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Behavioral effects of local microinfusion of pituitary adenylate cyclase activating polypeptide (PACAP) into the paraventricular nucleus of the hypothalamus (PVN)

Seth D. Norrholm, Mahasweta Das, and Gábor Légrádi*

Department of Anatomy, College of Medicine, University of South Florida, 12901 Bruce B. Downs Blvd., MDC 6, Tampa, FL 33612-4799, United States

Abstract

Pituitary adenylate cyclase activating polypeptide (PACAP) has been implicated in the regulation of several autonomic and neuroendocrine functions. In the hypothalamic paraventricular nucleus (PVN), for example, PACAP-immunoreactive fibers densely innervate corticotropin-releasing hormone (CRH)-containing neurons in the medial parvocellular region, suggesting that PACAP acts to mediate stress responses. Therefore, we examined the behavioral effects of an intra-PVN PACAP injection (25 pmol) in combination with a mild stressor. PACAP or artificial cerebrospinal fluid (aCSF) was microinjected into the PVN (0.25 μ l) and then animals were restrained or placed in their home cage for 5 min. Exploratory activity (total distance traveled) and scored behaviors (face washing, body grooming, wet dog shakes, and rearing) were observed in a familiar open field for 10 min. In animals receiving aCSF, there were no behavioral differences between restrained and unrestrained groups. For the entire 10-min observation period, animals receiving PACAP, whether restrained or not, displayed elevated face washing and body grooming with decreased locomotor activity and rearing. Among PACAP-injected animals, restrained animals displayed increased body grooming compared to unrestrained animals during the first 2 min in the open field suggesting a summation of the effects of peptide injection and stressor. The observed elevation in grooming is consistent with previous studies reporting similar increases following electrical-, NMDA-, CRH-, or stressor-induced activation of the PVN. Thus, at the level of the PVN, PACAP may act as an excitatory neuropeptide and augment behavioral responses to stressors.

Keywords

Stress; Restraint; HPA axis; PACAP; Grooming; Hypothalamus

1. Introduction

Pituitary adenylate cyclase activating polypeptide (PACAP), a member of the secretin/glucagon/VIP peptide superfamily, is a pleiotropic peptide that functions as a supplementary hypophysiotropic hormone, neuromodulator, vasodilator, and neurotrophic factor [1–3]. Although much has been learned about the molecular biology, cellular action, and the distribution of PACAP, its role in animal behavior is still unclear. Changes in several physiologic parameters and behaviors have been noted following central PACAP administration. Intracerebroventricular (i.c.v.) injection of PACAP suppresses food intake [4, 5], elevates plasma vasopressin and mean arterial blood pressure levels [6], induces

*Corresponding author. Tel.: +1 813 974 5956; fax: +1 813 974 2058. E-mail address: glegradi@hsc.usf.edu (G. Légrádi).

hyperthermia [7], and enhances grooming, rearing, and locomotor activity [4,8–10]. Collectively, these effects suggest participation of hypothalamic sites for the action of PACAP.

The hypothalamus exhibits abundant and widespread expression of PACAP and its receptors [11,12] particularly in the hypothalamic paraventricular nucleus (PVN) which serves as the anatomical core of the hypothalamic–pituitary–adrenocortical axis (HPA axis). Parvocellular neurons of the PVN act on stress-related neural circuits and integrate hormonal, autonomic, and behavioral responses to naturally-occurring stressors [13,14]. The PVN contains high levels of PACAP and its receptors [15,16]. More specifically, PACAP-immunoreactive fibers densely innervate CRH-containing neurons in the medial parvocellular region of the PVN [17]. At the cellular level, central PACAP administration elevates *c-fos*, vasopressin, and CRH gene expression in the PVN [18,19]. Further, we have recently shown that i.c.v. PACAP injection stimulates phosphorylation of the transcription factor CREB in PVN CRH neurons and elevates plasma corticosterone levels (Agarwal et al., submitted for publication). Thus, we hypothesized that PACAP may influence stress-induced behaviors through its activity within the PVN.

Central effects of PACAP mimic many of the behavioral effects of i.c.v. CRH administration or acute stress exposure [20]. Likewise, numerous stressful stimuli, including restraint and immobilization, enhance norepinephrine release from PVN terminals that originate in the lower medulla [21]. Local PACAP perfusion into the PVN also elicits enhanced norepinephrine release [22]. In addition, both mild restraint stress and i.c.v. PACAP administration evokes self-grooming behaviors during the period immediately after restraint or peptide infusion [4, 23], Agarwal et al., submitted]. The purpose of the present study was to investigate the behavioral effects of local PVN PACAP injection in the absence or presence of a mild stressor. Based on the abovementioned literature, we hypothesized that a mild restraint stressor would augment the behavioral effects of local PACAP infusion into the PVN.

2. Materials and methods

2.1. Subjects

Male Sprague Dawley rats (Taconic Farms; 225–250 g) were individually housed and maintained on a 12-hr light dark cycle with food and water available ad libitum. Animals were left undisturbed for 7 days after arrival to habituate them to environmental conditions within the vivarium. All procedures were conducted in strict accordance with the University of South Florida Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and the number of animals used.

2.2. Design and procedure

After the initial 1-week acclimation period, rats were randomly assigned to one of four experimental conditions: aCSF/NO RESTRAINT (control), PACAP injection/No restraint, aCSF injection/Restraint, and PACAP injection/ Restraint. Anesthetized (Ketamine, 90 mg/kg; Xylazine, 10 mg/kg; i.p.) animals were unilaterally implanted with an intracerebral guide cannula (Plastics One, Roanoke, VA, outer diameter 0.56 mm, inner diameter 0.29 mm) above the PVN (coordinates: 1.7 mm caudal to bregma, 0.5 mm lateral to the midline, and 5.4 mm below the skull surface) according to the stereotaxic atlas of Paxinos and Watson [24]. Guide cannulae were fixed to the skull surface with stainless steel anchor screws and cranioplastic cement (Plastics One). Following surgery, Ketoprofen (5 mg/kg) was administered to minimize post-surgical pain and inflammation. Animals were returned to the animal colony upon full recovery from surgical procedures. Experiments were conducted 7–9 days post-surgery and

during the light period of the cycle (1000 and 1430 h). Rats were habituated to the experimental room, infusion procedure, and open field for 5 days prior to the start of the experiments.

2.3. Microinfusion procedure

Animals were infused with PACAP (PACAP38; American Peptide Company, Sunnyvale, CA) or artificial cerebrospinal fluid (aCSF) [25] using a BAS Bee Syringe Pump system (West Lafayette, IN) connected to a 31-gauge internal cannula (Plastics One, outer diameter 0.25 mm, inner diameter 0.125 mm) that was lowered 2.5 mm below the end of the guide cannula to reach the dorsal PVN. PACAP was diluted in aCSF containing 0.05% bovine serum albumin (BSA, Sigma Chemicals, St. Louis, MO) and administered at a dose of 25 pmol in a volume of 0.25 µl injected over a 30-s period. The flow of the fluid was confirmed by observing the movement of a small air bubble (~1 mm) in the polyethylene tubing. Internal cannulae remained inserted for 1 min post-injection to prevent backflow and to allow for diffusion of the peptide.

2.4. Restraint procedure and behavioral testing

Immediately following local infusion of PACAP38 or vehicle into the PVN, animals were returned to their home cage and either exposed to a restraint stressor or left undisturbed for 5 min. The restraint apparatus was constructed from two 250 mL plastic beakers secured together into a tube assembly by large metal clips. Holes were drilled on the sides and at each end of the beakers to allow air flow into the apparatus. Non-restrained control animals were left in their home cage for 5 min. Rats were then placed into a familiar open field (49 cm wide, 46 cm long, 29 cm high) for 10 min. The open field was thoroughly cleaned with 20% EtOH in between each test session.

The movement of the rats during the 10-min open field session was continuously measured by EthoVision 3.0 software (Noldus Information Technology, Leesburg, VA) and simultaneously recorded by a digital video camera. Scored behaviors including face washing, body grooming, rearing, and wet dog shakes were identified using digital video files by an observer blind to the experimental conditions. Face washing was defined as licking of the forepaws followed by repetitive stroking of the face and head. Body grooming was defined as scratching or licking of the back, trunk, abdomen, urogenital region, or tail of the animal. Any vertical movements in which both forepaws of the animal were off of the ground or against the chamber wall were defined as rearing. Behavioral frequencies were measured according to the 15th second sampling procedure previously described [26]. Using this method, the videotaped 10-min post-injection period is divided into 15-s time intervals and the observer determines whether or not a specific behavior occurred during each 15-s interval; thus, the maximum possible score is 40 for the 10-min observation period.

2.5. Histological evaluation of injection sites

Standard Nissl staining by cresyl violet and immunolabeling for PACAP were used to determine both the accuracy of internal cannula placements as well as the spread of the injected synthetic peptide. Tissue preparation for transcardial perfusion and immunohistochemistry was performed according to previously described methods [17]. Upon removal from the open field, animals were deeply anesthetized with sodium pentobarbital (Nembutal, 90 mg/kg, i.p.) and perfused transcardially with heparinized saline followed by solution containing 2% paraformaldehyde and 2.5% acrolein in 0.1 M phosphate buffer (PB). Brains were sectioned at 30 µm on a cryostat and stored in phosphate buffered saline (PBS, pH 7.4). Sections were treated with 1% sodium borohydride in 0.05 M PB, then placed in 0.5% hydrogen peroxide in PBS, preincubated in 10% normal horse serum, and then incubated for 2 days at 4 °C in PACAP antibody (Peninsula Laboratories, Inc., San Carlos, CA) diluted 1:10,000 in 2% normal horse serum containing 0.08% sodium azide and 0.2% Kodak PhotoFlo. PACAP immunoreactivity was visualized with biotinylated donkey anti-rabbit IgG followed by the ABC Elite Kit (Vector,

Burlingame, CA) with diaminobenzidine as the chromogen. Injection sites were evaluated in serial sections of both Nissl stained and immunolabeled tissue. Data from animals with improperly placed cannulae were excluded from statistical analysis. Exclusionary criteria included abnormalities such as bleeding or necrosis, or aberrant injection into surrounding brain regions or ventricular system. Injection sites were also rejected if tissue damage exceeded 0.5 mm in width along or at the end of the cannula tract, as the outer diameter of the injection cannula was 0.25 mm.

2.6. Data analysis

Behavioral data for the entire 10-min open field session following intracerebral injection and restraint procedures were analyzed by two-way analysis of variance (ANOVA) with injection and stressor as between-subjects factors. Repeated measures ANOVAs were used to analyze the time course of the observed user defined behaviors in the open field. As we hypothesized that the effects of intra-PVN PACAP injection would be augmented by presentation of a mild restraint stressor, we established three a priori planned comparisons based on this hypothesis. Specifically, we sought to compare the rearing and grooming behaviors of PACAP-injected, restrained (PACAP/RESTRAINT) animals to aCSF-injected, unrestrained (aCSF/NO RESTRAINT) control animals and PACAP-injected, unrestrained (PACAP/ NO RESTRAINT) animals. These planned comparisons were included because the design of this study is not a 2×2 factorial design per se; the mild restraint stressor was not expected to intrinsically produce robust behavioral effects. The experiment was designed to examine the augmentation of PACAP-induced behavioral effects and, therefore, it was necessary to use a brief restraint session in which the potential for a ceiling effect was reduced. A probability level of 0.05 was accepted as an indicator of statistical significance except in instances where planned comparisons were executed and the critical level was appropriately lowered (Bonferroni technique, 0.05/number of pairwise comparisons).

3. Results

Fig. 1A illustrates a Nissl stained section of a typical injection site. The data reported here reflect local effects of synthetic PACAP on the PVN as confirmed by immunolabeling of injected PACAP using an antibody specific for PACAP38 [27]. This technique also afforded visualization of the spread of injected PACAP into major PVN subdivisions as shown in Fig. 1B. Individual animal data were excluded from analysis if immunoreactivity for injected PACAP spread into surrounding brain regions or the ventricular system.

Local unilateral microinfusion of PACAP (25 pmol) into the PVN increased grooming behaviors and suppressed locomotion and rearing in freely moving rats placed in a familiar environment for 10 min (Fig. 2, panels A–D). Two-way ANOVA demonstrated a main effect of injection on total face washing during the 10-min observation period ($F_{1,25}=13.6, p=0.001$; Fig. 2A). Face washing was significantly elevated in PACAP-injected animals as compared to aCSF-injected animals, regardless of the absence or presence of restraint stressor. In addition, there was a main effect of injection on total body grooming over the 10-min observation session ($F_{1,25}=49.0, p<0.001$; Fig. 2B). Intra- PVN PACAP injection significantly increased total body grooming compared to aCSF-injected control animals irrespective of whether or not animals were restrained. A main effect of injection was also observed with respect to rearing behavior during the 10-min post-injection observation period ($F_{1,25}=32.4, p<0.001$; Fig. 2C). PACAP-injected animals displayed significantly less rearing compared to aCSF-injected control animals over the 10-min observation period. Two-way ANOVA also revealed a main effect of injection on total locomotor activity during the 10-min post-injection period ($F_{1,25}=13.3, p=0.001$; Fig. 2D). Local microinfusion of PACAP into the PVN suppressed locomotor activity in all PACAP-injected animals regardless of exposure to restraint.

In the present study, we hypothesized that the behavioral effects of an intra-PVN PACAP injection would be augmented by exposing injected animals to a mild stressor immediately following peptide injection. For this reason, we analyzed the time course of changes in self-grooming and exploratory behaviors (locomotion, rearing) during the 10-min observation period following release from restraint or home cage. In order to perform a repeated measures analysis, the 10-min open field session following PVN injection and restraint procedures was divided into five 2-min bins. Face washing behaviors were analyzed by repeated measures ANOVA with injection and stressor as between-subjects factors and time as a within-subjects factor. There were no significant two- or three-way interactions with regard to face washing as indicated by this repeated measures analysis. Because our initial hypothesis proposed an additive effect of intra-PVN PACAP injection and mild restraint stress, planned comparisons were made between the aCSF/NO RESTRAINT, PACAP/ NO RESTRAINT, and PACAP/ RESTRAINT groups. The critical value for p was adjusted to account for these three planned comparisons (i.e., $0.05/3=0.017$ according to Bonferroni technique). Face washing was significantly elevated in the PACAP/NO RESTRAINT and PACAP/ RESTRAINT groups compared to the aCSF/NO RESTRAINT control group ($p=0.013$ and $p<0.001$, respectively; Fig. 3A). The PACAP-induced elevation in face washing observed during this initial 2-min time bin was consistent with the main effect of injection on face washing during the entire 10-min session as illustrated by two-way ANOVA.

Repeated measures ANOVA demonstrated a significant time $\times$ injection $\times$ stressor 3-way interaction with respect to body grooming behaviors across the 10-min open field session ($F_{4,100}=2.810$, $p=0.029$). Subsequent two-way ANOVA of body grooming during the first 2 min in the open field indicated main effects for injection ($F_{1,25}=24$, $p<0.001$) and stressor ($F_{1,25}=5.18$, $p=0.032$). A summation effect of PACAP injection and restraint stress was apparent during this time bin as PACAP/RESTRAINT animals groomed significantly more than aCSF/NO RESTRAINT control animals and PACAP/NO RESTRAINT animals ($p<0.001$ and $p=0.025$, respectively; Fig. 3B). Repeated measures ANOVA also indicated a significant three-way time $\times$ injection $\times$ stressor interaction with respect to rearing behavior during the 10-min session following injection and restraint procedures ($F_{4,100}=2.67$, $p=0.037$). A subsequent two-way ANOVA revealed a significant main effect of injection on rearing behavior during the initial 2-min time period in the open field ($F_{1,25}=18.76$, $p<0.001$; Fig. 3C). PACAP-injected animals, regardless of restraint exposure, displayed significantly less rearing during the first 2 min in the open field than aCSF-injected animals. Similarly, two-way ANOVA illustrated a main effect of injection during the first 2 min in the open field with respect to total locomotor activity ($F_{1,25}=11.01$, $p=0.003$; Fig. 3D). PACAP-injected animals, regardless of restraint exposure, traveled significantly less during the first 2 min in the open field than aCSF-injected animals.

The frequency of wet dog shakes was not significantly different between any of the experimental conditions (data not shown).

4. Discussion

4.1. Activation of the PVN by PACAP mimics stress effects

Recent work from our laboratory has shown that intra-cerebroventricular (i.c.v.) PACAP administration increases self-grooming behaviors, such as face washing and body grooming, and elevates exploratory rearing, [Agarwal et al., submitted for publication]. We have further hypothesized that some of these behaviors and the neuroendocrine activation following i.c.v. PACAP infusion are exerted through the PVN, since i.c.v. PACAP at 150 and 300 pmol doses induced activation of parvocellular CRH neurons by the phosphorylation of the transcription factor CREB, and markedly elevated plasma corticosterone levels [Agarwal et al., submitted for publication]. It has also been demonstrated that infusion of PACAP into the PVN evokes

local release of norepinephrine [22], similar to the effect of a number of stressors which act through the biosynthesis and release of CRH from parvocellular neurons in acute stress [21].

Using local microinjections of PACAP into the PVN, the present study demonstrated significant increases in grooming behavior, while locomotion was suppressed. Evaluation of the time course of post-restraint behaviors revealed that the combination of the stressor with intra-PVN PACAP injection further augmented these effects. The current results reinforce the concept that PACAP acts as an excitatory neuropeptide in the PVN capable of producing behavioral effects similar to those evoked by stressor exposure or intra-PVN CRH infusion. Therefore, we suggest that increased PACAPergic transmission in the PVN, as modeled by local microinfusion of synthetic PACAP in the current study, may drive certain behavioral responses to stress. It should also be pointed out that i.c.v. PACAP administration in our studies as well as by others produced a broader spectrum of behavioral manifestations than those reported here following local PVN microinjections. For instance, significant increases were found in wet dog shakes following i.c.v. PACAP at 150 and 300 pmol [Agarwal et al., submitted for publication], which resembles the activation of tachykinin, glutamate, opioid, dopamine, norepinephrine and serotonin systems [28–31], presumably not via the PVN but other periventricular brain regions, such as hippocampus, raphe nuclei and locus coeruleus [31,32]. Likewise, rearing and exploratory activity were enhanced by high (1–2 nmol) [8] or moderate (100–200 pmol) i.c.v. doses of PACAP [9] [Agarwal et al., submitted for publication] as opposed to the inhibited locomotor activity found by the current study following intra-PVN PACAP injections. Hence, our findings further underscore the importance of the exploration of brain-site specific effects of PACAP.

4.2. Grooming response elicited by intra-PVN PACAP and its relation to stress

The elevated grooming behavior induced by intra-PVN PACAP injection is consistent with previous reports of increased face washing and body grooming following electrical-, neurochemical-, or neuropeptide-induced stimulation of the PVN [33–37]. In the present study, the concurrent elevations in both face washing and body grooming elicited by PACAP injections reflect the qualitative nature of the observed grooming bouts. Grooming bouts in PACAP-injected rats began with rapid licking of the forepaws and washing movements over the head, followed by progressive licking of the trunk, anogenital region, hindpaws, and tail, such that grooming was organized in a characteristic cephalocaudal pattern [37]. Bouts of grooming behavior were interrupted by short bursts of locomotor activity and rearing. Conversely, vehicle-injected control animals circled the open field and often displayed rearing interrupted by short grooming bouts, which did not exhibit the aforementioned cephalocaudal pattern. Thus, the decreased locomotor activity observed in PACAP-injected animals appears to be compensated by increased levels of grooming such that local injection of PACAP sets behavioral priorities to grooming over other behaviors in a manner similar to the behavioral patterns elicited by PVN activation.

Previous reports have proposed alternative hypotheses regarding the time course and amount of stressor-induced grooming [38]. Grooming is described as either a necessary condition to reduce arousal following stressor exposure or a rebound of a response that was postponed by the stressor. An evaluation of the time course in the present study indicated that body grooming was significantly elevated in restrained rats receiving intra-PVN PACAP injection during the first 2 min of the open field session. According to van Erp et al. [38], grooming produced by restraint alone begins at a low level and reaches its maximum several minutes after stressor exposure. The current results, however, demonstrate an immediate increase in post-restraint grooming. This most likely reflects PACAP-induced grooming that was delayed, suppressed, or undetected during the restraint procedure. Unrestrained PACAP-injected animals displayed increased face washing and body grooming immediately upon release from restraint as well.

Yet, the early body grooming effect seen in PACAP-injected unrestrained animals was enhanced by co-administration of intra-PVN PACAP and mild restraint. The enhanced early body grooming response seen in restrained PACAP-injected animals is associated with a concurrent decrease in locomotor activity during this period. Thus, it appears that mild restraint augmented the effects of local PACAP infusion into the PVN.

A main objective of the current study was to expose rats to a mild stressor immediately following an intra-PVN injection of PACAP or aCSF. The restraint apparatus used here should be considered mild based on previous reports using restraint stress and due to its short duration [39,40]. Although the restraint session was brief, elevated plasma corticosterone levels were reported following a similar 5-min restraint period [41]. In addition, restrained animals consistently defecated in the restraint indicating increased emotionality in rodents [42].

Enhanced self-grooming behavior has been observed following exposure to many types of stressors including restraint [38,43]. It has been suggested that grooming underlies “dearousal” processes following stressor exposure [23]. In the present study, however, mild restraint stress did not elicit any significant differences in locomotor activity or self-grooming in aCSF-injected animals for the entire 10-min observation period. The lack of a stressor effect was anticipated and reflects a deliberate aspect of the experimental design. We sought to expose the rats to a “subthreshold” restraint stressor to prevent a potential ceiling effect in which the additive effects of peptide injection and stressor were unobservable.

4.3. Open field activity

In the present study, animals were exposed to the open field environment for 5–7 days before the start of experiments during the light (inactive) period of their circadian cycle to reduce the degree of novelty associated with the open field arena. Nevertheless, increased exploratory behavior in otherwise familiar environments reflects a change in environmental conditions (from home cage to open field) and not necessarily a response to novelty [38]. Despite the familiarity of the open field and the time of day, aCSF-injected control animals displayed a high level of exploratory activity that was characterized by predominant rearing and locomotion. Therefore, our experimental design using a familiar open field provided a higher level of baseline locomotor activity to which peptide- and stress-induced behavioral alterations could be compared and alleviated concerns regarding the floor effect often seen when assessing behavior in a familiar environment.

4.4. Dosage specificity

A primary objective of the present study was to determine if PACAP microinjected into the PVN alters behavioral responses to restraint stress. Dosage specificity is crucial to understanding the stress-related effects of PACAP. In order to effectively evaluate an interaction between the neuropeptide injection and stress exposure, it was necessary to use a low dose of PACAP and a mild stressor. In preliminary studies, higher doses of PACAP (≥ 50 pmol) induced a robust grooming effect that masked all other behaviors during the 10-min post-injection period as animals remained in one corner of the open field and groomed continuously. Grooming elicited by higher PACAP doses was characterized by strong cephalocaudal self-grooming that included increased bouts of penile licking. The increased occurrence of penile licking may be indicative of oxytocinergic neuronal activation [44] and suggests that at higher doses, perhaps the oxytocinergic effects of PACAP dominate over other behaviors. Grooming bouts following higher doses of PACAP were qualitatively similar to previous reports describing behavioral sequences produced by hypothalamic stimulation described as “difficult to interrupt” [13]. For these reasons, a lower dose of 25 pmol was used in our experiments.

4.5. Potential targets of PACAP in the PVN area

4.5.1. Parvocellular CRH neurons of the PVN—Intra-PVN infusion of PACAP induced similar, but not identical, behavioral effects to those produced by local infusion of CRH into the PVN. Increases in grooming were observed after CRH injection into the PVN of rats maintained in a familiar environment [36,45]. Monnikes and coworkers [36] observed a dual effect of intra-PVN CRH injection on locomotor activity, as total locomotor activity was elevated following lower doses of CRH but higher doses suppressed movement and increased episodes of freezing. In the present study, we also observed suppressed locomotion, which appeared to be compensated for by increased bouts of grooming. In addition, previous investigation evaluated the effects of local CRH injection in the home cage of the animal whereas we employed a familiar open field to assess post-injection behaviors. The similar effects of intra-PVN injections of PACAP and CRH raise an interesting question with regard to the activation of CRH neurons and PACAP-induced grooming. For example, is the activation of PVN CRH neurons an obligate requirement for PACAP-induced behavioral (and neuroendocrine) effects? To further explore this possibility, future studies may investigate the behavioral and neurobiological results of intra-PVN PACAP administration in combination with central administration of CRH receptor antagonists.

4.5.2. Other targets within the PVN—While local microinjection of PACAP into the PVN affords anatomically quite precise localization and characterization of the peptide's central effects, the grooming response evoked by intra-PVN PACAP administration is expected to be a complex phenomenon. The PVN is divided into magnocellular and parvocellular subdivisions with intrinsic cell groups expressing numerous peptides including vasopressin, oxytocin, CRH, enkephalin, neurotensin and galanin [21,46–48]. PVN regions expressing these peptides also contain PACAP binding sites [16,17,49–51]; which may include the high affinity PACAP type I receptor and the PACAP type II, or VPAC, receptor that exhibits equal affinity for both VIP and PACAP [3,16]. In addition, the PVN and its immediate vicinity contains high numbers of glutamatergic, GABAergic and magnocellular nitric oxide synthase positive neurons which have been implicated in neuroendocrine mechanisms and responses to physiologic challenges, including various types of stressors [52–54]. Therefore, the behavioral effects observed following intra-PVN PACAP injections likely involve the participation of these cell groups and their expressed transmitters, whose exact contribution is not fully known and should become a target of further pharmacologic investigation. For example, previous electrophysiological data have shown that PACAP modulates the activity of vasopressin- and oxytocin-expressing magnocellular cells in the PVN [55].

5. Summary

In conclusion, data from the present study show that microinjection of PACAP into the PVN elevates self-grooming behaviors and suppresses exploratory activity (locomotor activity and rearing). Therefore, PACAP appears to act as an excitatory neuropeptide mimicking previously demonstrated behavioral effects of electrical or neurochemical PVN activation. These effects are also characterized by shifting of behavioral priorities towards self-grooming over other behaviors such as exploration. Evaluation of the time course of PACAP-induced behaviors indicated a cumulative effect of intra-PVN PACAP injection and restraint stress. Thus, PACAP stimulates grooming behaviors likely through activation of the PVN and elevated PACAP levels in this hypothalamic nucleus may amplify behavioral responses to stress exposure. In addition to these novel behavioral findings, the widespread expression of PACAP receptors in the PVN, coupled with the dense innervation of parvocellular CRH neurons by PACAP-immunoreactive fibers support the notion that PACAP's actions may be mediated through CRH neurons. Future studies using selective PACAP receptor antagonists will provide further insight into the putative role of this peptide in regulating neurobiological responses to stress.

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Abbreviations

3V	third ventricle
aCSF	artificial cerebrospinal fluid
BSA	bovine serum albumin
HPA axis	hypothalamic–pituitary–adrenal axis
PVN	paraventricular nucleus of the hypothalamus
PACAP	Pituitary adenylate cyclase activating polypeptide

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Fig 1.

Representative photomicrographs of (A) a Nissl stained tissue section illustrating the PVN corresponding to 1.80 mm posterior to Bregma and (B) an adjacent PACAP-immunolabeled section illustrating an accurate injection site in the PVN. The observed immunoreactivity of the injected synthetic PACAP covered all major subdivisions of the PVN. The efficacy of PACAP antibodies was determined based on the degree of endogenous PACAP labeling on the opposite, uninjected side of the PVN (small arrows). 3V=third ventricle; large arrows indicate the core of the injection site; small arrows indicate endogenous PACAP-immunoreactive nerve fibers in the uninjected side of the PVN.

Fig 2.

Behavioral effects of an intra-PVN injection of aCSF or PACAP (25 pmol) in the absence or presence of mild restraint stress. Events/10 min represents the total number of 15-s time intervals in which the behavior was observed. Acute PACAP injection into the PVN elevated (A) face washing and (B) body grooming while suppressing (C) rearing and (D) locomotor activity. *Significant main effect of injection, $p < 0.05$, $n = 5-11$ animals/group.

Fig 3.

Behavioral effects of an intra-PVN injection of aCSF or PACAP (25 pmol) during the initial 2-min time period following 5-min exposure to restraint session or home cage. Acute PACAP injection into the PVN elevated (A) face washing and (B) body grooming while suppressing (C) rearing and (D) locomotor activity. The presentation of a mild restraint stressor augmented the effects of PACAP injection on body grooming (B). Events/2 min represents the total number of 15-s time intervals in which the behavior was observed. (RES=Restraint); ⁺ $p < 0.05$ vs. aCSF/No RES (CONTROL); [#] $p < 0.05$ vs. PACAP/No RES, $n = 5-11$ animals/group.