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ORIGINAL ARTICLE

Presence of substance P positive terminals on hypothalamic somatostatinergic neurons in humans: the possible morphological substrate of the substance P-modulated growth hormone secretion

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Abstract
Substance P is an undecapeptide affecting the gastrointestinal, cardiovascular, and urinary systems. In the central nervous system, substance P participates in the regulation of pain, learning, memory, and sexual homeostasis. In addition to these effects, previous papers provided solid evidence that substance P exhibits regulatory effects on growth. Indeed, our previous study revealed that growth hormone-releasing hormone (GHRH) neurons appear to be densely innervated by substance P fibers in humans. Since growth hormone secretion is regulated by the antagonistic actions of both GHRH and somatostatin, in the present paper we have examined the possibility that SP may also affect growth via the somatostatinergic system. Therefore, we have studied the putative presence of juxtapositions between the substance P-immunoreactive (IR) and somatostatinergic systems utilizing double label immunohistochemistry combined with high magnification light microscopy with oil immersion objective. In the present study, we have revealed a dense network of substance P-IR axonal varicosities contacting the majority of somatostatin-IR neurons in the human hypothalamus. Somatostatinergic perikarya are often covered by these fiber varicosities that frequently form basket-like encasements with multiple en passant type contacts, particularly in the infundibular nucleus/median eminence and in the basal periventricular area of the tuberal region. In addition, numerous substance-P-somatostatinergic juxtapositions can be found in the basal perifornical zone of the tuberal area. If these contacts are indeed functional synapses, they may represent the morphological substrate of the control of substance P on growth. Indeed, the frequency and density of these juxtapositions indicate that in addition to the regulatory action of substance P on GHRH secretion, substance P also influences growth by regulating hypothalamic somatostatinergic system via direct synaptic contacts.

Keywords Substance P · Somatostatin · Juxtaposition · Immunohistochemistry · Human hypothalamus

Introduction

Substance P is an eleven amino acid neuropeptide discovered almost a century ago due to its ability to cause prompt intestinal contraction (von Euler and Gaddum 1931). Substance P, along with neurokinin A (NKA) and neurokinin B (NKB), is a member tachykinin neuropeptide family and functions as a neurotransmitter and/or neuromodulator. Substance P activates neurokinin 1 (NK1) receptors with high affinity (Mantyh 2002), although it also exhibits low affinity to neurokinin 2 (NK2) and neurokinin 3 (NK3) receptors, which are the targets of NKA and NKB, respectively.

The role of substance P in nociception is well established (Zubrzycka and Janecka 2000), however, substance P is also involved in the physiology and pathophysiology of mood disorders, anxiety, stress (Ebner and Singewald 2006), reinforcement (Huston et al. 1993), neurogenesis (Park et al. 2007), respiratory rhythm (Bonham 1995), neurotoxicity, nausea/emesis (Hesketh 2001), vasodilation (Meeking et al. 2000; Schrauwen and Houvenaghel 1980; Bossaller et al. 1992). Substance P also participates ininflammatory processes [for review see (O'Connor et al. 2004)] that appear

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to be involved in the pathogenesis of Parkinson's disease (Thornton and Vink 2015). Due to the presence of substance P in the hypothalamo-hypophyseal axis (Chawla et al. 1997; Dudas and Merchenthaler 2002, 2006), it is conceivable that substance P may also modulate neuroendocrine functions. Indeed, our previously reported morphological findings suggest that substance P may be involved in the regulation of gonadal functions by direct synaptic contacts formed with gonadotropin-releasing hormone (GnRH) neurons (Dudas and Merchenthaler 2002). Similarly, substance P may also regulate growth, possibly at the hypothalamic levels, by affecting growth hormone (GH) secretion (Arisawa et al. 1989; Aronin et al. 1986; Chihara et al. 1978; Eckstein et al. 1980; Sheppard et al. 1979). Since substance P does not affect basal or stimulated GH release from rat primary pituitary cells in vitro (Cheng et al. 1997), this regulatory action appears to involve the hypothalamus. Indeed, non-peptide substance P antagonist, CP-96,345, fails to influence basal GH release at low concentrations in vitro (Houben and Denef 1993). Moreover, incubation of synthetic substance P with dispersed anterior pituitary cells does not affect GH release (Arisawa et al. 1989), further supporting the concept that substance P modulates GH secretion at the hypothalamic level, via growth hormone-releasing hormone (GHRH) and/or somatostatin secretion.

In our recent studies, we provided evidence that substance P communicates with GHRH neurons in the human hypothalamus and therefore, may regulate GH secretion via modulating GHRH neuronal activity (Uhlman et al. 2019). This conclusion was also supported by physiological findings indicating that substance P modulates GHRH secretion in numerous species (Bitar et al. 1991; Lemamy et al. 2012; Coiro et al. 1992). However, it is conceivable that substance P may also exert regulatory function on growth via the somatostatinergic system. Indeed, substance P dose-dependently increases somatostatin release from hypothalamic blocks in vitro (Sheppard et al. 1979). Decreased plasma GH levels following intracerebroventricular administration of substance P (Arisawa et al. 1989; Aronin et al. 1986; Chihara et al. 1978; Eckstein et al. 1980; Sheppard et al. 1979) can also be explained with the effect of substance P on the periventricularly arranged somatostatinergic neurons via volume transmission. Administration of a substance P antiserum or a specific substance P antagonist, [D-Pro2,D-Trp7,9]-substance P into the 3rd ventricle considerably elevates GH secretion in rats, while intracerebroventricular or intravenous injection of synthetic substance P significantly decreases plasma GH levels (Arisawa et al. 1989). Moreover, the suppressive effect of substance P on serum GH levels, when administered to the lateral ventricle of anesthetized rats, is blocked by somatostatin antiserum (Chihara et al. 1978). The GH-suppressing effect of intraventricularly-administered substance P has also been confirmed in female rhesus monkeys (Eckstein et al. 1980). In contrast, some studies report unaffected somatostatin levels in the portal blood after intracerebroventricular administration of substance P in rats under anesthesia (Abe et al. 1981). These results, coupled with finding that administration of substance P into the 3rd ventricle of conscious rats increases serum GH concentrations (Vijayan and McCann 1980) indicates that the mechanism via somatostatin is involved in the substance-P-regulated GH secretion is complicated and may depend on multiple factors [for review see (Aronin et al. 1986)]. The finding that neurokinin-1 (NK1) knockout mice reproduce and develop normally (Santarelli et al. 2001) raises the possibility of species differences in this regulatory function. To further investigate these apparently contradictory data, and explore the role of substance P on somatostatin release in humans, in the present study we have examined the possibility that substance P fiber varicosities directly innervate somatostatinergic neurons in the human hypothalamus. These hypothetical juxtapositions, in accord with the previously reported substance P-GHRH associations, may represent the morphological substrate of the involvement of substance P in the regulation of growth.

Methods

Hypothalamic samples

Human hypothalami of 1 adult man and 3 women, 75–87 years of age, were harvested from cadavers within less than 12 h post mortem period, in accordance with the regulations of the Institutional Review Board of Lake Erie College of Osteopathic Medicine (LECOM). None of the individuals had neurological or neuroendocrinological disorders in their clinical records.

Tissue preparation

Hypothalami were fixed by immersion in 0.1 M phosphate-buffered 4% formaldehyde at 4 °C for 2–8 weeks. Then, the tissue blocks were trimmed, and the diencephalon was cut in half along the midsagittal line. The blocks were cryoprotected with 30% sucrose in phosphate buffer containing 0.9% sodium chloride (PBS) and 0.15% sodium-azide. After the blocks were sunk in the sucrose solution, they were sectioned on a freezing microtome (American Optical Corp. Model 880) and 35 µm thick sections were prepared. The sections were collected in sequential order into 24-well plastic tissue culture plates containing PBS with 0.2% sodium-azide then stored at 4 °C until processing. Adjacent sections were processed as follows: (1) single label immunohistochemical detection of substance P, (2) single label detection of somatostatin, (3) double label immunohistochemical

detection of substance P (first label) and somatostatin (second label).

Single label immunohistochemistry

Following blocking of non-specific staining in 2.5% normal horse serum (NHS, Vector Laboratories, Burlingame, CA, USA), the sections were incubated in one of the primary antisera: rabbit anti-somatostatin antiserum (Chemicon, Temecula, CA, USA; dilution 1:10,000) and rabbit anti-substance P (Millipore Sigma, Burlington, MA, USA; dilution 1:1000) for 24 h at 4 °C. Subsequently, the sections were incubated in secondary antiserum containing peroxidase-labeled ready-to-use horse anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA; ImPRESS HRP reagent kit) for 1 h at room temperature. Following several changes of wash in phosphate-buffered saline (PBS), the sections were immersed into the chromogen solution until the sufficient staining was achieved (Vector SG chromogen kit; Vector Laboratories, Burlingame, CA, USA).

Double label immunohistochemistry

Double-label immunohistochemistry was carried out by simultaneous detection of the substance P- and somatostatin. The first signal (substance P immunoreactivity) was visualized using the black Vector SG chromogen (Vector Laboratories, Burlingame, CA, USA), and then NovaRed (Vector Laboratories, Burlingame, CA, USA) was utilized to reveal the second signal (somatostatin immunoreactivity). The reason for using these chromogens over fluorescence markers for these studies was due to their stability that makes it possible to store the stained sections for prolonged periods of time. In control sections, where the primary antibodies were omitted or replaced by non-immune rabbit serum at the dilution used for the primary antibodies, no immunoreaction was observed.

Computer-assisted mapping

Maps of the substance P-immunoreactive (IR) and somatostatin-IR structures were utilized to reveal the overlapping areas between the two neurotransmitter systems. The pattern of these systems was also described in preceding studies (Chawla et al. 1997; Dudas and Merchenthaler 2002, 2006; Dudas et al. 2013; Dudas 2017). The accuracy of the distribution of somatostatinergic and substance P-IR and elements has been verified, and the distribution of these neurotransmitter systems showed no significant discrepancy between the published and the present data. To create the maps, the mounted and coverslipped sections were scanned on a digital slide scanner (PathScan Enabler IV, Meyer Instruments Inc.) and the somatostatinergic and substance P-IR neurons were denoted on these hypothalamic slices using an Olympus BX45 microscope equipped with camera lucida and Adobe Photoshop software (Adobe Creative Suite 2.0; CS2). 3D models of the human somatostatin-IR and substance-P-IR systems were created by the computer-generated stacking of the maps of consecutive sections using VoxBlast NT/9 × Version 3.0 Light (Vaytek, Image Analysis Facility, University of Iowa) and then the 3D maps were superimposed to reveal the overlapping areas.

Microscopic analysis

The micrographs of the substance-P-somatostatinergic juxtapositions were taken with an Olympus BX45 microscope equipped with a digital camera (Olympus DP12) and with 100 × oil immersion objective. Adobe Photoshop software (Adobe Creative Suite 2.0; CS2) was used to create composite images from the consecutive micrographs if the neurons were larger than the frame of the camera. Representative coronal sections from each tissue sample containing the somatostatin-IR neuronal subpopulations in the infundibular nucleus and the basal part of the periventricular area of the tuberal region were used to count the somatostatin-IR neurons and analyze their relationship with the substance P-IR fibers. The level of the coronal sections was identical in each sample. Over 1000 neurons were counted in the 4 hypothalamic samples and subsequently they were subdivided into 3 subclasses: densely innervated (more than 5 contacting substance P fiber varicosities) and lightly innervated (1–5 contacts) neurons as well as perikarya that do not appear to receive any abutting substance P fibers.

Results

Diencephalic substance P-IR elements

The distribution and morphology of the substance P-IR elements have been detailed by our previous studies (Dudas and Merchenthaler 2002, 2006; Dudas et al. 2013; Dudas 2017) that have confirmed earlier data (Chawla et al. 1997). Briefly, the vast majority of the substance P-IR perikarya are located in the tuberal region of the human hypothalamus (Fig. 1); few scattered cells can be detected in the periventricular zone of the preoptic area and in the basal part of the posterior hypothalamus. Substance P-IR neurons form several clusters in the tuberal region; subgroups located in the infundibular nucleus/median eminence, the basal part of the periventricular area, the dorsomedial subdivision of the ventromedial nucleus, as well as the basal perifornical area of the tuberal region (Fig. 1).

Substance P-IR perikarya are generally fusiform-shaped with two processes emanating from the opposite poles of

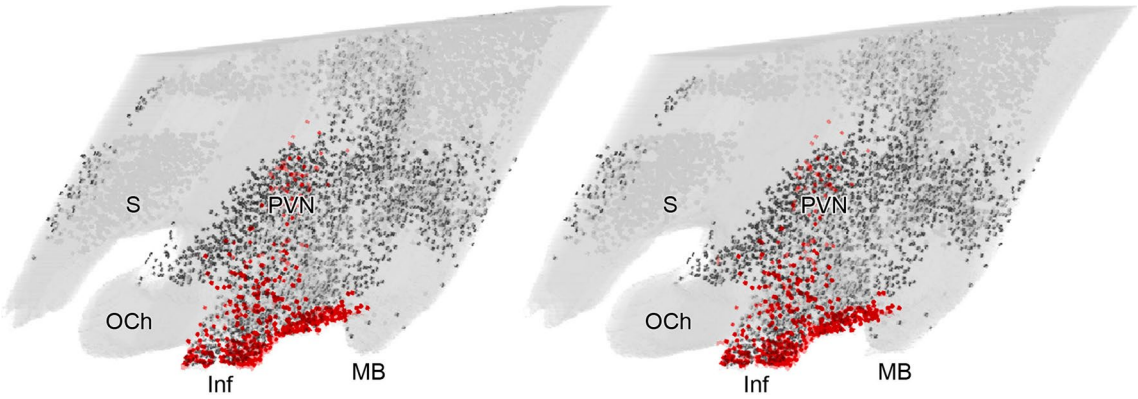

Fig. 1 Stereoscopic images of the human diencephalon illustrating somatostatinergic neurons (black dots) and substance P-IR perikarya (red dots). Stereoscopic images are visualized using U or parallel vision; the eyes are relaxed to look into the distance until the pair of the images fuse, and then refocused by the brain to form a 3D image. Using the parallel vision technique a 3D hypothalamus can be observed to float in front of the paper, with the immunolabeled perikarya (marked by blue and red dots) distinguishable at different depths. *Inf* infundibulum, *MB* mamillary body, *ME* median eminence, *OCh* optic chiasm, *S* septal area

the spindle-shaped cell body. Numerous multipolar cells can also be observed in the dorsomedial subdivision of the ventromedial nucleus. In the infundibulum/hypophyseal stalk, dense network of substance P-IR fiber varicosities surrounds the portal vessels. At the preoptic and tuberal regions, substance P-IR axonal varicosities are typically periventricularly arranged, running parallel with the surface of the 3rd ventricle; numerous substance P-IR fibers can also be detected over the optic tract in the basal part of the lateral hypothalamus as well as surrounding the anterior commissure and the fornix and passing along the diagonal band of Broca. The paraventricular nucleus also contains occasional fibers. Numerous associations can be observed between the substance P neural elements in the human hypothalamus; substance P-IR fiber varicosities occasionally abut on the surface of substance P-IR perikarya forming multiple contacts; in addition, fusiform neurons that are apparently not substance P-IR are sporadically surrounded by substance P-IR fibers in the periventricular zone.

Human somatostatinergic system

The pattern and morphology of the somatostatin-IR neural elements have been described in our previous studies (Dudas et al. 2013; Dudas 2017; Proudan et al. 2015). Briefly, a considerable majority of the somatostatin-IR perikarya are located in the infundibulum/median eminence (arcuate nucleus; Figs. 1, 2c, d, f, j, k) and in the periventricular zone of the preoptic and infundibular area (Figs. 1, 2a, b, g). Somatostatinergic cell bodies also populate the ventromedial and suprachiasmatic nuclei as well as the nucleus of the diagonal band of Broca. Several somatostatinergic perikarya can be observed in the paraventricular nuclei while the supraoptic nucleus contains only insignificant amount of somatostatinergic axonal varicosities without detectable perikarya. Somatostatin-IR cell bodies are also located perifornically in the tuberal region (Figs. 1, 2e, i, h) and at the mammillary and the supramammillary nuclei. The lateral hypothalamus contains scattered somatostatinergic cells, predominantly at the infundibular and posterior hypothalamic regions. Occasional perikarya along with fiber varicosities can be observed in the lamina terminalis.

Somatostatin-IR axonal varicosities populate the periventricular zone of the preoptic and infundibular regions, where these fibers are typically arranged parallel with the ependymal surface of the 3rd ventricle forming a discrete bundle that appears to originate from the periventricular perikarya and extends towards the infundibulum. Indeed, somatostatinergic axons densely populate the infundibulum/median eminence where they can be often observed in intimate relationship with the portal vessels. In the periventricular region, somatostatinergic fibers also form occasional, well-defined terminal fields around neurons that are obviously not immunoreactive for somatostatin. Towards the lateral zones of the hypothalamus somatostatin-IR axonal varicosities are less frequent; medial hypothalamic regions contain only few somatostatin-IR fibers.

Substance P-somatostatinergic associations

Substance P-IR fiber varicosities often form contacts with somatostatinergic neurons. Most of these juxtapositions are located in the infundibulum/median eminence (Fig. 2c, d, f, j, k). These contacts are typically en passant type associations, where substance P fibers form multiple contacts with somatostatinergic neurons while passing by. Substance P axonal varicosities often surround the entire somatostatinergic perikaryon and the emanating axons/dendrites while

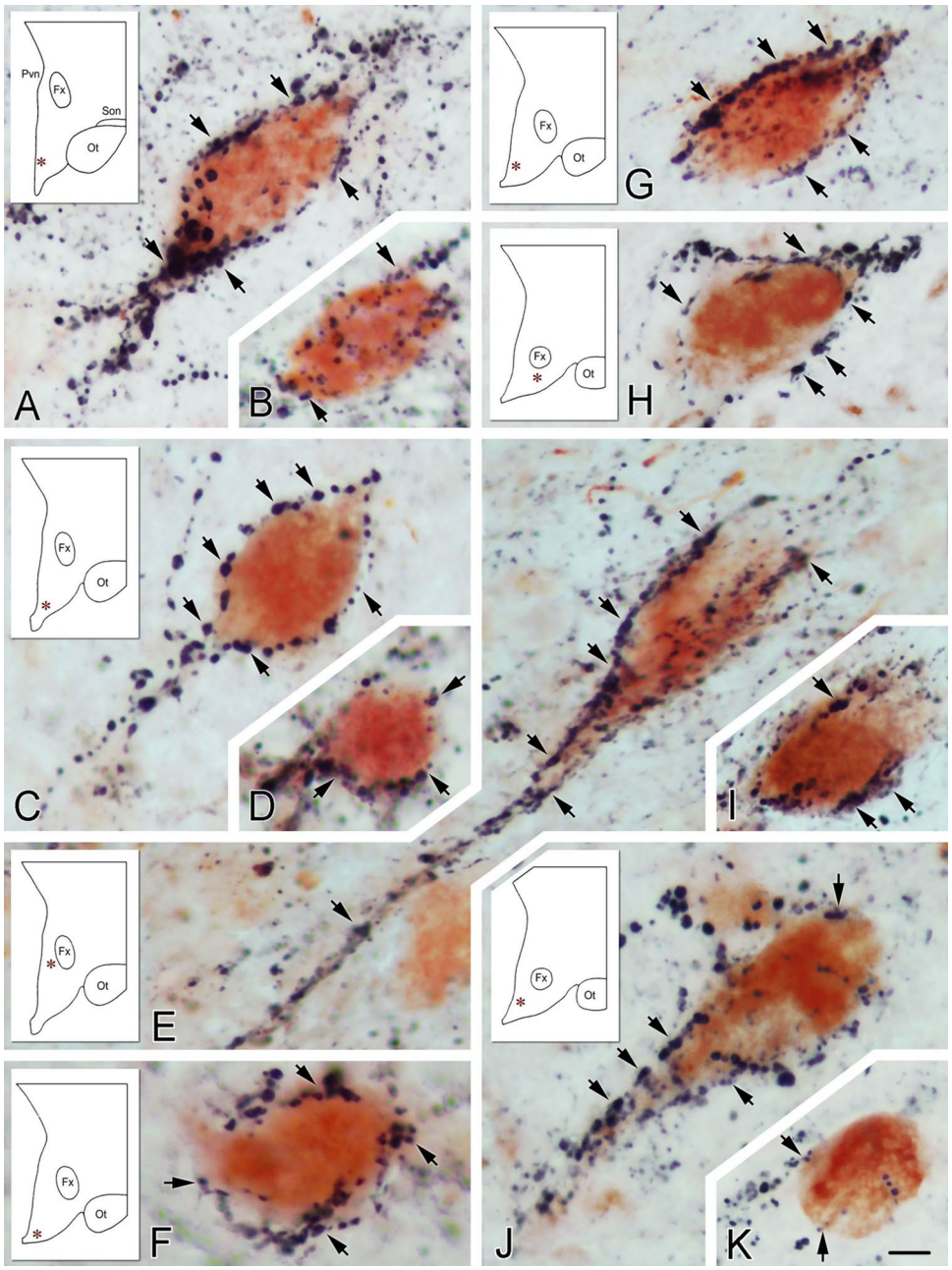

Fig. 2 Juxtapositions between the substance P-IR (black) and somatostatinergic (brown) neural elements are revealed using double-label immunohistochemistry in the human diencephalon. The substance P-IR fiber varicosities often abut on the somatostatinergic neurons forming numerous en passant type contacts. Asterisks on the coronal sections of the diencephalon in the corners of the micrographs denote the positions of the demonstrated somatostatinergic cells. The thickness of the sections is 30 µm. Scale bar 20 µm. *Fx* fornix, *Mb* mammillary body, *Pvn* paraventricular nucleus, *Ot* optic tract, *Son* supraoptic nucleus

passing by, characteristically following the contours of somatostinergic neurons and covering a significant amount of surface area with basket-like encasements (Fig. 2). This dense innervation with more than 5 contacts/cell is characteristic for the 32% of the counted somatostatinergic neurons, while the remaining somatostatinergic cells appear to be lightly innervated, receiving 1–5 contacting substance P-IR fibers (43% of the counted neurons), or did not appear to be associated with substance P-IR fibers at all (25% of the counted neurons).

Somatostatinergic neurons associated with dense network of substance P-IR axonal varicosities are present characteristically in the infundibular nucleus/median eminence (Fig. 2c, d, f, j, k) and in the basal periventricular area of the tuberal region (Fig. 2a, b, g). Several substance-P-somatostatinergic juxtapositions can be observed in the basal perifornical area of the tuberal zone (Fig. 2e, i, h) while the sparse substance P-IR fibers in the posterior hypothalamus typically avoid somatostatinergic perikarya. By analysing of the detected juxtapositions with high magnification utilizing oil immersion objectives, no gaps can be observed between the contacting elements. The patterns of innervation of the individual samples do not show significant gender/age differences.

Discussion

Substance P is a neurotransmitter/neuromodulator that appears to regulate pivotal hypothalamic functions including gonadal homeostasis and growth. Despite the significant amount of data, however, the exact mechanism by which substance P modulates GH secretion remains elusive. In our previous study, we have reported that substance P-IR axonal varicosities appear to directly innervate GHRH perikarya in the human hypothalamus (Uhlman et al. 2019). Although the data regarding the involvement of somatostatin in the substance P-regulated GH release are rather contradictory, in the present study we examined the possibility that substance P fibers, similarly to the GHRH-IR cells, may also directly innervate somatostatinergic neurons in humans.

Indeed, we observed that somatostatin-IR neurons, similarly to GHRH-IR neurons, were often heavily contacted by substance P-IR fiber varicosities that have covered significant area of their surface, often forming fiber baskets around the somatostatinergic cells, primarily in the periventricular area of the hypothalamus and in the infundibulum/median eminence (Fig. 2). Substance P-IR axons typically form en passant type contacts with somatostatin-IR perikarya with multiple juxtapositions while passing by, following the contours of the perikarya and often the emanating axons as well.

Although substance P may regulate GH secretion through multiple sites and mechanisms (see below), our present study provides a strong support for an action of substance P via not only the GHRH system but also via the somatostatinergic system in humans. These regulatory mechanisms may counteract each other and indicate the pivotal role of substance P in the control of somatic growth.

Regulation of GH secretion by substance P at the level of the hypophysis

Although previous data are rather contradictory, former studies suggest that substance P could directly act on hypophyseal somatotropic cells to induce GH release. Substance P-IR axons and nerve terminals have been found close to somatotrop cells in rats (Mikkelsen et al. 1989) and monkeys (Ju and Liu 1989). Moreover, substance P and its mRNA were detected in rat anterior pituitary (Mikkelsen et al. 1989) and substance P-IR cells were found in the anterior pituitary gland in sheep (Skinner et al. 2009). A hypophyseal action of substance P is also supported by the presence of NK1 receptors in human hypophysis (Wormald et al. 1989) and more recently, tachykinin NK2 receptors have also been characterized in the anterior pituitary gland in rats (Pisera et al. 2003). These studies indicate a paracrine interaction between substance P and somatotrops. However, there are serious contradictions outlined by former data that do not support the action of substance P at the level of the hypophysis; substance P does not affect basal or stimulated GH release from rat pituitary cells in vitro (Arisawa et al. 1989; Cheng et al. 1997) and the nonpeptide substance P antagonist, CP-96,345, does not influence basal GH release at low concentrations in vitro (Houben and Denef 1993), suggesting that substance P influences GH secretion at the hypothalamic level.

Regulation of GH secretion by substance P at the hypothalamic level

Although the action of substance P at the hypohyseal level remains elusive; there are several studies supporting the action of substance P on GH secretion via the hypothalamus by interacting with GHRH-IR and/or somatostatinergic systems. For example, intravenously administered substance P in rams caused an elevation in GHRH levels in hypophyseal portal blood (Lemamy et al. 2012) suggesting that the GH release by substance P could occur via a central, i.e. hypothalamic mechanism, by stimulating GHRH release. Such a hypothesis is supported by the presence of substance P-IR fibers in close proximity to GHRH neurons in the rat hypothalamic arcuate nucleus (Magoul et al. 1993) and the median eminence (Mikkelsen et al. 1989) as well as in the primate hypothalamus (Hokfelt et al. 1978). We have found a morphological substrate of this connection in the human hypothalamus where substance P-IR nerve terminals densely surrounded GHRH-IR perikaryal in the infundibulum (Uhlman et al. 2019). Interestingly, in the studies of Lemamy et al. (2012) intravenous injection of substance P elevated the levels of GHRH but decreased the levels of somatostatin in the hypophysial portal blood. It is known that the activity of GHRH neurons is inhibited by somatostatinergic afferents (Yamauchi et al. 1991) and it is possible that the levated GHRH levels were due to reduced action of somatostatin caused by substance P in the hypothalamus. Surprisingly, in the studies of Lemamy et al. (2012), the substance P stimulating action on GH and GHRH release was blocked by a specific non-peptidic NK2 tachykinin receptor antagonist (SR48968-Sanofi, Montpellier, France), whereas a specific non-peptidic NK1 tachykinin receptor antagonist (SR140333, Sanofi, Montpellier, France) failed to modify GH and GHRH secretions when it was infused in the same conditions as NK2 receptor antagonist. These data suggest that the action of substance P on GH and GHRH release is mediated, at least in part, by tachykinin NK2 receptor in sheep. Unfortunately, no data can be found on the presence

of neurons containing NK1 receptor in the human hypothalamus, despite the expression of NK1 receptors in cortex, striatum and brainstem (Tooney et al. 2000a, b).

In summary, in the present study we have provided evidence that substance P-IR nerve terminals occupy a large surface area of somatostatin-IR neurons in the human hypothalamus, suggesting that the substance P-IR nerve terminals function as synapses regulating the activity of somatostatin neurons and subsequently controlling hypophyseal GH secretion. These data strongly indicate that substance P controls growth through GH secretion not only via stimulating GHRH secretion but also via exhibiting inhibitory action on somatostatin release in humans.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants The brain utilized in these studies was harvested 12 h post mortem period in accordance with the regulations of the Institutional Review Board of Lake Erie College of Osteopathic Medicine (LECOM). See materials and methods.

References

Abe H, Chihara K, Chiba T, Matsukura S, Fujita T (1981) Effect of intraventricular injection of neurotensin and other various bioactive peptides on plasma immunoreactive somatostatin levels in rat hypophysial portal blood. Endocrinology 108:1939–1943

Arisawa M, Snyder GD, De PL, Ho RH, Xu RK, Pan G et al (1989) Role of substance P in suppressing growth hormone release in the rat. Proc Natl Acad Sci USA 86:7290–7294

Aronin N, Coslovsky R, Leeman SE (1986) Substance P and neurotensin: their roles in the regulation of anterior pituitary function. Annu Rev Physiol 48:537–549

Bitar KG, Bowers CY, Coy DH (1991) Effect of substance P/bombesin antagonists on the release of growth hormone by GHRP and GHRH. Biochem Biophys Res Commun 180:156–161

Bonham AC (1995) Neurotransmitters in the CNS control of breathing. Respir Physiol 101:219–230

Bossaller C, Reither K, Hehlert-Friedrich C, uch-Schwelk W, Graf K, Grafe M et al (1992) In vivo measurement of endothelium-dependent vasodilation with substance P in man. Herz 17:284–290

Chawla MK, Gutierrez GM, Young WS, McMullen NT, Rance NE (1997) Localization of neurons expressing substance P and neurokinin B gene transcripts in the human hypothalamus and basal forebrain. J Comp Neurol 384:429–442

Cheng K, Wei L, Chaung LY, Chan WW, Butler B, Smith RG (1997) Inhibition of L-692,429-stimulated rat growth hormone release by a weak substance P antagonist: L-756,867. J Endocrinol 152:155–158

Chihara K, Arimura A, Coy DH, Schally AV (1978) Studies on the interaction of endorphins, substance P. and endogenous somatostatin in growth hormone and prolactin release in rats. Endocrinology 102:281–290

Coiro V, Volpi R, Capretti L, Speroni G, Bocchi R, Caffarri G et al (1992) Intravenously infused substance P enhances basal and growth hormone (GH) releasing hormone-stimulated GH secretion in normal men. Peptides 13:843–846

Dudas B (2017) Central regulation of growth, 1st edn. NOVA Biomedical, New York

Dudas B, Merchenthaler I (2002) Close juxtapositions between LHRH immunoreactive neurons and substance P immunoreactive axons in the human diencephalon. J Clin Endocrinol Metab 87:2946–2953

Dudas B, Merchenthaler I (2006) Three-dimensional representation of the neurotransmitter systems of the human hypothalamus: inputs of the gonadotrophin hormone-releasing hormone neuronal system. J Neuroendocrinol 18:79–95

Dudas B, Peroski MS, Merchenthaler I (2013) Hypothalamic regulation of growth. In: Dudas B (ed) The human hypothalamus: anatomy, functions and disorders. Nova Biomedical, New York, pp 173–190

Ebner K, Singewald N (2006) The role of substance P in stress and anxiety responses. Amino Acids 31:251–272

Eckstein N, Wehrenberg WB, Louis K, Carmel PW, Zimmermann EA, Frantz AG et al (1980) Effects of substance P on anterior pituitary secretion in the female rhesus monkey. Neuroendocrinology 31:338–342

Hesketh PJ (2001) Potential role of the NK1 receptor antagonists in chemotherapy-induced nausea and vomiting. Support Care Cancer 9:350–354

Hokfelt T, Pernow B, Nilsson G, Wetterberg L, Goldstein M, Jeffcoate SL (1978) Dense plexus of substance P immunoreactive nerve terminals in eminentia medialis of the primate hypothalamus. Proc Natl Acad Sci USA 75(2):1013–1015

Houben H, Denef C (1993) Unexpected effects of peptide and non-peptide substance P receptor antagonists on basal prolactin and growth hormone release in vitro. Peptides 14:109–115

Huston JP, Hasenohrl RU, Boix F, Gerhardt P, Schwarting RK (1993) Sequence-specific effects of neurokinin substance P on memory, reinforcement, and brain dopamine activity. Psychopharmacology 112:147–162

Ju G, Liu S (1989) Relationship of Substance P-lmmunoreactive Nerve Fibers with Somatotropes of the Anterior Pituitary in the Monkey. J Neuroendocrinol 1(6):397–400

Lemamy GJ, Guillaume V, Ndeboko B, Mouecoucou J, Oliver C (2012) Substance P stimulates growth hormone (GH) and GH-releasing hormone (GHRH) secretions through tachykinin NK2 receptors in sheep. Peptides 35:60–64

Magoul R, Onteniente B, Benjelloun W, Tramu G (1993) Tachykinergic afferents to the rat arcuate nucleus. A combined immunohistochemical and retrograde tracing study. Peptides 14:275–286

Mantyh PW (2002) Neurobiology of substance P and the NK1 receptor. J Clin Psychiatry 63(Suppl 11):6–10

Meeking DR, Allard S, Munday J, Chowienczyk PJ, Shaw KM, Cummings MH (2000) Comparison of vasodilator effects of substance P in human forearm vessels of normoalbuminuric Type 1 diabetic and non-diabetic subjects. Diabet Med 17:243–246

Mikkelsen JD, Larsen PJ, Moller M, Vilhardt H, Saermark T (1989) Substance p in the median eminence and pituitary of the rat: demonstration of immunoreactive fibers and specific binding sites. Neuroendocrinology 50:100–108

O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F (2004) The role of substance P in inflammatory disease. J Cell Physiol 201:167–180

Park SW, Yan YP, Satriotomo I, Vemuganti R, Dempsey RJ (2007) Substance P is a promoter of adult neural progenitor cell proliferation under normal and ischemic conditions. J Neurosurg 107:593–599

Pisera D, Candolfi M, De Laurentiis A, Seilicovich A (2003) Characterization of tachykinin NK2 receptor in the anterior pituitary gland. Life Sci 73(19):2421–2432

Proudan N, Peroski M, Grignol G, Merchenthaler I, Dudas B (2015) Juxtapositions between the somatostatinergic and growth hormone-releasing hormone (GHRH) neurons in the human hypothalamus. Neuroscience 297:205–210

Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P, Hen R et al (2001) Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. Proc Natl Acad Sci USA 98:1912–1917

Schrauwen E, Houvenaghel A (1980) Substance P: a powerful intestinal vasodilator in the pig. Pflugers Arch 386:281–284

Sheppard MC, Kronheim S, Pimstone BL (1979) Effect of substance P, neurotensin and the enkephalins on somatostatin release from the rat hypothalamus in vitro. J Neurochem 32:647–649

Skinner DC, Lang AL, Pahl L, Wang Q (2009) Substance P-immunoreactive cells in the ovine pars tuberalis. Neuroendocrinology 89(1):3–8

Thornton E, Vink R (2015) Substance P and its tachykinin NK1 receptor: a novel neuroprotective target for Parkinson's disease. Neural Regen. Res. 10:1403–1405

Tooney PA, Au GG, Chahl LA (2000a) Localisation of tachykinin NK1 and NK3 receptors in the human prefrontal and visual cortex. Neurosci Lett 283:185–188

Tooney PA, Au GG, Chahl LA (2000b) Tachykinin NK1 and NK3 receptors in the prefrontal cortex of the human brain. Clin Exp Pharmacol Physiol 27:947–949

Uhlman D, Nguyen T, Grignol G, Merchenthaler I, Dudas B (2019) Substance P appears to affect growth via growth hormone-releasing hormone (GHRH) neurons in the human hypothalamus. Brain Struct Funct 224(6):2079–2085

Vijayan E, McCann SM (1980) Effects of substance P and neurotensin on growth hormone and thyrotropin release in vivo and in vitro. Life Sci 26:321–327

von Euler US, Gaddum JH (1931) An unidentified depressor substance in certain tissue extracts. J. Physiol 72:74–87

Wormald PJ, Millar RP, Kerdelhue B (1989) Substance P receptors in human pituitary: a potential inhibitor of luteinizing hormone secretion. J Clin Endocrinol Metab 69(3):612–615

Yamauchi N, Shibasaki T, Ling N, Demura H (1991) In vitro release of growth hormone-releasing factor (GRF) from the hypothalamus: somatostatin inhibits GRF release. Regul Pept 33:71–78

Zubrzycka M, Janecka A (2000) Substance P: transmitter of nociception (Minireview). Endocr Regul 34:195–201