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SOMATOSTATIN (SRIF) LIKE IMMUNOREACTIVITY IN MEDIAN EMINENCE (ME) OF FEMALE RAT BRAIN: EVIDENCE OF COMPARTMENTALIZATION IN ME

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Abstract : Somatostatin (SRIF) immunoreactivity was observed in rostro-caudal extent (Bregma levels-1.8 mm to -3.8 mm) of the median eminence (ME) in female rat brain using Avidin-Biotin Complex (ABC) method (Hsu et al, 1981). SRIF immunoreactivity (IR) was observed in entire-rostro-caudal extent of both internal (IZ) and external zone (EZ) of ME. Image analysis of SRIF stained sections showed that in rostral ME (Bregma -1.8 to -2.3 mm) dense immunoreactive nerve terminals were observed in EZ. In medial ME (Bregma-2.3 mm to 3.3 mm) SRIF-IR was low in IZ and dense in EZ. In this region dense immunoreactive nerve terminals were observed in lateral margin of EZ. In caudal ME (Bregma -3.3 mm to -3.8 mm) nerve terminals in lateral EZ and median IZ and EZ showed dense reactivity in nerve terminals. These results led us to hypothesize that each region-lateral IZ and EZ and medial IZ and EZ are independent functional units in ME. Six functionally independent compartments could be identified-Compartment I and III of IZ and II and IV in EZ (Lateral margins in ME), Compartment V (IZ) and Compartment VI (medial EZ).

Key words : SRIF ME compartments

INTRODUCTION

Pioneering work of Harris (1) provided evidence that secretion of anterior pituitary hormones is controlled by chemical messengers which are released from nerve endings into hypophyseal portal vessels in median eminence (ME). Conrad and Pfaf (2), Hofman and Hayes (3), Kawano and Diakoku (4), Makara et al (8), Ibata et al (6), Ishikawa et al (7), Leshin et al (8), Romero and Phelps (9), Bhatnagar et al (10), Bhatnagar et al (11) and Bhatnagar et al (12) have demostrated that the specific cell bodies located around periventricular zone (PeV) send their processes to IZ (Internal zone) and EZ (External zone) in ME. These cell bodies are known to secrete large number of physiotropic factors (release and release inhibiting factors), which are discharged by the nerve endings in both the zones of ME and are then carried away by portal circulation to anterior lobe of pituitary (13, 14). Earlier, immunocytochemical studies have demonstrated that factors which are

synthesized in hypothalamic cell bodies and reach portal vessels via nerve terminals are distributed differentially in ME. DYN (Dynorphin). TH (Tyrosine hydroxiase) and NT (Neurotensin; Meister et al, 15), NPY (Neuropeptide Y; Ciofi et al, 16), SRIF (Leshin et al, 17) and GHRH (Growth hormone release hormone; Liposits et al, 18) were localized in lateral EZ white NT (Meister et al, 15) GAD (Glutamic acid decarboxyase; Meister and Hokfelt, 19) and GHRH (Vandepol, 20) immunoreactive terminals were reported in medial EZ. Presence of GHRH (6, 18) and GAL (21) positive terminals were reported in IZ. LHRH (Leuteotrophic release hormone; Merchanthaler et al, 21) immunoreactive terminals were found throughout the anterior and posterior extentions of ME. Such a distribution of the physiotropic factors in ME led us to hypothesize that ME is divided into specific, functionally independent compartments.

In the present study, using SRIF immunoreactivity as an index, attempts have been made to elucidate differential distribution of SRIF immunoreactivity in specific locales (Compartments) in the rostro-caudal extent of ME.

METHODS

Adult female rats (n=5) Charls River, BW 250 ± 5 gm were maintained (in plastic cages) in complete NS (non stress) conditions (12 hrs light and 12 hrs dark cycle at 22 ± 2°C) for one week after receiving them from supplier. After 7 days, animals were placed in separate cages and animal room was locked for 24 hrs. Just after opening the animal room, rats were killed by decapitation (within 20 sec.) without anesthesia. Brain was dissected out, placed on chilled glass plate and desired portion was cut. Tissue was immersed in freshly prepared, chilled, 0.4% paraformaldehyde prepared in Phosphate buffer saline (0.1M, PBS, pH 7.5). Fixation was carried out at 4°C for 12 hrs. Perfusion fixation was avoided as this method is known to cause stress to animals which release excess SRIF from nerve terminals. Brains were rinsed in buffer several times and 50 µm thick sections were cut on Vibratome. Sections were rinsed several times in PBS and were subjected to SRIF immunolocalization according to Avidin-Biotin Complex (ABC) method of Hsu et al (23). The SRIF antiserum was generated (by Prof. G. Nilaver, OHSU, Portland) by immunizing rabbits with synthetic SRIF (1-14) conjugated with bovinethyroglobulin. Preliminary studies to carry out antibody titration assay to determine optimum dilution for obtaining specific reactivity was carried out on sections of the brain of the one control rat. 1 : 1000 dilution of the antisera in TBS (containing Triton X and BSA) was accepted for immunostaining. The sections were incubated for 12 hr at 4°C in primary antisera at dilution of 1 : 1000; the incubation medium consisted of TBS, pH 7.6; 0.1% Triton X 100 and 0.1% bovine serum albumin. After incubation, the section were rinsed in TBS, pH 7.6, three times for 10 minutes and transferred to biotinylated goat anti-rabbit serum (Vector Labs, Burlingame, CA), diluted 1 : 400 and incubated for 1 hr at room temperature. The sections were then rinsed three times for 10 min in TBS, pH 7.6 and incubated in ABC complex (diluted 1 : 1000 in TBS) for 1 hr at room temperature. The ABC complex was

prepared 5 min prior to use by mixing together equal parts (dilution 1 : 1000) of stock Avidin DM and Biotinylated peroxidase reagents provided in the Vectastatin ABC kit (Vector Labs, Burlingame, CA). Reaction products were then developed with 0.15 mg% 3'-3' diaminobenzidine hydrochloride (DAB, Sigma, USA). Following the development of brown reaction product, the sections were thorughly washed with TBS, pH 7.6, dehydrated in xylene and mounted under coverslip in permount.

Several control procedures were used to determine the specificity of the SRIF immunoreactivity. A 1 : 1000 dilution of SRIF antiserum was incubated with 10 (g of synthetic SRIF, (Peninsula Labs, USA) at 4°C for 24 hrs prior to incubation of the sections. Staining in these sections was completely abolished. Other controls included substitution of normal rabbit serum for the primary antiserum and also the omission of primary antibody in the incubating medium. Again staining was absent in both cases. As a final control reaction, sections from all experimental groups were processed together in all of the ICC steps.

Image analysis : Immunostained sections were subjected to image analysis by using IBAS 2000 (Carl Zeiss Inc., Germany). This instrument detected the variation in SRIF immunostaining in various regions of ME and projected this variation in terms of grey tone scale on computer screen. Colour pattern of immunostaining appear on computer screen represented the dense (+++), weak (++) or negative (-) SRIF immunoreactive terminals in ME.

RESULTS

Distribution of SRIF immunoreactivity in the ME was studied in serial sections passing through rostro-caudal extent (Bregma level -2.3 mm to -3.3 mm) of ME (Figs. 2, 4, 6) in the hypothalamic region of the rat brain. Each SRIF immunostained section was subjected to image analysis to identify variation in SRIF immunostaining (Figs. 1, 3 and 5). Identification of various levels and SRIF immunostained areas were based on stereotaxic atlas of rat brain by Paxinos and Watson (21). Representative sections of the the brain passing through anterior-posterior extentions of ME are discussed here.

Bregma-1.8 mm to -2.3 mm

This section is passing through rostral ME. SRIF immunoreactive terminals were observed in both upper IZ and lower EZ (Fig. 2). Image analysis of the SRIF immunoreactivity revealed that dense plexesus of SRIF terminals were located in lateral and medial EZ. In IZ comparatively week immunoreactive terminals were observed (Fig. 1).

Bregma-2.3 mm to -3.3 mm :

SRIF immunoreactive terminals were observed in both IZ and EZ in ME (Fig. 4). Image analysis of the section demonstrated that dense accumulation of immunoreactive terminals was mainly in medial IZ and lateral EZ (Fig. 3).

Bregma-3.3 mm to -3.8 mm :

In this section as well, SRIF immunoreactive terminals were observed

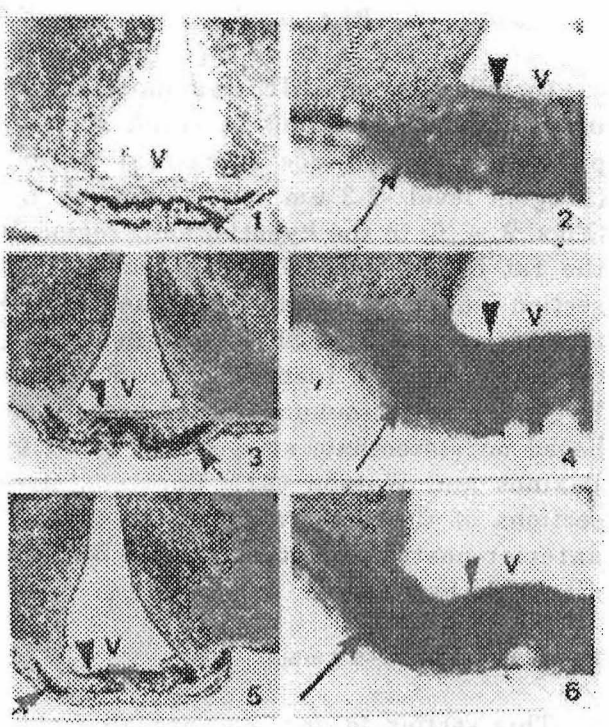

Fig. 1 : Photomicrograph demonstrating computer screen image of the SRIF immuno-staining in rostral level (Bregma level -1.8 mm) of ME, in image analysis. Note accumulation of dense nerve terminals in EZ (arrow) only 40 X.

Fig. 2 : Photomicrograph of the SRIF stained passing through postral ME (Bregma -1.8 mm). Note immunoreactive terminals in both IZ (arrow head) EZ (arrow). 60 X.

Fig. 3 : Photomicrograph demonstrating computer screen image of the SRIF immunostaining in medial level (Bregma level -2.3 mm) of ME in image analysis. Note dense immunoreactive terminals in both medial IZ (arrow head) and in lateral EZ (arrow). 40 X.

Fig. 4 : Photomicrograph of the SRIF immunostained section passing through medial ME (Bregma -2.3 mm). 60 X.

Fig. 5 : Photomicrograph demonstrating computer screen image of SRIF immunostaining in caudal level. (Bregma level -3.3 mm) of ME in image analysis. Note dense accumulation of nerve terminals in medial IZ (arrow head) and in lateral EZ (arrow). 40 X.

Fig. 6 : Photomicrograph of the SRIF immunostaining in section passing through caudal level of ME (Bregma -3.3 mm). Note dense immunoreactive nerve terminals in both IZ and EZ. 60 X.

TABLE I : SRIF immunoreactivity in various regions of ME.

S. No.	*Bregma level*	*Median Eminence*					
		IZ			*EZ*		
		Lateral	*Medial*	*Caudal*	*Lateral*	*Medial*	*Caudal*
1	−1.8 mm to −2.3 mm	++	++	+++	+++	+++	
2.	−2.3 mm to −3.3 mm		+++		+++		
3.	−3.3 mm to −3.8 mm	+++	++		+++		

throughout the ME (Fig. 6). Image analysis revealed dense accumulation of SRIF positive terminals in lateral IZ and EZ only (Fig. 5). Weak SRIF immunoreactive terminals were observed in median IZ.

DISCUSSION

Results of the present study suggest that SRIF immunoreactivity is differentially distributed in rostral-caudal extent (Bregma-1.8 mm to -3.8 mm) of ME. SRIF immunoreactive nerve terminals were in both IZ and EZ but dense immunoreactive terminals were distributed in EZ. In median ME, moderately reactive terminals were observed in medial IZ and in lateral EZ but dense accumulation of intense SRIF positive terminals were observed in lateral EZ. In caudal ME, few nerve terminals were

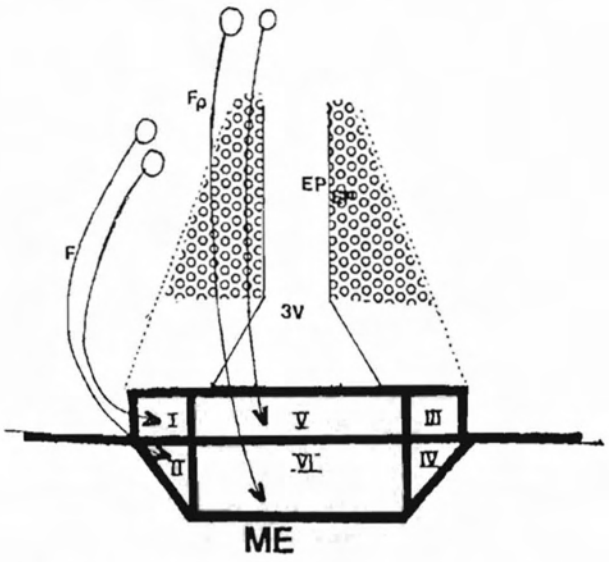

Fig. 7: Diagram to represent the various compartments in ME.

observed in both lateral and median IZ, while in lateral and median EZ dense accumulation of nerve terminals were observed. Image analysis studies carried out on immuno-stained preparations to elucidate variation in immunoreactivity substantiated the above observations. These results suggest that six regions could be localized in ME. We hypothesize that these are six functionally independent compartments in ME-Compartment I and III of both lateral IZ; Compartment II and IV of both lateral EZ; Compartment V and VI of both medial IZ and EZ (Fig. 7). Immunohistochemical studies on the distribution of large number of peptides in hypothalamic area and ME also supported the above assumption because of their differential distribution in lateral of medial IZ and EZ. Dense accumulation of DYN, TH and NT (15), NPY (16), SRIF (17) and GHRH (18) was reported in lateral zone of EZ only. While NT (15), GAD (19) and GHRH (20) nerve terminals were accumulated densely in median EZ. GHRH (18) and GAL (21) immunoreactive terminals were localized densely in median IZ. LHRH (22) immunoreactive terminals were densely localized in entire ant.-posterior extent of ME. The above observations and our results suggest that these peptides which are synthesized in specific cell groups (Pa, Pe, PaPc, DM, ARC etc.) located in hypothalamic area around PeV zone, reach lateral or medial IZ and EZ through separate processes. Large number of tracing studies (3, 5, 7, 9, 25) have shown that cell bodies in and around PeV zone in hypothalamus project to various regions of ME via specific pathways. These observations establish the functional independence of lateral or medial IZ and EZ in ME. Although anterograde or retrograde tracing studies have not been attempted so far to understand the differential distribution of nerve terminals in ME but lesion studies in ME and SRIF immunocytochemistry using five specific antibodies recognizing different fragments of preprohormone provide strong evidence in favour of differential accumulation of these SRIF peptides in rostro-caudal extent of ME (Unpublished data, submitted else where).

In conclusion it is proposed that ME is divided into six compartments, each is specific and functionally independent.

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