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Ghrelin amplifies the nicotine-induced dopamine release in the rat striatum

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ABSTRACT

The orexigenic peptide ghrelin plays a prominent role in the regulation of energy balance and in the mediation of reward mechanisms and reinforcement for addictive drugs, such as nicotine. Nicotine is the principal psychoactive component in tobacco, which is responsible for addiction and relapse of smokers. Nicotine activates the mesencephalic dopaminergic neurons *via* nicotinic acetylcholine receptors (nAchR). Ghrelin stimulates the dopaminergic neurons *via* growth hormone secretagogue receptors (GHS-R1A) in the ventral tegmental area and the substantia nigra pars compacta resulting in the release of dopamine in the ventral and dorsal striatum, respectively. In the present study an *in vitro* superfusion of rat striatal slices was performed, in order to investigate the direct action of ghrelin on the striatal dopamine release and the interaction of ghrelin with nicotine through this neurotransmitter release. Ghrelin increased significantly the dopamine release from the rat striatum following electrical stimulation. This stimulatory effect was reversed by both the selective nAchR antagonist mecamylamine and the selective GHS-R1A antagonist GHRP-6. Nicotine also increased significantly the dopamine release under the same conditions. This stimulatory effect was antagonized by mecamylamine, but not by GHRP-6. Ghrelin further stimulated the nicotine-induced dopamine release and this effect was abolished by mecamylamine and was partially inhibited by GHRP-6. The present results demonstrate that ghrelin stimulates directly the dopamine release and amplifies the nicotine-induced dopamine release in the rat striatum. We presume that striatal cholinergic interneurons also express GHS-R1A, through which ghrelin can amplify the nicotine-induced dopamine release in the striatum. This study provides further evidence of the impact of ghrelin on the mesolimbic and nigrostriatal dopaminergic pathways. It also suggests that ghrelin signaling may serve as a novel pharmacological target for treatment of addictive and neurodegenerative disorders.

1. Introduction

Ghrelin is a 28-amino acid orexigenic peptide, which was originally isolated from the rat stomach (Asakawa et al., 2001; Kojima et al., 1999). It acts on the growth hormone secretagogue receptor (GHS-R), which has two isoforms (GHS-R1A and GHS-R1B) (Guan et al., 1997). Activation of GHS-R1A plays role in the regulation of food intake and energy balance, as well as in reward, addiction, memory, arousal and neuroprotection (Ferrini et al., 2009). GHS-R1B may play role in the regulation/inhibition of GHS-R1A (Chan and Cheng, 2004).

Nicotine is the principal psychoactive component in tobacco, which is responsible for addiction and relapse of smokers (Wonnacott et al., 2005). Nicotine exerts its effects on the central nervous system (CNS) by the activation of nicotinic acetylcholine receptors (nAchRs) (Glennon and Dukat, 2000). The dopaminergic neurons of the mesencephalon express a variety of nAchR subtypes. These upon being activated by nicotine, promote a sustained increase in dopamine release in the target areas that is considered essential to the development of nicotine addiction (Balfour, 2004; Pidoplichko et al., 2004). Nicotine stimulates the dopamine release in rodent striatal slices *in vitro* (Giorguieff-Chesselet et al., 1979; Teng et al., 1997) and this effect can be inhibited by the non-selective, competitive nAchR antagonist mecamylamine (Haikala and Ahtee, 1988; Lecca et al., 2000).

Nicotine, infused directly into the dopaminergic target areas, elevates dopamine release from the nerve terminals and this effect is blocked by mecamylamine (Lecca et al., 2000). The principle dopaminergic pathways arise from the mediobasal hypothalamus (tuberoinfundibular pathway), ventral tegmental area (mesolimbic/mesocortical pathways) and the substantia nigra (nigrostriatal

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pathway) (Narayanan et al., 2010). The mesolimbic pathway originates from the ventral tegmental area (VTA) and innervates the amygdala and the ventral striatum (represented by the nucleus accumbens or NAcc). The mesocortical pathway also begins in the VTA and connects to the prefrontal cortex (Oades and Halliday, 1987). The mesolimbic pathway is involved in the control of motor behavior as well as in motivation, emotions and reward, whereas the mesocortical pathway is involved in the higher cognitive functions such as working memory, as well as in learning and reward (Janhunen and Ahtee, 2007). The nigrostriatal pathway projects from the substantia nigra pars compacta (SNc) to the dorsal striatum (consisting of caudate nucleus and putamen, CPu) (Janhunen and Ahtee, 2007). The nigrostriatal pathway is mainly involved in the control of posture and motor behavior, learning of motor habits and programs, as well as decision-making and reward (Balleine et al., 2007; Janhunen and Ahtee, 2007).

Ghrelin has a prominent impact on the cholinergic-dopaminergic reward link (Dickson et al., 2010; Disse et al., 2011; Jerlhag et al., 2007, 2008, 2009, 2012). This reward link encompasses the cholinergic afferent projection from the laterodorsal tegmental area (LDTg) to the VTA and the dopaminergic mesolimbic pathway projecting from the VTA to the NAcc (Blaha et al., 1996). There is growing body of evidence that this link mediates the reward and reinforcement for both natural and artificial rewards and addictive drugs, such as nicotine (Jerlhag et al., 2006; Lanca et al., 2000; Larsson and Engel, 2004; Pidoplichko et al., 2004). Ghrelin presumably, *via* activation of this reward link, increases the incentive value of motivated behaviors such as reward seeking (Jerlhag et al., 2012). Ghrelin has an impact on the dopaminergic nigrostriatal pathway, as well. It activates the dopaminergic neurons of SNc *via* GHS-R1A, which results in the increase of dopamine concentration in the dorsal striatum (Andrews et al., 2009). In addition, exogenous ghrelin administration decreased SNc dopaminergic cell loss and restricted striatal dopamine loss after 1-methyl-4-phenyl-1,2,5,6 tetrahydropyridine (MPTP) treatment in mice (Moon et al., 2009). This neuroprotective effect of ghrelin is mediated *via* the activation of UCP2-dependent mitochondrial mechanisms (Andrews et al., 2009). Thus, ghrelin plays an important role in the maintenance and protection of normal nigrostriatal dopamine function.

Recently published studies about ghrelin primarily focus on its action on the cholinergic-dopaminergic reward link and its interaction with addictive drugs. In these studies ghrelin was administered peripherally, intracerebroventricularly or locally, into the LDTg or the VTA link (Dickson et al., 2010; Disse et al., 2011; Jerlhag et al., 2007, 2008, 2009, 2012). These studies provide little evidence of the direct effect of ghrelin on the dopaminergic target areas. Therefore, the aim of the present study was to investigate the effects of ghrelin on the striatal dopamine release, *in vitro*. In the present experiments, ghrelin was administered locally into the striatum, alone or in combination with nicotine.

2. Materials and methods

Male Wistar rats weighing 150–250 g were sacrificed and *in vitro* superfusion method (Gaddum, 1953; Harsing and Vizi, 1984) was utilized to determine the effects of ghrelin and nicotine on striatal dopamine release. During the experiments every effort was made to limit the number of animals used, and to minimize animal suffering. The rats were decapitated and their brains were rapidly removed. The striatum was isolated and dissected in a Petri dish filled with ice-cold Krebs solution (composition: 113 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM NaHCO3, 11.5 mM glucose, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, pH 7.4; Reanal, Hungary). According to the Stereotaxic atlas of the rat brain (Pellegrino et al., 1979), after the following coordinates: rostro-caudal +4.0 to −1.0 mm, medio-lateral +1.0 to +5.0 mm, dorso-ventral +3.0 to +8.0 mm with the bregma taken as point of reference. The extracted striatum was cut with a McIlwain tissue chopper and slices of 300 μM were produced. The slices were incubated for 30 min in 8 ml of Krebs solution, submerged in a water bath at 37 °C and gassed through a single use needle with a mixture of 5% CO_2 and 95% O_2. During the incubation, 15 mM [3H]dopamine (PerkinElmer Inc., USA) with a specific activity of 14.0 Ci/mM was injected into the incubation medium. Two tritiated slices were transferred to each of the four cylindrical perspex chambers of the superfusion system (Experimetria Ltd., Hungary). A multichannel peristaltic pump (Gilson Minipuls 2) was used to maintain a constant superfusion rate of 300 μl/min. The superfusion was performed for 30 min to allow tissue equilibrium, then the samples were collected for 32 min in Eppendorf tubes by a multichannel fraction collector (Gilson FC 203B). 20 min prior to sample collecting, the slices were pretreated with 100 μM mecamylamine, a non-selective nAch receptor antagonist (Sigma–Aldrich Inc., USA) or with 1 μM [D-Lys3]-Growth Hormone Releasing Peptide-6 (GHRP-6), a selective GHS-R1A antagonist (Sigma–Aldrich Inc., USA). The antagonists were administered in equimolar doses with the agonists. 10 min prior to sample collecting, the slices were treated with 1 μM ghrelin (Bachem Inc., Switzerland), 100 μM nicotine (Bachem Inc., Switzerland) or combined (1 μM ghrelin + 100 μM nicotine). The dose selection of nicotine and ghrelin were made according to previous *in vitro* studies (Cruz et al., 2013; Grilli et al., 2008). Gold electrodes were attached to both halves of the superfusion chambers and connected to an ST-02 electrical stimulator (Experimetria Ltd., Hungary). Two minutes after the sample collection had started, electrical stimulation consisting of square-wave impulses (total duration: 2 min, voltage: 100 V, pulse length: 5 ms, frequency: 10 Hz) was delivered to each of the four chambers. The remnants of the superfused brain slices were solubilized in 200 ml of Krebs solution, using an ultrasonic homogenizer (Branson Sonifier 250). The radioactivity in the fractions and the homogenized tissue samples was measured with a liquid scintillation spectrometer (Tri-carb 2100TR, Packard, USA) after the addition of 3 ml of scintillation fluid (Ultima Gold, Perkin Elmer Inc., USA). The fractional [3H]dopamine release was calculated as a percentage of the radioactivity present in the collected sample relative to the total radioactivity of the corresponding tissue. Statistical analysis of the results was performed by analysis of variance (ANOVA, Statistica v5.0, StatSoft Inc.). Differences between samples were determined by two-way ANOVA with repeated measures, and a probability level of 0.05 or less was accepted as indicating a statistically significant difference.

3. Results

Ghrelin increased significantly the fractional [3H]dopamine release from rat striatum slices following electrical stimulation [$F_{14min}(1,10) = 24.6409$, $p < 0.05$ for ghrelin *versus* control]. This increasing effect was abolished by both mecamylamine [$F_{14min}(1,10) = 22.0428$, $p < 0.05$ for ghrelin + mecamylamine *versus* ghrelin] and GHRP-6 [$F_{14min}(1,10) = 21.0244$, $p < 0.05$ for ghrelin + GHRP-6 *versus* ghrelin] (Fig. 1).

Nicotine enhanced significantly the fractional [3H]dopamine release from rat striatum slices following electrical stimulation [$F_{14min}(1,10) = 36.9874$, $p < 0.05$ for nicotine *versus* control]. This enhancing effect was reversed completely by mecamylamine [$F_{14min}(1,10) = 54.4473$, $p < 0.05$ for nicotine + mecamylamine *versus* nicotine], but it was not influenced considerably by GHRP-6 [$F_{14min}(1,10) = 10.1076$, $p = 0.3384$ for nicotine + GHRP-6 *versus* nicotine] (Fig. 2).