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The area postrema (AP) and the parabrachial nucleus (PBN) are important sites for salmon calcitonin (sCT) to decrease evoked phasic dopamine release in the nucleus accumbens (NAc)

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Abstract

The pancreatic hormone amylin and its agonist salmon calcitonin (sCT) act via the area postrema (AP) and the lateral parabrachial nucleus (PBN) to reduce food intake. Investigations of amylin and sCT signaling in the ventral tegmental area (VTA) and nucleus accumbens (NAc) suggest that the eating inhibitory effect of amylin is, in part, mediated through the mesolimbic ‘reward’ pathway. Indeed, administration of the sCT directly to the VTA decreased phasic dopamine release (DA) in the NAc. However, it is not known if peripheral amylin modulates the mesolimbic system directly or whether this occurs via the AP and PBN. To determine whether and how peripheral amylin or sCT affect mesolimbic reward circuitry we utilized fast scan cyclic voltammetry under anesthesia to measure phasic DA release in the NAc evoked by electrical stimulation of the VTA in intact, AP lesioned and bilaterally PBN lesioned rats.

Amylin (50 µg/kg i.p.) did not change phasic DA responses compared to saline control rats. However, sCT (50 µg/kg i.p.) decreased evoked DA release to VTA-stimulation over 1 h compared to saline treated control rats. Further investigations determined that AP and bilateral PBN lesions abolished the ability of sCT to suppress evoked phasic DA responses to VTA-stimulation. These findings implicate the AP and the PBN as important sites for peripheral sCT to decrease evoked DA release in the NAc and suggest that these nuclei may influence hedonic and motivational processes to modulate food intake.

Keywords

Obesity; Amylin; Reward; Dopamine; Mesolimbic; Food intake

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1. Introduction

Obesity is a serious concern because it is associated with co-morbidities such as type 2 diabetes mellitus, heart disease, stroke, and some types of cancer. Obesity results from a combination of causes but the ease of access to palatable energy-dense food and its overconsumption are considered major factors [1,2]. These palatable foods are considered pleasurable and stimulate brain reward systems independent of their caloric value [1,3,4]. One system implicated in the reinforcing effects of palatable food is the mesolimbic system, consisting of dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) [5]. Peripheral hormones that affect food intake, such as leptin, ghrelin, glucagon-like peptide 1 (GLP-1) and insulin have also been found to affect the mesolimbic system [6,7].

Amylin is co-secreted with insulin from the pancreatic beta cells [8]. The teleost peptide salmon calcitonin (sCT) is structurally and functionally related to amylin and is used as an amylin agonist with a prolonged duration of action compared to endogenous or exogenous amylin [9]. Amylin, sCT and other related peptides have been established to reduce food intake when given peripherally; the effect of amylin and sCT is primarily mediated by activating noradrenergic neurons in the area postrema (AP) [10–12]. Activation of amylin receptors (AmyR) by sCT in the VTA reduces sugar-evoked dopamine (DA) release in the NAc of rats compared to saline-treated controls [13]. Furthermore, the inhibition of food intake with administration of sCT in the VTA is attenuated by dopamine receptor agonists administered in the NAc [13]. These findings have been interpreted in a way that amylin signaling in the VTA may reduce eating by influencing hedonic/motivational processes relating to food intake [13].

While there is evidence to suggest that sCT in the VTA modulates the mesolimbic system, it is not known whether peripherally administered sCT, or indeed amylin, could also mediate these effects via primary action in the AP or elsewhere. Indirect evidence to support the involvement of AP neurons in food seeking behavior comes from rats with lesions of the AP [14,15]. These rats consume significantly greater quantities of palatable food than control rats in a short amount of time and work harder under operant conditions for sucrose rewards [14,15]. However, while peripheral amylin reduces food intake by primary action in the AP, it is not known whether activation of amylin receptors (AmyR) in the AP by peripheral amylin or sCT also modulates dopamine signaling. Using fast scan cyclic voltammetry (FSCV), we therefore tested whether peripheral amylin or sCT alters DA release in the NAc, and whether the AP or downstream lateral parabrachial nucleus (PBN) are required for these effects.

2. Methods

2.1. Animals

Adult male Wistar rats were housed in individual cages under an artificial 12 h/12 h light/dark cycle (lights off at 1900) and at a room temperature of 21 ± 2 °C. Rats were maintained on water and chow (4.5% fat, 18.5% protein, 54.2% N-free extracts; #3430, Provimi Kliba

AG Kaiseraugst, Switzerland) available ad libitum. All experiments were approved by the Veterinary Office of the Canton Zurich, Switzerland.

2.2. Fast scan cyclic voltammetry (FSCV)

Rats underwent surgical procedures and FSCV recordings as reported [16]. Rats were not fasted prior to recordings. Briefly, rats weighing between 300 and 400 g were anesthetized by i.p. injection of ketamine (50 mg/kg; Narketan, Vetoquinol AG, Ittingen, Switzerland) and xylazine (2.5 mg/kg, Xylazin; Streuli Pharma AG, Uznach, Switzerland). A guide cannula (MD 2251, Bioanalytical Systems, Indiana, USA) was implanted dorsal to the right NAc (1.3 mm anterior and 1.3 mm lateral to bregma). A chlorinated silver reference electrode was placed in the left forebrain. Stainless steel skull screws and dental cement secured implants to the skull.

A glass-insulated carbon fiber recording electrode housed in a micromanipulator (UIC Biologic Research Center) was inserted in the cannula guide allowing the carbon fiber electrode to be lowered to the NAc and a bipolar stainless steel stimulating electrode was positioned above the VTA (Plastics One; 0.20 mm diameter, 5.2 mm posterior and 1.0 mm lateral to bregma). The DV positions of both electrodes were adjusted until maximum evoked DA release was observed (6.0–6.4 mm DV for NAc and 7.2–8.7 mm DV for VTA). Dopamine detection was accomplished by FSCV performed with a computer-controlled potentiostat (custom FSCV interface, Electronics and Materials Engineering (EME) Shop, Seattle, WA) and the software package, TarHeel CV (UNC Electronics Facility, Chapel Hill, NC). The VTA was stimulated (60 Hz, 24 pulses, 150 μ A) every 5 min until the magnitude of evoked DA release was stable ($< 10\%$ variation over 20 min, baseline). Rats were injected during this time with the same mixture of anesthetic at half the previous dose to maintain anesthesia for the rest of the experiment. Once four baseline release measurements were collected (time 0), rats were injected with amylin (50 μ g/kg), sCT (50 μ g/kg) or saline i.p. and the stimulations continued every 5 min for 60 min. Peak DA release in the NAc following each stimulation was recorded. Amylin (H9475) and sCT (H2260) were purchased from Bachem (Bubendorf, Switzerland). At the end of 60 min, the DA receptor antagonist raclopride (2 mg/kg; R121, Sigma Chemie GmbH, Buchs, Switzerland) was administered i.p. to check for dopaminergic responsiveness. By inhibition of autoreceptors, raclopride increased the magnitude of evoked dopamine in all animals tested (data not shown). Following experiments, recording electrodes were calibrated in a flow cell as described previously [17].

2.3. Area postrema lesions

Rats weighing between 190 and 250 g underwent AP lesions as reported [18]. Briefly, animals were anesthetized as described above and the rats' head was flexed ventrally at an approximate 110° angle in a stereotaxic frame. Skin and three layers of neck musculature were cut and retracted for visualization of the foramen magnum. Under visual control with a surgical microscope, the cranial dura mater was penetrated with an angled cannula tip and the cerebrospinal fluid was blotted. AP lesions were performed by aspirating the AP with a blunted cannula tip fixed to a flexible tube attached to a vacuum pump [19,20]. In sham-lesioned rats, the AP was exposed but left intact. Musculature and skin were sutured

respecting the individual muscle layers. Rats received subcutaneous injections of 5 mL sterile saline, antibiotics (7.5 mg/kg enrofloxacin, Baytril 2.5%; Pro-vet AG, Lyssach, Switzerland) and an analgesic (1 mg/kg flunixin, Flunixinim; Graeub AG, Bern, Switzerland) daily for at least 3 days post-surgery. Animals were left to recover for 8 days, after which lesions were verified using a within-subject crossover test of 1-h food intake in response to an injection of 20 µg/kg amylin or saline i.p. after 12 h food restriction [18]. FSCV was then performed on rats between postoperative days 14–18. At the end of the experiments, AP lesions were also confirmed histologically.

2.4. Electrolytic PBN lesions

Rats weighing between 190 and 250 g were electrolytically lesioned in the PBN as reported [21]. Briefly, rats were anesthetized and fixed in a stereotaxic apparatus. A 2- to 3-cm midline skin incision was made from the level of the orbita to approximately 1 cm caudally from the occipital crest. The periosteum was detached and retracted laterally. The skull was removed above the area of the PBN (coordinates: 9.2 mm caudal, and bilaterally 2.5 mm lateral from [48]). An insect pin served as the electrode and was attached to the anode of the electrolytic lesion-making device (Ugo Basile, Italy). The pin was lowered 6.0 mm ventrally from dura at a 15° angle, 2.5 mm lateral from Bregma and an anodal current (0.5 mA for 10 s) was passed on each side to produce the electrolytic lesions. Sham lesions were made by the same procedure but without passing the current. Rats received subcutaneous injections of 5 mL sterile saline, antibiotics (7.5 mg/kg enrofloxacin, Baytril 2.5%; Provet AG, Lyssach, Switzerland) and an analgesic (1 mg/kg flunixin, Flunixinim; Graeub AG, Bern, Switzerland) daily for at least 3 days post-surgery. Rats were allowed to recover for 8 days, after which lesions were verified by testing food intake in response to 5 µg/kg amylin or saline i.p. after 12 h food restriction. This dose was chosen because a previous study had shown that the anorectic effect of 5 µg/kg but not 20 µg/kg amylin was attenuated in PBN-lesioned (PBN-X) rats [21]. At the end of the experiments, PBN lesions were also confirmed histologically and it was found that all rats with successful bilateral lesions did not reduce food intake in response to amylin. FSCV was then performed on rats between postoperative days 14–18.

2.5. Statistics

A one-way ANOVA was performed to analyze differences in body weight after surgery. A two-way ANOVA was performed to analyze differences in 1-hr food intake in response to amylin administration after surgery. Data sets are reported as mean ± SEM. Peak DA concentration after each stimulation in each group was normalized to the average of the four baseline phasic DA measurements and expressed as a percentage of the average baseline. Differences in DA as a percentage of baseline were then analyzed by two-way repeated measures ANOVA with time as within-subjects factor and treatment as between-subjects factor. Tukey post hoc tests were used to investigate multiple comparisons. All statistics were performed with Graphpad Prism 7 software and significance was predefined as $p < 0.05$.

3. Results

3.1. Peripheral sCT but not amylin reduces phasic DA release in the NAc

To test whether peripheral sCT and amylin decrease DA release, we performed VTA-stimulated FSCV in anesthetized rats and measured the NAc dopamine response to electrical stimulation of the VTA. Stimulation reliably evoked a phasic increase in DA in the NAc (Fig. 1A–F) and the magnitude of phasic DA released in response to VTA-stimulation within session before (0 min) and 60 min after administration of saline ($n = 8$), amylin ($n = 8$) or sCT ($n = 6$) is shown in Fig. 1G–I. Analysis of phasic DA responses found modulation by drug administration (Treatment $\times$ Time interaction, $F(30, 285) = 1.941$, $p < 0.05$, Fig. 1J). Further analysis found that saline and 50 $\mu\text{g/kg}$ amylin did not alter DA responses ($p > 0.05$) but sCT significantly reduced DA ($p < 0.05$; Fig. 1J). A representative image showing the typical placement of the recording electrode in the NAc (Fig. 1K) and the stimulating electrode in the VTA (Fig. 1L) is shown.

3.2. The AP is involved in the reduction of phasic DA release in the NAc by peripheral sCT

To test whether the AP is involved in the decrease of phasic DA release by peripheral sCT, we measured the NAc dopamine response to electrical stimulation of the VTA in rats with (AP-X) and without (SHAM) lesion of the AP. AP-X animals in both saline and sCT groups showed reduced body weight 14 days after surgery (AP-X-saline 300 ± 14.4 g, AP-X-sCT 305.7 ± 14.5 g; $n = 9$) compared to the SHAM groups (SHAM-sal 346 ± 8.5 g, SHAM-sCT 346 ± 11.0 g; $n = 8$, $F(3, 30) = 5.965$, $p < 0.05$). The AP lesion was first verified by feeding tests after 12 h of fasting. Total food intake after 1 h was lower in AP-X rats than in SHAM rats injected with saline however overall this was not significant ($F(1, 62) = 2.56$ p value > 0.05). Administration of 20 $\mu\text{g/kg}$ amylin affected food intake ($F(1, 62) = 15.61$ $p < 0.05$ and post hoc tests revealed that SHAM rats showed a reduction of 1 h-food intake after systemic amylin compared to saline (4.65 ± 0.40 g vs 2.23 ± 0.43 g; $p < 0.05$). In contrast, and consistent with previous studies [12,18,20], amylin did not produce an anorectic response in AP-X rats (3.26 ± 0.47 g vs 2.96 ± 0.48 g; p value > 0.05). A representative image of an AP lesion and the respective brain area in a SHAM rat is shown in Fig. 2N–O.

VTA-stimulation reliably evoked a phasic increase in DA in SHAM (Fig. 2A–D) and AP-X rats (Fig. 2E–H), and the magnitude of phasic DA released in response to VTA-stimulation within session before (0 min) and 60 min after the administration of sCT is shown in Fig. 2I–L. DA in response to VTA-stimulation significantly decreased to approximately 80% of baseline over 60 min in SHAM rats that received 50 $\mu\text{g/kg}$ sCT compared to SHAM rats receiving saline (Main treatment effect, $F(3, 30) = 4.068$ p value < 0.05 and Treatment $\times$ Time interaction, $F(48, 480) = 1.65$, $p < 0.01$; Fig. 2M); this was consistent with the results obtained in the first experiment in naïve, unoperated rats. There was no change in phasic DA in response to VTA-stimulation in AP-X rats that received 50 $\mu\text{g/kg}$ sCT compared to AP-X rats that received a saline injection (Fig. 2M). DA in response to VTA stimulation was slightly lower in AP-X rats receiving saline compared to SHAM animals. AP-X rats receiving sCT did not show a reduction in evoked DA release and DA release was not significantly different compared to both SHAM and AP-X rats receiving saline (Fig. 2M).

3.3. The PBN is involved in the reduction of phasic DA release in the NAc by peripheral sCT

To test whether the PBN is involved in the decrease DA release by peripheral sCT, we measured the NAc dopamine response to electrical stimulation of the VTA in rats with (PBN-X) and without (SHAM) bilateral lesions of the PBN. There were no significant differences in body weight 14 days after surgery across all groups (SHAM-saline 347.5 ± 14.3 g, SHAM-sCT 352.2 ± 15.2 g vs PBN-X-saline 351.7 ± 14.2 g PBN-X-sCT 350.2 ± 4.5 g; $n = 6$, $F(3, 20) = 0.03$, $p > 0.05$). The PBN lesions were first verified by assessing food intake after 12 h fasting in response to an i.p. injection of saline and amylin ($5 \mu\text{g/kg}$). Overall there were no differences in 1 h food intake between SHAM and PBN-X animals ($F(1, 36) = 0.96$ p value > 0.05). Administration of $5 \mu\text{g/kg}$ amylin modified 1 h food intake ($F(1, 62) = 15.61$ p value < 0.05) only in SHAM rats where there was a reduction in food intake 1 h after amylin injection compared to saline injection (3.80 ± 0.39 g vs 2.13 ± 0.41 g; $p < 0.05$). Amylin did not produce an anorectic response in rats with bilateral lesions (3.48 ± 0.24 g vs 3.19 ± 0.43 g, p value > 0.05). A representative image of bilateral lesions in the PBN and of a control rat with intact PBN is shown in Fig. 3N/O/P/Q.

VTA-stimulation reliably evoked a phasic DA increase in SHAM (Fig. 3A–D) and PBN-X rats (Fig. 3E–H), and the magnitude of phasic DA released in response to VTA-stimulation within session before (0 min) and after 60 min administration of sCT is shown in Fig. 3I–L. When comparing DA as a percentage of baseline, DA phasic responses were modulated by $50 \mu\text{g/kg}$ sCT over time (Time $\times$ Treatment interaction ($F(45, 300) = 4.58$, p value < 0.05 , Main treatment effect $F(15, 300) = 1.71$ p value < 0.05 , Time effect $F(3, 20) = 9.35$ p value < 0.05). DA in response to VTA-stimulation significantly decreased to approximately 80% of baseline over 60 min in SHAM rats that received $50 \mu\text{g/kg}$ sCT compared to SHAM rats receiving saline (p value < 0.01 ; Fig. 3M), similar to the first two experiments. There was no change in DA in response to VTA-stimulation in PBN-X rats that received $50 \mu\text{g/kg}$ sCT compared to rats that received a saline injection (Fig. 3M). DA in response to VTA-stimulation was slightly lower in both groups of PBN-X rats compared to SHAM animals receiving saline. However, both PBN-X groups retained a DA response that was similar to their respective baselines.

4. Discussion

We found that peripheral sCT, but not amylin, decreases VTA-stimulated DA release in the NAc of rats. Furthermore, the reduction in DA release in the NAc was mediated through the AP and PBN because lesioning these areas blocked the inhibitory effect of peripheral sCT on VTA-stimulated phasic DA release. The control of food intake by amylin signaling via the AP/nucleus tractus solitarius (NTS)-PBN-central amygdala (CeA) neuronal network has been extensively investigated [10,12,18,21]. Feeding and functional c-Fos studies show ablation of the AP reduces the anorectic effect of peripheral amylin and sCT. Furthermore, ablation of the AP blocks amylin-induced c-Fos expression in the NTS, PBN and CeA, and ablation of the PBN blocks amylin-induced c-Fos in the CeA [12,21]. The neurochemical phenotypes that mediate the inhibitory effect of amylin and sCT on food intake have been

shown to be similar [18]. Therefore, the effect of sCT on phasic DA release may use the same neuronal pathway as the eating inhibitory effect of both amylin and sCT.

Pharmacokinetic differences between amylin and sCT may have contributed to the contrasting effects observed on evoked DA release. sCT displays irreversible receptor binding and this is reflected in a prolonged eating inhibitory effect in studies measuring food intake [18,22]. The administration of sCT culminates in a stronger and longer anorectic response compared to amylin administered at the same dose [18]. Therefore, the effect of sCT may produce changes in neural activity over a prolonged period to eventually influence the VTA. Both sCT's and amylin's inhibitory effects on eating have been shown to be dose dependent [18]; we therefore expect that, if the same neuronal pathway were involved, the effect on phasic DA release would also be dose dependent, however this remains to be tested formally and was beyond the scope of this proof of concept study. Another important distinction between the two peptides is that, while sCT is often used as an amylin agonist, it is also a calcitonin receptor (CTR) agonist [22]. Amylin receptors are heterodimers of the CTR, which represents the core receptor protein, and one of three receptor accessory modifying proteins (RAMPs) [22–24]. In pharmacological studies, RAMPs alter CTR pharmacology from calcitonin preferring to amylin preferring receptors [22]. Unlike amylin, sCT binds to the CTR and all three AmyR subtypes with high affinity [8]. Therefore, it is also possible sCT's inhibition on food intake and reduction in phasic DA is mediated not only by AmyR but additionally by CTRs.

The current results broaden the possibility that AP and PBN nuclei are also important in hedonic/motivational processes, at least indirectly. This finding is important as it supports the idea that homeostatic signals also modulate the perception of food reward (for example, the hedonic value and the motivation to work for food). The role of DA in reward-related behaviors has received much attention because it has recently become accepted that obesity may be due to an imbalance within the mesolimbic system [25]. Like drugs of abuse, it has been reported that rewarding food stimulates dopaminergic transmission in the NAc [16,26,27]. Studies on the neurobiology of food reward have traditionally focused on neurocircuitry involving taste receptor cells projecting to the PBN and subsequently engaging the NAc [28,29]. However there is increasing evidence that the satiating negative feedback signals acting in the hindbrain also play a role in the balance of food reward [7]. For example, GLP-1 neurons have been found to project from the NTS to the VTA and NAc. Furthermore, GLP-1R activation in the VTA and NAc decreases sucrose palatability and reduces operant responding for sucrose reinforcement on a progressive ratio (PR) schedule [7,30]. It was proposed that this circuit provides a negative feedback on reward processes in the VTA and NAc in periods of satiation [7].

Our data suggest that the eating inhibitory effect of sCT uses the same neuronal pathway to influence reward processes in the VTA. Taken together with previous studies showing that AP-X rats consume significantly greater quantities of palatable food than control rats in a short amount of time and work harder under operant conditions for sucrose rewards [14,15,31], it could be suggested that the AP may also be an important nucleus to sense negative feedback and provide input on the mesolimbic system. Further, and similar to lesions of the AP, rats with lesions in the PBN also did not respond to peripheral sCT to

reduce evoked DA release. Hence, the AP-PBN axis may not only mediate amylin's and sCT's effects on eating [12,20,21] but also sCT's influence on the mesolimbic system. In other words, and even though peripheral amylin itself did not have an effect on phasic DA release, our results show the reduction of DA release in the NAc by sCT is mediated through the AP and PBN which provides potential targets for therapeutic anti-obesity agents specifically directed at reward-related eating behavior.

Our results have been obtained in male rats. However, given the prevalence of morbid obesity being higher in women than men [32–34] and that sex related differences in dopaminergic function are believed to play an important role in the observed sex differences in the vulnerability to eating disorders [32,33,35], it will be important in the future to consider the effect of sCT on DA release in females. Estradiol, the main type of estrogen, is a major regulator of both physiological and pathophysiological aspects of eating [32]. Estradiol has also been demonstrated to have significant modulatory effects on DA systems in females but has not been shown to enhance striatal DA release in males [35,36]. Two rat studies investigating the estrogenic modulation of amylin's eating-inhibitory effects have produced contradictory results. In one study, chronic subcutaneous amylin infusion reduced eating more in ovariectomized rats [37]. In the other study, estradiol cyclically increased the satiating potency of acute amylin administration and the desatiating potency of amylin antagonism [38]. Estrogen's effect on the suppression of food intake by sCT has yet to be investigated. However, given that sCT activates the same signaling pathway as amylin and estrogen enhances striatal DA release, it is possible that estrogen may modulate the suppression effect of sCT on both food intake and DA release [18,35]. It is clear there is a need to investigate sex differences in amylin sensitivity in feeding and reward-related eating behavior.

Our study suggests that the primary site of sCT action on the reward system may be via the AP. This finding is in contrast to studies suggesting that sCT may exert a direct action on VTA to modulate reward behaviors [13,39]. First, sugar-evoked DA release in the NAc of rats was reduced in response to sCT administered into the VTA compared to controls [13]. Second, sCT reduced intake of a palatable 15% sucrose solution and reduced the motivation to obtain a sucrose pellets, as measured by operant responding in a progressive ratio task [13,39]. Third, the anorectic effect of peripheral sCT was attenuated by administration of the amylin receptor antagonist, AC187, into the VTA and also by knocking down the CTR in the VTA using adenovirus vectors [39]. Finally, administration of AC187 in the VTA was also found to increase food intake [39].

The following model may perhaps reconcile these apparently contradictory findings between our study and previous studies. A population of PBN neurons has recently been shown to express calcitonin-like gene related peptide (CGRP) [40]. CGRP is another member of the calcitonin family and has also been shown to decrease food intake by decreasing meal size [8,20]. Pharmacological studies show that CGRP also has a high affinity to one subtype of AmyR, the Amy1 receptor that results from the co-expression of CTR and RAMP1 [41,42]. We therefore hypothesize that sCT activated AP neurons project to CGRP-ergic PBN neurons which may then project directly to the VTA or indirectly via the CeA [43]. Because the amylin receptor antagonist AC187 is unable to distinguish between the different

subtypes of AmyR and because sCT potentially activates all subtypes of the AmyR [8,22], this model reconciles our findings with prior reports and may indicate that endogenous CGRP acts as a neurotransmitter on VTA neurons that carry the Amy1R to modulate DA release into the NAc.

Anatomically, the majority of neuronal projections from the NTS-PBN-CeA are predominately ipsilateral [21,44]. Our results show that sCT inhibited DA release in the NAc, ipsilateral to VTA stimulation. The question then arises as to whether sCT would also affect DA release if the PBN lesion were ipsi- or contralateral to the recording site. While the connections involving sCT/amylin signaling may be ipsilateral to the PBN, we expect that the laterality of PBN connections would not affect sCT inhibition on stimulated DA release in the NAc as, ultimately, VTA projections to the NAc are both contralateral and ipsilateral [45,46]. Furthermore, it has recently been shown that dopamine neurons can release dopamine in the contralateral hemisphere and that there is a synchrony of dopamine fluctuations between hemispheres [45]. Therefore, unilateral PBN lesions would be expected to affect dopamine responses to sCT in both hemispheres.

VTA-stimulated phasic DA was found to be less in saline treated PBN-X animals compared to saline treated SHAM rats. However, electrically-evoked release via VTA stimulation can vary widely across animals [16,26,47]. The magnitude of release is dependent on numerous factors including placement of the stimulating electrode, number of release sites that the electrode is capable of detecting, density of dopamine transporters in the vicinity of the electrode, as well as the characteristics of the recording electrode. For this reason, we analyzed data within-animal rather than between animals to interpret sCT effects on DA phasic release. Given the variable baselines found in each study, it is very challenging to conclude the PBN has an excitatory effect on the VTA which results in reduced VTA-stimulated phasic DA release if ablated. This possibility is beyond the scope of the present set of studies but it may be an important point to consider, in particular for future studies in a model of awake and behaving rats, exposed to natural stimuli (e.g. sucrose pellets) rather than electrically evoked DA release.

To summarize, our data support a role for the AP and PBN in influencing food intake and reward signaling. This is important in regards to the effect of obesity for the amylinergic feedback system and its influence on the mesolimbic systems. Given that obesity is fueled in part by a failure to decrease consumption of palatable foods due to their rewarding properties, further studies are warranted to investigate differences in amylin receptor signaling in response to amylin and sCT and utilize these differences for therapeutic interventions.

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Fig. 1.

sCT decreases phasic dopamine (DA) evoked by VTA-stimulation in anesthetized rats.

Representative colorplots depicting current changes (color) as a function of electrode potential (y-axis) and time (x-axis) just before (baseline; 0 min, A,C,E) and 60 min (B,D,F) after injection with saline (A,B), amylin (C,D) or sCT (E,F). The VTA was stimulated 5 s into recording resulting in dopamine release identified by its oxidation ($\sim +0.6$ V; green) and reduction (~ -0.2 V; light yellow). Average dopamine concentration at 0 min and at 60 min after injection of saline (G) amylin (H) and sCT (I) was extracted from individual color plots. Time course showing peak DA (normalized as a percentage of baseline) evoked by VTA-stimulation every 5 min after injection of saline, amylin and sCT (J), $*p < 0.05$ saline vs sCT and $\$p < 0.05$ amylin vs sCT. A representative coronal brain slice showing the typical position of a recording electrode (arrow) in the NAc (K) and the position of the VTA stimulating electrode placement (arrows) (L). Abbreviations: aca, anterior commissure; AMY, amylin; NAc, nucleus accumbens; SAL, saline; sCT, salmon calcitonin; SNR, substantia nigra, VTA, ventral tegmental area. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2.

AP lesions block the decrease in phasic dopamine induced by sCT in anesthetized rats. Representative colorplots depicting current changes (color) as a function of electrode potential (y-axis) and time (x-axis) in SHAM (A,B,C,D) and AP-X (E,F,G,H) rats at 0 min (A,C,E,F) and 60 min (B,D,F,G) after injection with saline (B,F), or sCT (D,H). Average dopamine concentration at 0 min and at 60 min after injection of saline (I,K) and sCT (J,L) was extracted from individual color plots from SHAM (I,J) and AP-X (K,L) rats. The VTA was stimulated 5 s into recording resulting in dopamine release identified by its oxidation (~ 0.6 V; green) and reduction (~ -0.2 V; light yellow). (M) Time course with SHAM and AP-X rats showing peak dopamine (normalized to baseline) evoked by VTA-stimulation every 5 min after injection of saline or sCT. $*p < 0.05$ SHAM/saline vs SHAM/sCT, $\$p < 0.05$ AP-X/sCT vs SHAM/ sCT and $\Phi p < 0.05$ AP-X/saline vs SHAM/sCT. Representative coronal brain slices showing the intact AP of a SHAM rat (N) and the lesioned area of the AP in an AP-X rat (O). Abbreviations: AP, area postrema; AP-X, AP lesioned rats; NTS, nucleus of the solitary tract; SAL; saline; sCT, salmon calcitonin. (For interpretation of the

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Fig. 3.

Bilateral PBN lesions block decrease in phasic dopamine induced by sCT in anesthetized rats. Representative colorplots depicting current changes (color) as a function of electrode potential (y-axis) and time (x-axis) in SHAM (A,B,C,D) and AP-X (E,F,G,H) rats before (0 min, A,C,E,G) and 60 min (B,D,F,H) after injection with saline (B,F), or sCT (D,H). The VTA was stimulated 5 s into recording resulting in dopamine release identified by its oxidation ($\sim +0.6$ V; green) and reduction (~ -0.2 V; light yellow). Average dopamine concentration before (0 min) and at 60 min after injection of saline (I,K) or sCT (J,L) as extracted from individual color plots. (M) Time course with SHAM and PBN-X rats showing peak dopamine (normalized to baseline) evoked by VTA-stimulation every 5 min after injection of saline or sCT. $*p < 0.05$ SHAM/saline vs SHAM/sCT, $\$p < 0.05$ PBN-X/saline vs SHAM/sCT, $\$p < 0.05$ PBN-X/sCT vs SHAM sCT. Representative coronal brain slices showing the intact PBN of a SHAM rat (N, left; O, right) and bilateral lesion areas of the PBN in a PBN-X rat (P, left; Q, right). Abbreviations: PBN, parabrachial nucleus; PBN-X, PBN lesioned rats; SAL, saline; sCT, salmon calcitonin; scp, superior cerebellar peduncle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)