Activation of the amylin pathway modulates cocaine-induced activation of the mesolimbic dopamine system in male mice

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ABSTRACT

Besides food intake reduction, activation of the amylin pathway by salmon calcitonin (sCT), an amylin and calcitonin receptor agonist, inhibits alcohol-mediated behaviors in rodents. This involves brain areas processing reward, i.e. the laterodorsal (LDTg), ventral tegmental area (VTA) and nucleus accumbens (NAc). However, the effects of stimulation of the amylin pathway on behaviors caused by cocaine and the brain areas involved in these processes have not yet been investigated. We therefore explored in male mice, the effects of systemic administration of sCT on cocaine-induced locomotor stimulation, dopamine release in the NAc and cocaine reward, as well as reward-dependent memory of cocaine, in the conditioned place preference (CPP) paradigm. Moreover, the outcome of systemic sCT and cocaine co-administration for five days on locomotor activity was investigated. Lastly, the impact of sCT infusions into the LDTg, VTA, NAc shell or core on cocaine-evoked locomotor stimulation was explored. We found that sCT attenuated cocaine-induced locomotor stimulation and accumbal dopamine release, without altering cocaine’s rewarding properties or reward-dependent memory retrieval in the CPP paradigm. Five days of cocaine administration caused locomotor stimulation in mice pre-treated with vehicle, but not with sCT. In mice infused with vehicle into the aforementioned reward-related areas, cocaine caused locomotor stimulation, a response that was not evident following sCT infusions. The current findings suggest a novel role for the amylinergic pathway as regulator of cocaine-evoked activation of the mesolimbic dopamine system, opening the way for the investigation of the amylin signalling in the modulation of other drugs of abuse.

1. Introduction

Emerging data suggest common neurochemical mechanisms behind homeostatic feeding and reward caused by drugs of abuse (for review (Blanco-Gandía et al., 2020)), where gut-brain peptides appear to be important modulators (for review (Jerlhag, 2019)). Indeed, the orexigenic peptide ghrelin enhances natural and artificial reward-related behaviors (Dunn et al., 2019; Egecioglu et al., 2010; Leggio et al., 2014), whereas the anorexigenic glucagon-like peptide-1 (GLP-1) or neuropeptide U (NMU) attenuate such behaviors (Dickson et al., 2012; Erreger et al., 2012; Vallof et al., 2016). The common denominator of this regulation appears to be the ability of the aforementioned gut-brain peptides to act on brain structures responsible for reward processing, like the laterodorsal tegmental area (LDTg), the ventral tegmental area (VTA) and the nucleus accumbens (NAc) (Jerlhag et al., 2007; Vallof et al., 2019). The NAc is composed by two distinct subregions, namely shell and core; these two subregions show increased dopamine release after reward stimuli and differentially regulate reward-related behaviors (for review (Di Chiara, 2002)).

Amylin, a gut-brain peptide produced in the pancreas has been well established to decrease food intake and body weight (for review (Hay et al., 2015)). It exerts its anorexigenic properties through activation of amylin receptors (AMYRs) in the central nervous system (Mollet et al., 2004). The AMYR is composed by the core calcitonin receptor (CTR) and one of three receptor activity-modifying proteins (RAMPs), thus creating the AMYR1, 2 or 3 respectively (Bower and Hay, 2016). Beyond homeostatic food intake regulation, amylin signalling is implicated in the attenuation of natural rewards, like sexual behavior (Clementi et al., 1999) and palatable foods (Mietlicki-Baase et al., 2015). Specifically, reduction of palatable sucrose solution intake by amylin involves areas such as the VTA (Mietlicki-Baase et al., 2013) and the LDTg (Reiner et al., 2017). However, the involvement of amylin signalling in the
regulation of artificial rewards, like drugs of abuse, was only recently identified. On that note, activation of the amylin pathway, by administration of the AMYR (and CTR) agonist salmon calcitonin (sCT), attenuates both acute and chronic alcohol-mediated behaviors in rodents (Kalafateli et al., 2019a; Kalafateli et al., 2019b). In addition, chronic high alcohol consumption alters the gene expression profile of the components of the AMYR in the NAc of male rats (Kalafateli et al., 2019a). Moreover, AM1213, another amylin receptor analogue selective for the AMYR, also attenuates chronic alcohol-mediated behaviors in rats (Kalafateli et al., 2020b), demonstrating the importance of amylin signalling in the regulation of alcohol-mediated reward. Nevertheless, the role of amylinergic pathways in regulating reward induced by other drugs of abuse which activate the mesolimbic dopamine system, like cocaine (Kalivas and Duffy, 1990), remains unknown to date. We therefore investigated the effects of systemic administration sCT, on cocaine-induced behaviors in male mice and attempted to identify brain areas involved in modulating cocaine-induced locomotor stimulation. We firstly examined the effects of systemic sCT administration on the ability of cocaine to activate the mesolimbic dopamine system as reflected by cocaine-induced locomotor stimulation and accumbal dopamine release. We also investigated the effects of sCT on cocaine reward and reward-dependent memory retrieval in the conditioned place preference (CPP) paradigm in male mice. Furthermore, the influence of systemic sCT and cocaine co-administration for five days on locomotor activity in male mice was investigated. Lastly, we explored the effects of locally infused sCT in the areas of the LD tg, VTA, NAc shell or core on cocaine-induced locomotor stimulation in male mice.

2. Materials and methods

2.1. Animals

For the present experiments, adult post pubertal, age-matched male NMRI mice (8–12 weeks old and 25–30 g body weight; Charles River; Sulzfeld, Germany) were used. This strain was used as it displays robust locomotor stimulation, accumbal dopamine release and expression of CPP in response to cocaine and other addictive drugs (Jerlhag et al., 2010; Kalafateli et al., 2019b). Moreover, sCT attenuates alcohol-induced reward in this strain (Kalafateli et al., 2019b), and the latter is responsive to the reduction of cocaine and alcohol reward caused by other gut-brain peptides (Egecioglu et al., 2013; Vallot et al., 2015). The mice were group housed, fed ad libitum and maintained at a 12/12 hour light/dark cycle and at 20 °C with 50% humidity.

All experiments were approved by the Swedish Ethical Committee on Animal Research in Gothenburg. Each experiment used an independent set of mice. We follow the 3Rs (refine, reduce, replace) according to the EU Directive 2010/63/EU for animal experimentation, by using the number of animals that prior power analysis has confirmed as necessary and by using the same group of animals when the experimental setup allows. We have followed and adapted to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) and PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines.

2.2. Drugs

For systemic administration of sCT (Tocris Bioscience; Bristol, UK), the peptide was diluted in vehicle (saline; 0.9% sodium chloride solution). It was injected intraperitoneally (IP), at a dose of 5 μg/kg for and at 30 min prior to cocaine administration. This dose of sCT was used as it has been previously established to attenuate alcohol-mediated behaviors (Kalafateli et al., 2019a; Kalafateli et al., 2019b). Moreover, lower doses of sCT reduce, but not significantly attenuate alcohol-induced locomotor stimulation (Kalafateli et al., 2019b), whereas higher doses affect the animals’ gross behavior (unpublished data). For intracranial bilateral infusions, sCT was diluted in vehicle (Ringer solution: NaCl 140 mM, CaCl₂ 1.2 mM, KCl 3.0 mM and MgCl₂ 1.0 mM, Merck KGaA; Darmstadt, Germany). It was infused at a volume of 0.5 μl per side into the LDtg (0.005 μg per side), VTA (0.4 μg per side) or NAc shell and core (0.02 μg per side), 30 min prior to cocaine. The aforementioned doses were the same per animal, as intracranial drug infusions are not dependent on the animals’ weight. The doses were selected as the highest doses not affecting baseline locomotor activity in separately conducted experiments for each brain region (unpublished data). These dose-response studies were based on previous studies showing that similar or higher doses in the same nuclei do not present adverse effects like nausea or malaise (Baisley et al., 2014; Mietlicki-Baase et al., 2013; Reiner et al., 2017). Cocaine hydrochloride (Apoteket AB, Gothenburg, Sweden) was diluted in vehicle (saline; 0.9% sodium chloride solution) and was injected IP in all cases at a dose of 10 mg/kg, 5 min prior to behavioral observation. This dose of cocaine was selected based on its ability to activate the mesolimbic dopamine system as seen by increased locomotor stimulation, accumbal dopamine release and CPP in mice (Egecioglu et al., 2013; Jerlhag et al., 2010) and has been extensively used in the literature (Abizaid et al., 2011; Harasta et al., 2015).

2.3. Surgical procedures and cannula placement verification

The same surgical procedure was followed for the insertion of guide and/or microdialysis probes. The mouse was anesthetized with isoflurane (Baxter International; Apoteket AB, Gothenburg, Sweden) using a pump (Univerit 400 Anaesthesia Unit, Univerton Ltd.; Zejting, Malta), kept on a heating pad to prevent hypothermia and placed in a stereotaxic frame (David Kopf Instruments; Tujunga, CA, USA). A mixture of the local anaesthetic xylocain (10 mg/ml) together with adrenalin (5 μg/ml) (Pfizer Inc.; Apoteket AB, Gothenburg, Sweden) was administered on the skin surface prior to incision, at an approximate dose of 0.3 mg/kg and 0.16 μg/kg respectively. The analgesic carprofen (Rimady®, 5 mg/kg subcutaneous; Astra Zeneca; Apoteket AB, Gothenburg, Sweden) was also administered prior to surgery. The skull bone was exposed and holes for guides/probe/anchoring screw were drilled. The mice were either implanted with bilateral guides (stainless steel, length 10 mm, with an o.d./i.d. of 0.6/0.45 mm) or a custom-made dialysis probe. The guides/probe were anchored to the screw and the skull bone with dental cement (DENTALON® plus, Agnths’ AB; Lidings, Sweden). The coordinates targeting the LDtg, VTA, NAc shell and NAc core in mice are described in Supplementary Table 1. In all experiments with local infusions, a dummy cannula was inserted through the guide, in order to remove clotted blood and dampen spreading depression, 1 h before the intracranial infusions. Vehicle or sCT was infused over 60 s using an injector cannula targeting the area of interest (Supplementary Table 1), which was retracted after an additional 60 s.

After termination of the experiments, the mice were euthanized after exposure to isoflurane and subsequently CO₂. After determination of the endpoint, the animals were decapitated and the brains were isolated. The brains were mounted on a vibroslice device, were cut in 50 μm sections and the location was determined by observation, using the brain atlas (Paxinos and Franklin, 2012). For the microdialysis probe, the observation was done by converting the length of the probe’s active space to the atlas scale, to reassure dopamine collection only from the area of the NAc shell and not from neighboring regions. Only data from animals with correct placements were included in the statistical analysis (Supplementary Fig. 1 A-D).

2.4. Locomotor activity

Locomotor activity was recorded in sound-attenuated, ventilated and dim lit locomotor boxes (420 × 420 × 200 mm; Open Field Activity System; Med Associates Inc.; Georgia, Vermont, USA). In this system, 15 × 15 infrared beams at the bottom of the floor detected and a computer program recorded distances travelled (cm per 5 min) of each mouse.
during the entire test session. In total, six different locomotor activity experiments were conducted and in each of them, the following treatment groups were created: vehicle-vehicle, vehicle-cocaine, sCT-vehicle, sCT-cocaine.

2.4.1. Effects of acute systemic sCT administration on cocaine-induced locomotion in mice

The first set of locomotor activity tests was performed to investigate the effects of acute systemic sCT administration (5 μg/kg, IP) on acute cocaine (10 mg/kg, IP)-induced locomotor stimulation in mice. The mice were allowed to habituate to the locomotor activity boxes for 60 min and then sCT or an equal volume of vehicle was injected (IP). After 30 min, cocaine or vehicle was injected IP and after 5 min, the 60-minute cumulative locomotor activity was registered.

2.4.2. Effects of repeated systemic sCT and cocaine co-administration on locomotion in mice

The second set of locomotor activity experiments was designed to investigate the link between five days of systemic sCT (5 μg/kg, IP) and cocaine (10 mg/kg, IP) co-administration on locomotor activity in mice. On the experimental day, the mice were allowed to habituate to the locomotor activity boxes for 30 min, then sCT or an equal volume of vehicle was injected (IP) and after 30 min, cocaine or vehicle was injected IP. Since in this setup all the mice (N = 32) were given both injections daily, locomotor activity was then registered for another 30 min, starting 5 min after the last vehicle or cocaine injection.

2.4.3. Effects of sCT infusions into reward-related areas on cocaine-induced locomotion in mice

The final four locomotor activity tests were performed to investigate the effects of local infusion of sCT into i) LDTg (0.005 μg per side), ii) VTA (0.4 μg per side), iii) NAc shell or iv) NAc core (0.02 μg per side for both subregions) on acute cocaine (10 mg/kg, IP)-induced locomotor stimulation in mice. In each of these four experiments, the mice were allowed to habituate to the locomotor activity boxes for 60 min and then sCT or an equal volume of vehicle was infused locally. After 30 min, cocaine or vehicle was injected IP and after 5 min, the 60-minute cumulative locomotor activity was registered.

2.5. Conditioned place preference

For both CPP experiments, a two-chambered conditioned place preference apparatus with distinct visual and tactile cues was used. The experiments consisted of the preconditioning phase (day 1), conditioning phase (days 2–5) and post-conditioning phase (day 6). Conditioning (20 min per session) was done using a biased procedure in which cocaine was paired with the initial less preferred compartment and IP vehicle administration was paired with the initial preferred compartment, thus creating a vehicle-cocaine and an sCT-cocaine group. The injections were altered between morning and afternoon in a balanced design, with an equal number of animals per group (N = 16 per group). CPP was calculated as the difference in % of the total time spent in the drug-paired (i.e., less preferred) compartment during the test and pre-conditioning sessions.

2.5.1. CPP expression of the rewarding properties of cocaine

The first CPP experiment was designed to evaluate the effects of systemic sCT (5 μg/kg, IP) administration on the rewarding properties of cocaine (10 mg/kg, IP). For this experiment, sCT or IP vehicle was injected 30 min prior to the cocaine or vehicle injection, on each of the four conditioning days. On the post-conditioning day, the mice were placed on the midline of the arena with free access to both compartments for 20 min.

2.5.2. CPP expression of the cocaine reward-dependent memory retrieval

The second CPP experiment was designed to assess the effects of systemic sCT (5 μg/kg, IP) on cocaine (10 mg/kg, IP) reward-dependent memory retrieval. For this experiment, a vehicle injection was administered IP during the pre-conditioning day in order to mimic the test (post-conditioning) day. Cocaine and vehicle were administered daily during the four conditioning days. On the post-conditioning day, sCT or vehicle was administered; 30 min following the injections, the mice were placed on the midline of the arena with free access to both compartments for 20 min.

2.6. In vivo microdialysis and dopamine release measurements

The present microdialysis experiments were designed to evaluate the effects of systemic sCT (5 μg/kg, IP) on cocaine (10 mg/kg, IP)-induced dopamine release in the NAc shell using a high-performance liquid chromatography-electrochemical detection (HPLC-ED) system. The mice were habituated to the microdialysis set-up for an hour, and subsequently dialysate samples were collected in 20-minute intervals across the entire test session (from ~40 to 200 min). The baseline dopamine levels were defined as the average of three consecutive samples (~40 to 90 min). At 10 min, sCT or an equal volume of vehicle was administered IP. Thirty minutes later (at 40 min), vehicle or cocaine was injected IP and eight additional samples were collected. Collectively, the following four treatment groups were created: vehicle-vehicle, vehicle-cocaine, sCT-vehicle, and sCT-cocaine.

During the experiment, the dialysis probe was connected to a micro-perfusion pump (U-864 Syringe Pump; Agnths’o AB) and washed with Ringer solution at a rate of 1.5 μl/min. Dopamine was separated and quantified using two different HPLC apparatuses with electrochemical detection as described previously (Kalafateli et al., 2019b).

2.7. Statistical analysis

The data from the acute locomotor activity experiments were analyzed using two-way ANOVA for comparison between treatments. The data from the repeated locomotor activity experiments were analyzed using two-way repeated measures ANOVA for comparison between treatments across time points. Tukey’s post hoc test was used for multiple pairwise comparisons for all locomotor activity experiments. The microdialysis experiments analysis was conducted using two-way repeated measures ANOVA with the correction of Geisser-Greenhouse’s epsilon to control for violations of sphericity, followed by Bonferroni’s post-hoc test for multiple comparisons between treatments and across time points. The CPP tests were analyzed with an unpaired, two-tailed t-test. The effect size was estimated as eta squared (η²) for ANOVA and Cohen’s d for pairwise comparisons (for magnitude of effect size interpretation see Cohen, 1988). Data are presented as mean ± SEM with a probability value of P < 0.05 considered as statistically significant.

3. Results

3.1. Effects of acute systemic sCT administration on cocaine-induced locomotor stimulation and accumbal dopamine release in mice

There was an overall effect of cocaine treatment (F(1, 27) = 16.92, P = 0.0003), sCT treatment (F(1, 27) = 4.36, P = 0.0464) and treatment combination (F(1, 27) = 5.66, P = 0.0247) on the scores of locomotor activity in mice (η² = 0.51; Fig. 1A). Cocaine (N = 8) increased locomotion in mice when compared to the vehicle (P = 0.0004, N = 8) group. Acute sCT administration attenuated cocaine-induced locomotor stimulation in mice when compared to the vehicle-cocaine receiving group (P = 0.0217, N = 7). Locomotor activity in the sCT-cocaine group did not differ from the vehicle group (P = 0.5054, N = 8). sCT did not have an effect per se on locomotor activity when compared to the vehicle group (P = 0.9967, N = 8).

Analysis of the microdialysis experiments showed an overall effect of
3.2. Effects of repeated systemic sCT and cocaine administration on cocaine-induced locomotor stimulation in mice

There was an overall effect of treatment (F(3, 25) = 15.43, P < 0.0001), time (F(28.21, 70.52) = 9.10, P < 0.0001) and time x treatment interaction (F(12, 100) = 3.43, P = 0.0003) on locomotor activity scores in mice (q² = 0.93; Fig. 2). Specifically, cocaine-induced (N = 8) locomotor stimulation at all tested days when compared to the vehicle-vehicle group (D1: P = 0.0002, D2: P = 0.0105, D3: P = 0.0020, D4: P = 0.0101, D5: P = 0.0022; N = 8). In mice treated with sCT prior to cocaine daily (N = 6), there was no significant increase in locomotor activity compared to vehicle-treated mice (D1-D5; P > 0.05). However, there was no significant difference in cocaine response in mice pre-treated with vehicle or sCT (D1-D5; P > 0.05). Moreover, there was no difference in locomotor activity in sCT-vehicle mice (N = 7) compared to vehicle-treated mice at any day (D1-D5: P > 0.05).

3.4. Effects of acute local sCT administration in reward-processing areas on cocaine-induced locomotor stimulation in mice

The analysis of the LDTg experiments showed an overall effect of
Effects of repeated systemic sCT and cocaine co-administration on cocaine-evoked locomotor stimulation in male mice.

Repeated cocaine (Coc) administration (10 mg/kg, IP) for five days increased locomotion in mice, when compared to the vehicle (Veh) group at all days of administration (D1-D5; *P < 0.05, **P < 0.01, ***P < 0.001 for Veh-Coc vs Veh-Veh comparisons). In mice pre-treated with sCT (5 µg/kg, IP) for five days prior to cocaine, no significant increase in locomotor activity was noted at any day, when compared to the vehicle group (n.s.: not significant, for sCT-Coc vs Veh-Veh comparisons). There was no difference in cocaine response between the sCT or vehicle pre-treated mice. (Data are presented as mean ± SEM).

The VTA data analysis showed an overall effect of cocaine (F(1, 26) = 18.10, P = 0.0002), but not of sCT treatment (F(1, 25) = 1.92, P = 0.1779) or their interaction (F(1, 25) = 1.26, P = 0.2711) on locomotor activity in mice (r² = 0.29; Fig. 3A). Post hoc analysis revealed that cocaine (N = 8) increased locomotion when compared to the vehicle group (P = 0.0299, N = 8). Cocaine did not cause locomotor stimulation in mice infused with sCT into the LDTg (P = 0.7798, N = 6) when compared to the vehicle group. No differences were noted in cocaine response on locomotion between mice pre-treated with either vehicle or sCT (P = 0.3957). sCT did not affect locomotor activity per se (P = 0.9972, N = 8) when compared to the vehicle-treated mice.

The VNA shell experimental scores showed an overall effect of cocaine (F(1, 26) = 14.31, P = 0.0009), but not of sCT treatment (F(1, 25) = 0, P = 0.9851) or treatment interaction (F(1, 25) = 0.05, P = 0.8318) on locomotion scores in mice (r² = 0.34; Fig. 3B). Cocaine administration (N = 7) increased locomotion when compared to the vehicle-treated mice (P = 0.0028, N = 8). Cocaine did not cause locomotor stimulation in mice treated with sCT in the VTA (P = 0.1534, N = 6) when compared to the vehicle group. However, there were no differences in cocaine response on locomotor activity between mice receiving vehicle or sCT (P = 0.4143). sCT did not have an effect on locomotor activity per se (P = 0.9214, N = 8).

The NAc shell experimental scores showed an overall effect of cocaine treatment (F(1, 25) = 13.41, P = 0.0009), but not of sCT treatment (F(1, 25) = 0, P = 0.9851) or treatment interaction (F(1, 25) = 0.1356, P = 0.7158) on locomotor activity in mice (r² = 0.34; Fig. 3C). Specifically, cocaine (N = 6) induced locomotor stimulation when compared to the vehicle-receiving mice (P = 0.0459, N = 7). Cocaine administration did not cause significant locomotor stimulation in mice treated with intra-NAc shell sCT (P = 0.534, N = 7) when compared to the vehicle group. No differences were noted in cocaine-induced locomotion between vehicle- and sCT receiving mice (P = 0.9931). sCT did not affect locomotor activity per se (P = 0.9942, N = 8) when compared to the vehicle group.

Lastly, analysis of the NAc core scores indicated an overall effect of cocaine (F(1,23) = 11.11, P = 0.0029; r² = 0.36; Fig. 3D), but not of sCT pre-treatment (F(1, 23) = 1.89, P = 0.1827) or treatment interaction (F (1, 23) = 1.90, P = 0.1809) on locomotor activity in mice. Post hoc analysis showed that cocaine increased locomotion (N = 7) when compared to the vehicle group (P = 0.0122, N = 7). Mice infused with sCT into the NAC core did not display locomotor stimulation evoked by cocaine (P = 0.5366, N = 6) when compared to vehicle-treated mice. However, there were no differences between the vehicle- and sCT- cocaine groups (P = 0.2518). sCT did not affect locomotion per se (P > 0.9999, N = 7) when compared to the vehicle-receiving group.

4. Discussion

To the best of our knowledge, these are the first preliminary studies presenting association between cocaine-induced behaviors and amylin signalling in mice. The present data also provide some indication of the brain regions participating in the cocaine-amylin association. In fact, we present that acute systemic administration of sCT, an AMYR and CTR agonist, attenuates locomotor stimulation and extracellular accumul dopamine release caused by cocaine administration. Contrarily, sCT does not affect the rewarding properties and reward-dependent memory of cocaine in the CPP paradigm in mice. Moreover, the elevated locomotor stimulation following repeated injections of cocaine was lower, but not attenuated, in mice injected repeatedly with systemic sCT for five days. Acute intracranial infusions of sCT into the LDTg, VTA, NAC shell and NAc core reduce, but do not inhibit cocaine-evoked locomotor stimulation in mice when compared to the vehicle group.

Older studies have reported that central sCT administration decreases amphetamine-induced locomotor stimulation (Twery et al., 1986) and amylin infused into the third ventricle reduces amphetamine-evoked hypermobility and stereotypies in rats (Clementi et al., 1996). More recent studies have established that amylin signalling modulates both acute and chronic alcohol-mediated behaviors in rodents (Kalafateli et al., 2019a; Kalafateli et al., 2019b). However, very little is known about the involvement of amylinerergic pathways in the modulation of other drugs of abuse, like cocaine. We here show that a single systemic injection of sCT reduces cocaine-induced locomotor stimulation in mice, possibly by decreasing dopaminergic neurotransmission in the NAc shell as shown by our microdialysis experiments. This is in accordance with our previous studies showing that the same dose of sCT (5 µg/kg, IP) attenuates alcohol-induced locomotor stimulation and accumul dopamine release in mice (Kalafateli et al., 2019b). Our results are similar to those obtained in studies using Exendin-4, an agonist for the GLP-1 receptor, which attenuates acute cocaine-induced locomotor stimulation and dopamine release in the NAC shell in mice (Egecioglu et al., 2013). On a similar note, infusions of NMU into the NAc shell reduced the increased locomotion caused by repeated cocaine administration in rats (Kasper et al., 2016). These results indicate commonality between different gut-brain peptides in their ability to modulate cocaine-induced activation of the mesolimbic dopamine system. It should be noted here that although the individual response to cocaine varies between mice in these locomotor activity experiments, we here observe that cocaine increases locomotion in the group receiving cocaine, when compared to vehicle-treated mice as a group. These individual differences in drug responses are commonly reported in behavioral studies (Kalafateli et al., 2018; Vallöf et al., 2019). Therefore, the statistical approach of mean comparison that is usually followed is suitable for the interpretation of the data.

Albeit we here show a protective effect of sCT prior to a cocaine stimulus, possibly by inhibiting cocaine’s ability to activate the mesolimbic dopamine system, the exact mechanisms by which sCT attenuates extracellular dopamine release evoked by cocaine remain to be elucidated. In the present studies, sCT was administered peripherally, but we suggest that it modulates the behavioral responses of cocaine through central mechanisms rather than peripheral. Supportively, alcohol-mediated behaviors do not fall under peripheral amylinerergic regulation, such as caloric (Kalafateli et al., 2019b) and sCT is suggested to act on AMYRs located in brain areas, e.g. the VTA, to regulate food intake (Mietlicki-Baase et al., 2013). To further identify whether the same reward-processing areas are involved in amylinerergic modulation of cocaine, we here pinpoint that all areas investigated, namely the LDTg, VTA, NAC shell and core, contribute to the ability of sCT to regulate cocaine-induced locomotor stimulation. Indeed, the cocaine response in mice infused with sCT into each of aforementioned area prior to cocaine,
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did not differ statistically from the vehicle receiving group; however, there were no statistically significant differences between the sCT-cocaine and vehicle-cocaine group. Activation of AMYRs in the LDTg (Reiner et al., 2017) and VTA (Mietlicki-Baase et al., 2017; Mietlicki-Baase et al., 2015) decreases food intake and complex motivated feeding behaviors in rats. Moreover, AMYR activation by amylin infusions into the NAc shell reduces μ-opioid receptor-driven feeding (Baisley and Baldo, 2014) and intra-VTA sCT administration attenuates food-induced dopamine release in the NAC core (Mietlicki-Baase et al., 2015).

In the present studies, the lack of a robust reducing effect of locally infused sCT on locomotor response to cocaine could be possibly attributed to that all the investigated areas participate to some degree and that other brain areas are involved in this modulation. Amylin is suggested to synergize with leptin to decrease eating in regions of the hypothalamus (Lutz et al., 2018), a structure where local amylin production has also been detected (Li et al., 2015). Moreover, the hypothalamus appears to play a role in cocaine-induced CPP and self-administration (Blacktop et al., 2017). Lesions in the nucleus of the solitary tract inhibits amylin’s anorectic effect (Lutz et al., 1998), rendering this area another interesting brain site for further investigation. We here suggest that AMYRs in these areas processing reward are important for cocaine-induced activation of the mesolimbic dopamine system; however, the possibility should also be considered that CTRs, which also are agonized by sCT (Lee et al., 2016), may participate. Thus, there is a need for additional experiments using pharmacological compounds more selective to AMYRs, which have been shown to attenuate alcohol-mediated behaviors (Kalafateli et al., 2020b) in order to define the sole involvement of amylinergic pathways in the modulation of cocaine reward.

In the CPP paradigm, we did not detect an effect of sCT on the rewarding properties of cocaine or on cocaine-dependent memory retrieval. This is in contrast to the mice data showing that systemic sCT administration attenuates both these CPP behaviors following alcohol injections (Kalafateli et al., 2019b). This discrepancy could be attributed to differential neurochemical mechanisms modulating cocaine- and alcohol-induced CPP in mice. Besides dopaminergic mechanisms which are crucial for the expression of alcohol-induced CPP (Young et al., 2014), in the expression of cocaine CPP, serotonergic systems also play an important role (Hnasko et al., 2007). Therefore, the effect of sCT on cocaine- and alcohol-induced CPP, could be tentatively controlled by different neurochemical systems. We here observed that cocaine

Fig. 3. Effects of sCT infusion in reward-related brain regions on cocaine-induced locomotor activity in male mice.

Acute systemic administration of cocaine (Coc; 10 mg/kg, IP) increased locomotor stimulation caused by cocaine in mice, when compared to the vehicle (Veh) group, in all the investigated brain areas (A-D; *P < 0.05, **P < 0.01 for Veh-Coc vs Veh-Veh comparisons). In mice pre-treated with bilateral sCT infusions into the (A) LDTg (0.005 μg per side), (B) VTA (0.4 μg per side), (C) NAc shell or (D) core (0.02 μg per side for both subregions), cocaine did not induce locomotor stimulation when compared to the vehicle group. No difference in cocaine response was noted between the sCT or vehicle pre-treated mice. (Data are presented as mean ± SEM; n.s.: not significant).
induced a CPP of approximately 15%, and a low cocaine response that might not have been possible to be reduced by sCT. However, this appears less likely as other gut-brain peptide such as those targeting the ghrelin or GLP-1 receptors, reduce cocaine-induced CPP response (Egecioglu et al., 2013; Jerlhag et al., 2010). It is therefore likely that the CPP-response to cocaine involved GLP-1 and ghrelin receptors, rather than sCT responsive receptors.

We here show that in mice treated with sCT and cocaine for five days, cocaine increased locomotor stimulation in vehicle-, but not sCT-treated mice at all experimental days. These results possibly indicate that the effects of sCT in response to cocaine do not differ over exposure time, tentatively involving decreased dopaminergic tone in each experimental trial. One possible explanation could be the differential modulation of alcohol and cocaine by sCT, possibly due to the variable neurobiological mechanism of action between these two addictive drugs (Koob and Weiss, 1992). The above could be also the case for the differences Nevertheless, as the present study focuses on acute cocaine-induced behaviors, how sub-chronic administration of sCT affects chronic cocaine behaviors in both male and female rodents remains to be investigated. Our present data support that sCT regulates only acute behavioral responses to cocaine. Thus, the investigation of amylin signalling in complex chronic cocaine behaviors is of great interest for the future. For example, studies of GLP-1 receptor activation have shown attenuation of cocaine self-administration in mice (Sorensen et al., 2015) and cocaine-seeking in rats (Hernandez et al., 2018; Hernandez et al., 2019), rendering sCT an interesting candidate for the modulation of such behaviors.

Notably, in the present experiments we have used doses of sCT that have been previously established as having an effect on alcohol-mediated behaviors (Kalafateli et al., 2020a; Kalafateli et al., 2019a; Kalafateli et al., 2019b). Therefore, different doses of sCT could have variable effects on cocaine-induced activation of the mesolimbic dopamine system and such experiments are warranted in the future. Additionally, in the current studies we used a single cocaine dose that causes robust behavioral responses (Abizaid et al., 2011; Egecioglu et al., 2013; Harasta et al., 2015; Jerlhag et al., 2010). Our previously published studies with other gut-brain peptides, like the GLP-1 analogue Ex4 (Egecioglu et al., 2013) and the ghrelin receptor antagonist JMV2959 (Jerlhag et al., 2010), were found to reduce behavioral responses to the currently used cocaine dose. Nevertheless, the effects of sCT on different cocaine doses would be an intriguing future study and will shed more light on the magnitude of sCT’s effect on cocaine behaviors. Lastly, the present study covers a preliminary approach on the effects of sCT on acute cocaine behaviors and future experiments including more complex self-administration paradigms in rodents will provide more understanding on the mechanisms involved in the regulation of chronic cocaine behaviors.

Interestingly, sCT attenuates some, but not all, of the currently studied cocaine-induced behaviors, adding to the previously established role of the amylin pathway in alcohol reward modulation. Additional investigation of the effects of AMYR agonists on the regulation of cocaine behavioral responses will bring new insights into how amylin signalling regulates cocaine-induced activation of the mesolimbic dopamine system and reward in general.

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Authors contribution

ALK performed hands on work, analyzed data, wrote the manuscript and managed literature search, CA performed hands on work and analyzed data, EJ designed the study, contributed to the conception and interpretation, managed literature search, analyzed data and wrote the manuscript. All authors contributed to and have approved the final manuscript.

Declaration of competing interest

EJ has received financial support from the Novo Nordisk Foundation. This does not alter the authors’ adherence to any of the journal’s policies on sharing data and materials. The remaining authors declare no conflict of interest.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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