but the concomitant dopamine release in the VTA and NAC shell might represent a common underlying characteristic induced by various reinforcing stimuli, including natural rewards such as food/water [28], drugs of abuse [29][30][31] and the orexigenic peptide ghrelin (included due to later results) [27]. When acyl-ghrelin was infused into the third ventricle (i.c.v.), the dopamine levels increased in both the (anterior) VTA and the NAC shell, while a systemic pretreatment with the JMV2959 fully blocked the ability of ghrelin (i.c.v.) to increase the dopamine levels in both areas. Furthermore, the dopamine D1 receptor antagonist (SCH23390) administered systemic or centrally into the VTA and NAC shell fully blocked the ability of ghrelin (i.c.v.) to increase locomotor activity in mice [27]. Interestingly, when the authors tested amphetamine's mesoaccumbal effects, systemic JMV2959 pretreatment reduced (rather than fully blocked) the systemic amphetamine-induced concomitant dopamine release in both the VTA and the NAC shell, without altering dopamine levels per se [27]. These results further indicated that the ghrelin/GHS-R1A signaling significantly modulates the ability of stimulants to activate the mesoaccumbal dopamine pathway, which is associated with their reinforcing effects.

2. Clinical Studies

Thus far, only two clinical studies have investigated the possible role of genetic variability of ghrelin signaling system in the (meth)amphetamine dependence, the severity of use, and/or the emotional problems associated with the use of the drugs. An initial study [32] showed no association between pre-proghrelin gene (GHRL) variations

and susceptibility to the development of methamphetamine dependence in a sample of the Korean population but found a significant correlation between carrying the GHRL single nucleotide (Leu72Met) polymorphism and emotional problems, such as depression or anxiety, which are associated with drug addiction. The second clinical study [33] found that the single nucleotide polymorphism (SNP) located on the ghrelin receptor gene GHSR (rs2948694) seemed to be associated with amphetamine dependence, because it was significantly more common among Swedish amphetamine-dependent individuals when compared to healthy controls. No significant differences were found in the pre-proghrelin gene GHRL SNP between the amphetamine-dependent and healthy populations; however, amphetamine-addicted patients carrying the minor allele of the GHRL SNP (rs4684677) showed significantly higher amphetamine-addiction severity scores (Addiction severity index, ASI).

In several clinical articles, ghrelin blood levels were analysed in connection with stimulant administration under miscellaneous conditions. A small study found no significant effects of intravenous cocaine on total or acyl-ghrelin blood levels in experienced cocaine users (the satiety status not mentioned) [34]. One study measured the ghrelin changes in children with attention deficit hyperactivity disorder (ADHD) before and after two month of treatment with methyphenidate, and the fasting total ghrelin blood levels were significantly increased; no differences were found between the baseline total ghrelin levels of ADHD patients and the corresponding healthy control group [35].

3. Summary

The relevant reviewed results are summarized in **Table 1**. The existing preclinical experiments in rodents suggest that ghrelin/GHS-R1A signaling is significantly involved in the mesoaccumbal effects of stimulants. Furthermore, acyl-ghrelin administration can enhance several behavioral correlates of stimulant abuse. In addition, GHS-R1A antagonism can attenuate the association between stimulants' effects and the surroundings (CPP), and it can significantly reduce spontaneous stimulant (methamphetamine, cocaine) intake, drug seeking, and the reinstatement of drug-seeking behavior. Moreover, the possible involvement of ghrelin/GHS-R1A interactions with other neural systems (e.g., the adrenergic system, sigma-1 receptor) in stimulants' reinforcing effects has been discussed in several studies. Scarce human genetic data indicated an association between a single nucleotide polymorphism in the GHS-R1A gene and amphetamine dependence. Existing human studies have measured ghrelin blood changes in connection with stimulant administration as additional information within research focused on other topics; therefore, further clinical studies aimed specifically at the problem of ghrelin and stimulants are warranted. The involvement of ghrelin/GHS-R1A signaling in stimulant reinforcement proaddictive effects seems to be clearly implied; however, additional preclinical and (mainly) clinical studies are required to further elucidate the relationship between ghrelin and stimulants and to test the relevance of the possible future use of ghrelin/GHS-R1A antagonism in the prevention and treatment of stimulant addiction.

Table 1. Ghrelin/GHS-R1As and stimulants—preclinical and clinical studies overview.

PRECLINICAL STUDIES—STIMULANTS			
Study Design	**Results**	**Drug of Abuse**	**Reference**

Drug effect on the ghrelin blood concentrations	Total ghrelin blood levels were increased following drug single dose administration (in rats)	Methamphetamine	Crowley et al. [1]
		Methamphetamine and high doses of MDMA	Kobeissy et al. [2]
	Drug IVSA and extinction/anticipation significantly increased both acyl- and des-acyl ghrelin blood levels (in rats) Blockade of peripheral adrenergic ß1 receptors by atenolol attenuated the elevation in circulating ghrelin induced by cocaine	Cocaine	You et al. [4] [5]
	Total ghrelin blood levels positively correlated with cue-induced cocaine-seeking behavior (in rats)	Cocaine (cue)	Tessari et al. [3]
Acyl-ghrelin administration effects	Systemic pretreatment with acute ghrelin augmented/sensitized acute cocaine-induced hyperlocomotion (in rats)	Cocaine	Wellman et al. [8]
	Systemic pretreatment with repeated ghrelin augmented/sensitized acute cocaine-induced hyperlocomotion (in rats)	Cocaine	Wellman et al. [9]
	Central (NAC-core) pretreatment with ghrelin augmented/sensitized acute drug-induced hyperlocomotion (in rats)	Cocaine	[14]
	Central (NAC-core) pretreatment with ghrelin augmented acute drug-induced hyperlocomotion and in co-administration with D1-agonist also the drug-induced behavioral senzitization (in rats)	Amphetamine	Jang et al. [15]
	Systemic pretreatment with ghrelin increased development of drug-induced place preference (CPP) (in rats)	Cocaine	Davis et al. [16]
	Central (VTA) pretreatment with ghrelin increased development of drug-induced place preference (CPP) (in rats)	Cocaine	Schuette et al. [17]
	Central (VTA and NAC) pretreatment with ghrelin increased development of drug-CPP and this was attenuated when JMV2959 was centrally co-administered with ghrelin (in rats)	Cocaine	Dunn et al. [18]
	Systemic and central (VTA) ghrelin augments cocaine-enhanced alcohol consumption (in rats)	Cocaine (alcohol)	Cepko et al. [20]

	Systemic JMV2959 pretreatment decreased the drug-induced hyperlocomotion (in mice)	Cocaine and Amphetamine	Jerlhag et al. [23]
	Systemic JMV2959 pretreatment decreases the expression of drug-induced conditioned place preference expression (CPP) and accumbens dopamine release (in mice)	Cocaine and Amphetamine	Jerlhag et al. [23]
	Repeated systemic JMV2959 pretreatment decreases the drug-induced hyperlocomotion (in mice)	Amphetamine	Suchankova et al. [24]
	Systemic JMV2959 reduced systemic drug-induced increase of dopamine in the NAC-shell and in the VTA (in rats)	Amphetamine	Edvardsson et al. [27]
	Systemic repeated JMV2959 together with cocaine decreased the drug-induced behavioral sensitization (in rats)	Cocaine	Clifford et al. [25]
GHS-R1A antagonist administration	Systemic JMV2959 pretreatment decreased the drug intravenous self-administration (IVSA) and drug-seeking behavior, plus the expression and also development of drug-induced conditioned place preference (CPP) (in rats)	Methamphetamine	Havlickova et al. [26]
	Systemic JMV2959 pretreatment decreased the drug-induced CPP, as well as the body weight gain. However, acyl-ghrelin antibodies administration attenuated the weight gain but not the cocaine-CPP, which indicated, that the GHS-R1A effects on reward are independent from peripheral acyl-ghrelin binding (in mice)	Cocaine	Wenthur et al. [19]
	Systemic JMV2959 pretreatment dose-dependently inhibited drug-IVSA, drug-seeking, and reinstatement of drug-seeking triggered by the drug (in rats)	Cocaine	You et al. [5]
	Systemic JMV2959 pretreatment inhibited the brain stimulation reward (BSR) and drug-potentiated BSR maintained by optogenetic stimulation of VTA dopamine (in mice)	Cocaine	You et al. [5]
GHS-R1A knockout animals	GHS-R1A gene knockouts show reduced drug-induced behavioral sensitization (rats)	Cocaine	Clifford et al. [25]
Ghrelin peptide gene (GHRL) knockout animals	GHRL gene knockouts showed reduced drug-induced hyperlocomotion as well as reduction of behavioral sensitization dopamine content in striatal dissections (30 min	Cocaine	Abizaid et al. [36]

	after cocaine) did not differ between GHRL knockouts and wild mice		
CLINICAL STUDIES—STIMULANTS			
Study Design	**Results**	**Drug of Abuse**	**Reference**
Genetic study	No association between pre-proghrelin gene (GHRL) variations and the drug dependence, but correlation between SNP on GHRL and emotional problems (depression, anxiety) was found (Korean population)	Methamphetamine	Yoon et al. [32]
	SNP located on the ghrelin receptor gene GHSR seemed associated with the drug dependence; no differences were found between drug-dependent and healthy participants in SNP on the pre-proghrelin gene (GHRL) (Swedish population)	Amphetamine	Suchankova et al. [33]
Ghrelin blood levels	In children with ADHD the total ghrelin blood levels were increased after two months treatment with the drug in comparison to basal pretreatment levels	Methylphenidate	Sahin et al. [35]
	No significant effect of intravenous drug administration in experienced cocaine users on the acyl- or total ghrelin blood levels was observed	Cocaine	Bouhlal et al. [34]

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