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Neuroendocrine plasticity in GnRH release is disrupted by valproic acid treatment of cycling rats

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Abstract

Valproic acid (VPA) has been used for > 30 years in the treatment of epilepsy and is now one of the most frequently prescribed anti-epileptic drugs (AEDs) worldwide. Its chronic use has been associated with hyperandrogenism and polycystic ovaries in women with epilepsy and thus suggests change in normal levels of estrogens – the gonadal steroids in females. We have tested the hypothesis whether AEDs that exert anticonvulsive effects via key molecules of the gamma amino butyric acid (GABAergic) system, have inhibitory effects on the hypothalamo-hypophyseal-gonadal (HPG) axis at the level of hypothalamic gonadotropin releasing hormone (GnRH) synthesis and/or release and thereby affect reproductive health. Three-month old female Wistar rats were given VPA (i.p.) at a dose of 300 mg/Kg once a day for 12 weeks; the control group received an equivalent volume of vehicle. Glutamic acid decarboxylase (GAD), glial fibrillary acidic protein (GFAP) and their mRNA expression in the median eminence arcuate region (ME-ARC) of the hypothalamus were upregulated in the VPA treated group. By contrast, polysialyltransferase (PST) mRNA which is the enzyme responsible for the polysialylation of neural cell adhesion molecule (NCAM), a plasticity marker, was found to be downregulated. These results support our hypothesis that VPA disrupts normal neuronal-glial plasticity in the hypothalamus and can thereby cause reproductive neuroendocrine disorders in female patients treated for epilepsy, bipolar disorder or migraine.

Key words: Valproic acid; hypothalamus; neuro-glial plasticity; GABA.

Introduction

Reproductive endocrine disturbances are one of the major consequences of using AEDs (1, 2, 3, 4). A very high occurrence of hyperandrogenism and polycystic ovaries has been found among women taking VPA for epilepsy (5). In females VPA decreased estradiol release and enhanced testosterone level (6). In addition, VPA may inhibit conversion of testosterone to estradiol (7) or induce ovarian androgen biosynthesis (8). Decrease in serum estradiol level increases testosterone/estradiol ratio in VPA treated rats (9).

Despite its widespread use, the exact mechanism of action of VPA on the neuroendocrine system and sites of action for inhibition of the reproductive axis remains to be clarified. However, it is generally agreed upon that VPA acts to potentiate GABA mediated postsynaptic inhibition within the CNS via some, as of yet, unknown cellular mechanism. The first major hypothesis for the cellular action of VPA was made by Godin *et al*. (1969) (10), who proposed that VPA produces an increase in the level of GABA in the CNS. Since that time, several mechanisms have been postulated to explain the GABA elevating effects of VPA, including inhibition of several degradative enzymes in the GABA shunt pathway; as well as an increase in the activity of GAD the rate limiting enzyme in the synthesis of GABA (11). Several lines of evidence support the inhibitory role of GABA in the modulation of the activity of GnRH cells. Ultrastructural data indicates that GABAergic neurons establish inhibitory synapses directly onto the GnRH neurons within the median preoptic area (mPOA) (12). Moreover, addition of GABA and GABA agonists directly into the mPOA have been shown to reduce GnRH gene expression (13), suppress the release of LH from the anterior pituitary (14), and block the estrogen-induced LH surge that normally occurs at the time of proestrous (15). In a previous study we have reported a significant increase in GABA-ir in the mPOA and ME-ARC region (16), the site of GnRH release in the perivascular capillaries for action on anterior pituitary LH and FSH release.

Classically considered as supporting cells of neurons, astrocytes display rapid electrical,

metabolic, and transcriptional responses to neural activity (17). Astrocytes participate in synaptic patterning via responsiveness to steroid hormones in steroid concentrating brain regions in mammalian brain (18). Specialized glial cells play key role in GnRH release by extension and retraction of their processes which are found interposed between GnRH synaptic terminals and the portal vasculature of the median-eminence (19, 20, 21) and this glial plasticity is a factor regulating GnRH cyclicity. GnRH neurons and their axon terminals are profusely apposed by astrocytes at the median eminence (19, 22). Their structural relationship is highly dynamic and regulated by gonadal steroids (18, 23). Astroglial plasticity in external zone of median eminence of female rats result in more GnRH neuronal terminals establishing physical contact with the pericapillary space in proestrous when estrogen levels are high in comparison to diestrous when estrogen levels are low (19, 24). Earlier report from our lab has reported astroglial processes association with the GnRH neuron terminals in ME-ARC region which vary otherwise in conjunction with cyclic changes in GnRH neuronal terminals (21). Although VPA has GABAergic mechanism of action, VPA mediated decrease in gonadal steroid levels may indirectly disrupt the glial plasticity in median-eminence and ultimately the GnRH release.

The present study was designed to explore whether VPA treatment changes the expression of some molecular markers of neuro-glial plasticity in the ME-ARC region of the hypothalamus and serum estrogen levels. Using dual immunostaining for GnRH and GFAP, we studied effect of VPA treatment on astroglial processes association with the GnRH neuron terminals in ME-ARC region. GAD and GFAP protein expression was also studied by western blotting. Neural cell adhesion molecule (NCAM) is the cell surface molecule and PST enzyme adds negatively charged polysialic acid (PSA) moieties onto NCAM. PSA-NCAM functions as a spacer that reduces adhesion forces between cells and allows dynamic changes in the membrane contacts facilitating outgrowth of axons (25). We earlier reported that PSA-NCAM expression in the vehicle treated proestrous rats was more pronounced and colocalized with GnRH immunoreactivity in the mPOA and external zone of the ME-ARC region, whereas a significant decrease in PSA-NCAM and GnRH dual staining was observed in VPA treated group (16). The alterations in neuro-glial plasticity markers expression by VPA treatment was further tested at transcriptional level and the expression of GFAP, PST, GnRH and GAD mRNA were studied by fluorescent In-Situ Hybridisation (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR).

Methods

Wistar strain female albino rats in the age group of 3-4 months were procured from National Institute of Nutrition (NIN) Hyderabad. Animals were housed three per cage in a temperature humidity controlled environment on a 12-h light –12-h dark cycle with free access to food and water. The estrous cyclicity was monitored by daily inspection of vaginal cytology. After at least 3-4 complete four-day cycles, the animals were used for further treatment with VPA. The use of animals for research work was approved by the Institutional Animal Ethical Committee for conducting experiments.

Drug administration

To study the effects of VPA, rats were given intraperitoneal injection of VPA (300 mg/kg) and control animals were injected vehicle solution between 9.00 to 10.00 AM for 12 weeks. This dosage of VPA in a previous study by Roste *et al*. (2002) (9) was calculated to correlate with their therapeutically relevant serum concentration when used as an anticonvulsant. We have shown development of polycystic ovaries with this dose in our previous study (16). The animals were sacrificed between 14.00 to 16.00 hours on proestrous day of control group, whereas, all VPA treated animals were in diestrous phase. It should be noted that on the start of VPA drug treatment both control and test groups were in proestrous phase, and VPA medication for 12 weeks caused reproductive dysfunction to turn them into diestrous phase.

Immunofluorescence staining

For dual immunofluorescent staining of GnRH + GFAP and GnRH + GAD in ME-ARC region of hypothalamus, brains (n = 5 for each group) were perfused transcardially with 4% paraformaldehyde in phosphate buffer saline (PBS) (0.1 M) between 15.00 and 16.00 hours followed by overnight fixation in same solution and cryo-preservation in 20% and 30% sucrose in PBS each for 24 hours at 4°C. 20 μm coronal sections from ME-ARC region of hypothalamus were cut on Thermo Shandon Cryostat Microtome set at -20°C, thaw-mounted onto poly-L-lysine-coated slides and immunostained according to our protocol reported previously (16). The antibody dilutions used were as follows: anti-GFAP (1:500, Sigma), anti-GAD (1:10,000, Sigma)

and anti-GnRH (1:10,000, Generous gift from Dr. Benoid).

ELISA for Estrogen levels in blood serum

Serum estrogen levels from control and VPA treated rats (n = 9 in each group) were determined by using competitive ELISA assay kit (Cayman Chemicals company, USA) following manufacturers standard protocol.

Western blot hybridisation

Tissue from ME-ARC region of control and VPA treated rats (n = 5 for each group) was pooled and homogenized in 5 volumes of chilled homogenizing buffer (50 mM Tris, 150 mM NaCl, 1 mM EDTA, 100 μM $NaVO_4$, 1 mM PMSF and 0.5 mM DTT) and centrifuged for 10 min at 10,000 rpm. Protein content in the supernatant was determined by the Bradford method. Protein lysate (20-30 μg) was resolved on one-dimensional 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE) under standard denaturing conditions according to the method of Laemmli (1970) (26). The protocol followed for western blotting was as described earlier (21).

FISH for PST, GFAP and GAD65/GAD67 mRNA expression

In situ hybridization was performed as described by Wood *et al*. (1997) (27) using DIG labeled probes for PST, GFAP, GAD65 and GAD67 mRNA (Table 1), synthesized and purified by Sigma Chemicals, USA.

Brain tissue sections from ME-ARC region were prepared and treated as described earlier (21). Sections were then incubated with hybridization mixture (Prehybridisation buffer with 600 ng/ml probe) containing DIG labeled oligonucleotide probes at 42°C for 60 h in humidified box. After stringency washings, sections were incubated in a humid chamber with FITC labeled anti-DIG antibody (1:200) for 2 h. Finally sections were mounted using Fluoromount (Sigma). A set of slides from each group were processed without exposure to DIG labeled probe to serve as negative control.

RT-PCR

The expression of PST, GFAP, GnRH, GAD65 and GAD67 mRNA were quantified by semi-quantitative RT–PCR analysis, using β-actin mRNA as an internal standard. Tissue from ME-ARC hypothalamus (n = 5) was pooled from VPA treated and control rats to isolate RNA using TRI reagent (Sigma). For GnRH mRNA analysis whole of hypothalamus was used (n = 3). Equal amounts of RNA were used for cDNA synthesis. cDNAs were synthesized in 20 μl reaction mixture containing 200 units M-MLV reverse transcriptase (Fermentas) according to standard protocol. 2 μl of cDNA was amplified in a 50 μl PCR reaction mixture containing 20pM respective primers. Cycling conditions comprised an initial denaturation of 3 min at 94°C followed by 35 cycles of amplification (at 94°C for 40 sec, 55°C for 45 sec and 72°C for 1 min) and final elongation step at 72°C for 10 min. The PCR products were electrophoresed in 2% agarose gel. The primer sequences and product sizes are given in Table 2. For quantification, the PCR bands on the photograph of the gel were scanned by a densitometer linked to a computer analysis system.

Data Analysis

The images were analyzed using Image Pro-Plus software version 4.5.1 from the Media Cybernetics. GnRH, GFAP and GAD immunoreactivity was quantified from 5-8 randomly selected fields in 4-5 consecutive sections in ME-ARC region from 3-4 animals of control and test groups, using the count/size command of image pro-plus software.

Statistical Analysis

Data of immunohistochemistry, western blotting, FISH and RT-PCR was analysed using Student's

Table 1

The length and the sequence of the DIG labeled probes

Marker	Length	Sequence
PST	39	5'ATC TCC GAT GAG TTG CGT CTC TTG GTG CTC CTC AGT TCT3'
GFAP	49	5'CGC ATC TCC ACC GTC TTT ACC ACG ATG TTC CTC TTG AGG TGG CCT TCT G3'
GAD-65	48	5'GGC GTC CAC ACT GCA AGG CCT TGT CTC CCG TGT CAT AGG ACA GGT CAT3'
GAD-67	48	5'TAG TAT TAG GAT CCG CTC CCG CGT TCG AGG AGG TTG CAG GCG AAG GCG3'

Table 2

The sequence and the expected product size of the primers used for different markers

Marker	Product size	Sequence
PST	500bp	forward 5' TAA GGT GCA ATC TAG CTC CTG TGG TGG 3' reverse 5' GCA TCC TGT GAG GAC TGG CGT TGG AAA 3'
GFAP	327	forward 5'GGC GCT CAA TGC TGG CTT CA 3' reverse 5'TCT GCC TCC AGC CTC AGG TT 3'
GnRH	124	forward 5'GGC AAG GAG GAG GAT CAA A 3' reverse 5'CCA GTG CAT TAC ATC TTC TTC TG3'
GAD65	437	forward 5' GGC TCT GGC TTT TGG TCC TT 3' reverse 5' TGC CAA TTC CCA ATT ATA CTC TTG A3'
GAD67	302	forward 5'GCT GGA AGG CAT GGA AGG TTT TA 3' reverse 5'AAT ATC CCA TCA CCA TCT TTA TTT GAC C 3'
β-actin	291	forward 5'TCA CCC ACA CTG TGC CCA TCT ACG A 3' reverse 5'CAG CGG AAC CGC TCA TTG CCA ATG G 3'

t-test (Sigma Stat 3.5). The values are expressed as mean ± SEM. The statistically significant change in test rats in comparison to control rats is denoted by '*' ($P < 0{\cdot}05$). Differences were considered statistically significant if $P < 0.05$.

Results

Estrogen levels in VPA treated test group

Serum estradiol concentrations measured by ELISA assay showed significant decrease in VPA treated group (19.4 ± 2.1 pg/ml) as compared to vehicle treated control proestrous phase rats (100.1 ± 6.9 pg/ml).

Effect of VPA treatment on GnRH and GFAP at protein and mRNA level in hypothalamus

Immunofluorescence data indicated that GnRH expression was significantly higher in the ME-ARC region of the hypothalamus in the vehicle treated control proestrous group as compared with the VPA treated test rats (Fig. 1C, 1G). GnRH axons were co-distributed with the glial elements in the internal zone of the ME-ARC region in the control group, whereas GFAP staining was observed in both internal and external zones of the ME-ARC region in test group rats (Fig. 1A, 1E), thus indicating reduced glial apposition with GnRH axon terminals in the parenchymatous space to facilitate GnRH release in the control group and higher glial apposition in the test group. Using quantitative immunofluorescence analysis of staining intensity measurements, we showed a statistically significant increase in the GFAP staining in the outer zone of ME-ARC region in the test group (Fig. 1B, 1F). The quantitative analysis of immunoblots also revealed a significantly higher GFAP protein content from the ME-ARC region of VPA treated test rats (Fig. 1H). FISH and RT-PCR data indicated that GFAP mRNA expression was significantly higher in the VPA treated rats (Fig. 2A, 2B, 2C and 2D). GnRH mRNA expression quantified by RT-PCR showed higher expression in the vehicle treated proestrous rats (Fig. 2E).

GAD65 and GAD67 protein and mRNA expression in hypothalamus

GABAergic neurons were found to show significantly higher immunoreactivity for GAD in ME-ARC region of the hypothalamus (Fig.3D) and lower GnRH-ir (Fig. 3E) in VPA treated test group as compared to vehicle treated control proestrous rats. A statistically significant increase ($P < 0.05$) in the GAD staining was observed in the ME-ARC region from the VPA treated test rats (Fig. 3C). Western blot hybridization signals from ME-ARC region revealed a significantly higher GAD65 and GAD67 protein content from test rats (Fig. 3F) which was further supported by FISH data (Fig. 4). GAD65 and GAD67 mRNA expression quantified by semi quantitative RT-PCR analysis also showed higher expression of GAD in the ME-ARC region of VPA treated test rats (Fig. 4D, 4H).

PST mRNA expression using FISH and RT-PCR

FISH data indicated that PST mRNA expression was significantly higher in the ME-ARC region in the vehicle treated control proestrous group (Fig. 5A) as compared to the VPA treated test rats

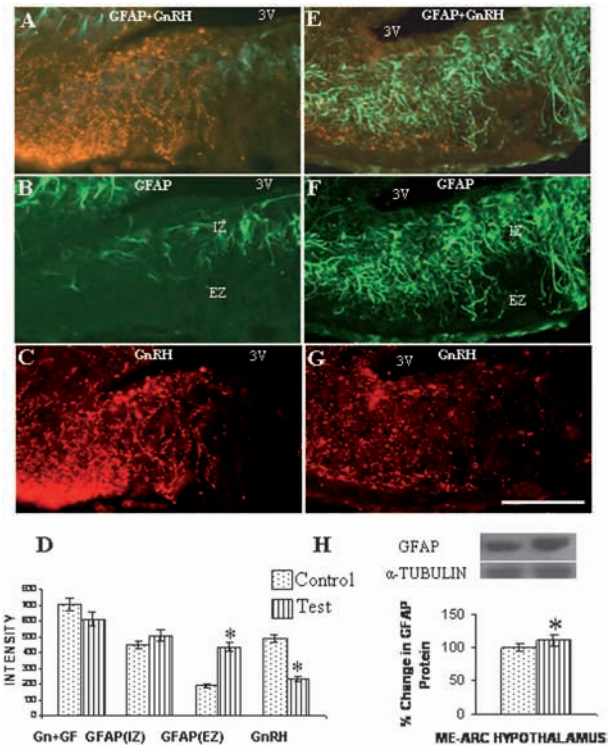

FIG. 1. — Photomicrographs of 20μm thick coronal section through the ME-ARC region of the hypothalamus; Immunostaining for GnRH and GFAP is shown for the vehicle treated control proestrous (A, B and C) and VPA treated test (E, F and G). GnRH-ir is higher in control proestrous (C). GFAP-ir is higher in test group in external zone of ME-ARC region (F). Intensity measurement data of the GFAP immunoreactivity associated with the GnRH axon terminals in the ME-ARC region of control proestrous (dotted bars) and test (lined bars) (D) depicts the staining intensity of GnRH and GFAP in control and test rats. GFAP+GnRH, GFAP and GnRH depict the intensity data of dual-stained sections, GFAP-ir and GnRH-ir in control and test rats respectively. Representative Western blot hybridisation signals using antibodies specific for GFAP and α-tubulin from ME-ARC region in hypothalamus from control proestrous and test rats are shown. Mean values of GFAP levels for each group is expressed as percentage of α-tubulin labeling (H). All statistical analyses were performed using Student's t-test, values are means ± S.E.M, *P < 0·05, IZ Internal Zone, EZ External Zone, 3V Third Ventricle, ir immunoreactivity, Scale bars = 100 μm.

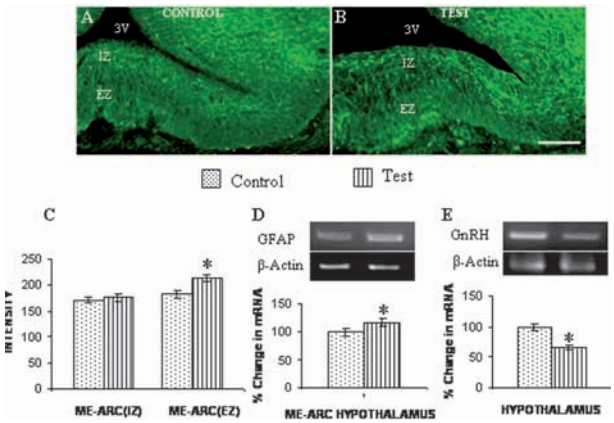

FIG. 2. — Representative Fluorescent in-situ hybridization images of 30 μm thick coronal sections showing staining for GFAP mRNA in ME-ARC region of the hypothalamus in brain. GFAP mRNA expression is increased in external zone of ME in test rats (2B) as compared to control proestrous rats (2A). Intensity measurement data also shows higher intensity in external zone of ME of test rats (2C). Representative semiquantitative RT-PCR product gel signals for GFAP and β-actin from ME-ARC region of hypothalamus shows increase in GFAP mRNA expression in test rats (2D). Representative semiquantitative RT-PCR product gel signals for GnRH and β-actin from whole hypothalamus region of brain shows decrease in GnRH mRNA expression in VPA treated test rats (2E). All statistical analyses were performed using Student's t-test, values are means ± S.E.M; *P < 0·05, IZ Internal Zone, EZ External Zone, 3V Third Ventricle, Scale bars = 100 μm.

(Fig. 5B). Quantitative immunofluorescence data is presented in Fig. 5C. RT-PCR data further confirmed higher expression of PST in the ME-ARC region of hypothalamus in control proestrous rats (Fig. 5D).

Discussion

Chronic VPA treatment had adverse effects on reproductive neuroendocrine system in nonepileptic female rats and these findings suggest direct drug mediated effects on reproductive dysfunction not related to epilepsy. Our present study demonstrated the VPA mediated changes both at protein and mRNA level in the key molecules which play an important role in regulation of estrous cyclicity. As estrogen is implicated in regulating the neuro-glial plasticity in ME-ARC region, decrease in estrogen levels as a result of VPA treatment is likely to result in disruption of neuronal-glial plasticity. A rise in circulating estradiol precedes the preovulatory LH surge and the ovarian cycle is also accompanied by a fall in the number of axosomatic synapses (28). Chronic treatment with VPA has been associated with a variety of endocrine related side effects. VPA treatment increases the testosterone/estradiol ratio, mainly because of marked reduction of serum estradiol levels (9). Previous report from our lab. has shown VPA mediated changes in ovarian histology with a significantly higher number of ovarian cysts per mid ovarian section as compared to vehicle treated control rats (16).

GnRH is synthesized in neuronal cell bodies distributed diffusely in the hypothalamic preoptic area and secreted from the external zone of the median eminence by an increase in the length of axons (29, 20). A rise in circulating estradiol

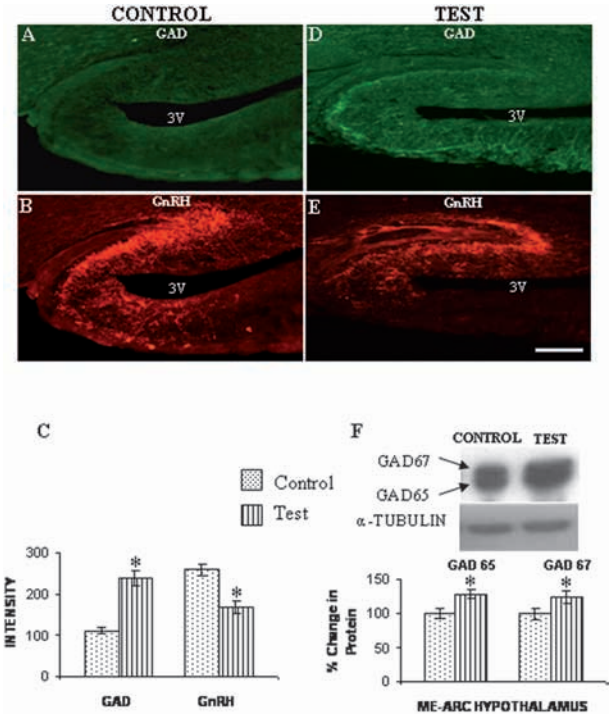

FIG. 3. — Photomicrographs of 20 μm thick coronal section through the ME-ARC region of the hypothalamus; Immunostaining for GAD and GnRH is shown for the vehicle treated control proestrous (A and B) and VPA treated test (D and E). GnRH-ir is higher in control proestrous (B) while GAD-ir is higher in test group (D). Intensity measurement data of the GAD and GnRH immunoreactivity in the ME-ARC region of control proestrous (dotted bars) and test (lined bars) depicts the staining intensity of GAD and GnRH respectively (C). Representative Western blot hybridisation signals of GAD65 and GAD67 from ME-ARC region in hypothalamus from control proestrous and test rats are shown. Mean values of GAD65 and GAD67 levels for each group is expressed as percentage of α-tubulin labeling (F). All statistical analyses were performed using Student's t-test, values are means ± S.E.M, *P < 0·05, 3V Third Ventricle, ir immunoreactivity, Scale bars = 100 μm.

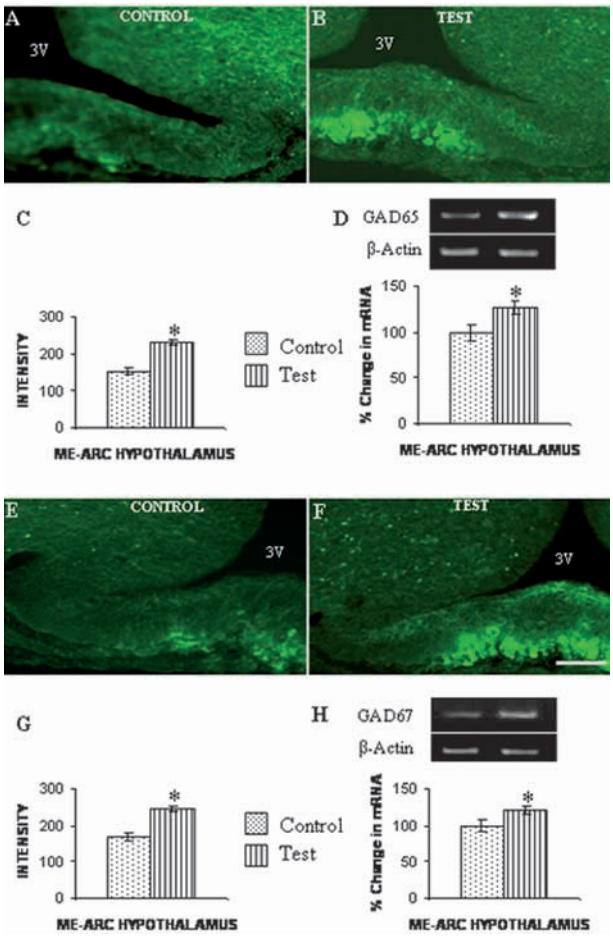

FIG. 4. — Representative Fluorescent in-situ hybridization images of 30 μm thick coronal sections showing staining for GAD65 (4A and 4B) and GAD67 (4E and 4F) mRNA in ME-ARC region of the hypothalamus in brain. Intensity measurement data (4C and 4G) shows higher GAD65 and GAD67 mRNA expression in VPA treated test rats (4B and 4F) as compared to vehicle treated control proestrous rats (4A and 4E). Representative semiquantitative RT-PCR product gel signals for GAD65, GAD67 and β-actin from ME-ARC region of hypothalamus shows increase in GAD65 and GAD67 mRNA expression in VPA treated test rats (4D and 4H) as compared to control rats. All statistical analyses were performed using Student's t-test, values are means ± S.E.M, *P < 0·05, 3V Third Ventricle, Scale bars = 100 μm.

precedes the preovulatory LH surge accompanied by a fall in the number of axosomatic synapses on randomly selected arcuate nucleus neurons by the morning of estrous, with a return to the usual levels by the morning of the next day (28). Previous work has documented action sites of estrogen (30, 31) within the human hypothalamus. However, we know little about the key signaling components used by astroglial cells to modulate its interactions with the GnRH neurons in adult brain hypothalamus. By using antibodies to intermediate filament proteins such as GFAP, we could visualize VPA mediated changes in the anatomical relationship between GnRH neurons and astrocytes in median eminence. Our fluorescent microscopy results show that GnRH axons were co-distributed with the glial elements in the internal zone of the median-eminence in the control group, whereas GFAP staining was observed in both internal and external zones of the median-eminence in VPA treated rats, thus indicating reduced glial apposition with GnRH axon terminals in the parenchymatous space to facilitate GnRH release in the control group. GFAP staining in external zone of median eminence in VPA treated animals reveals increased apposition of glial processes to the axon terminals of GnRH neurons thus suggesting that VPA treatment may disrupt GnRH release by promoting

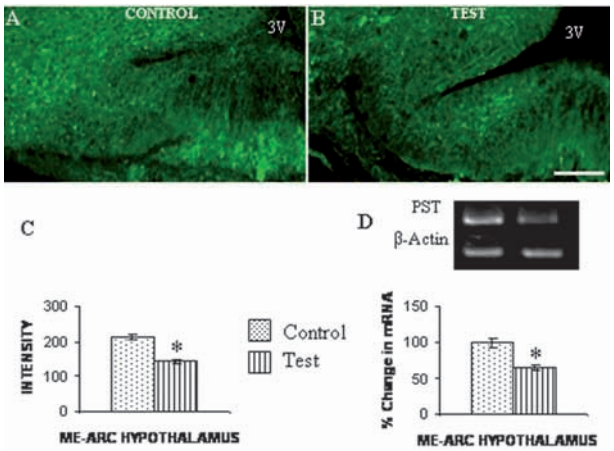

FIG. 5. — Representative Fluorescent in-situ hybridization images of 30 μm thick coronal sections showing staining for PST mRNA in ME-ARC region of the hypothalamus in brain. PST mRNA expression is decreased in VPA treated test rats (5B) as compared to vehicle treated control proestrous rats (5A). Intensity measurement data shows lower PST mRNA expression in test rats (5C). Representative semiquantitative RT-PCR product gel signals for PST and β-actin from ME-ARC region of hypothalamus again shows decrease in PST mRNA expression in test rats (5D). All statistical analyses were performed using Student's t-test, values are means ± S.E.M, *P < 0.05, 3V Third Ventricle, Scale bars = 100 μm.

physical interference of glial processes between GnRH axon terminals and perivascular space. This is further confirmed by western blot results. VPA mediated marked reduction of serum estradiol levels may be one of the factors as the structural relationship between GnRH terminals and glial processes is highly dynamic and is regulated by gonadal steroids (9, 19). Moreover, GFAP mRNA expression also revealed a similar trend confirming that VPA mediates changes in glial plasticity at transcriptional level as well. This increased apposition in the external zone of median eminence offered by extension of astroglial processes may be the linked to increased levels of GFAP both at protein and mRNA levels.

GAD67 mRNA expression was high during the early hours of proestrous and then declined around the time of the GnRH-induced LH surge (32), which mark the two E2-dependent signals required for GnRH and LH surge release (33). Present data shows that GAD-ir was significanty higher and GnRH-ir and mRNA expression was significantly lower in VPA treated test group in comparison to control proestrous rats and supports previous work from our lab. where we reported significantly higher GABA-ir and decrease in GnRH-ir in the ME-ARC region of the hypothalamus in VPA treated groups as compared to the vehicle treated control proestrous rats (16). GAD protein and mRNA expression were both found to be higher in VPA treated test rats, which supports the hypothesis that GABA elevating effect of VPA may be due to enhanced activity of GAD which is the rate limiting enzyme for GABA synthesis. VPA is an inhibitor of histone deacetylases (HDACs), enzymes that remove the acetyl group from histones in nucleosomes and thus modulating transcription of specific genes (34, 35). Moreover VPA has been shown to upregulate GAD67 mRNA expression via HDAC inhibition mechanism as VPA hyperacetylates the brain nucleosomal histones (36, 37). Thus enhanced GAD expression by VPA induced HDAC inhibition may be another plausible mechanism to explain the VPA mediated increase in GABAergic neurotransmission, which in turn decreases GnRH release and reproductive failure in rats given long term VPA treatment.

Further we have studied the effect of VPA medication on GnRH neuronal plasticity. At puberty, GnRH is released in distinct pulsatile pattern which drives pulsatile gonadotropin secretion that is critical for the activation and proper functioning of gonads (38, 39, 40). For carrying out function of pulsatile release of GnRH, dynamic transformation of individual GnRH axon terminals occurs in the ME-ARC region of hypothalamus during pulsatile release of GnRH and this restructuring of ME-ARC region during proestrous phase is orchestrated by endocrine events occurring in a cyclic manner (21). This earlier report from our lab. had shown that ME-ARC retains the capacity to express PSA-NCAM which may be responsible for structural reorganization of GnRH neuron terminals in proestrous phase. NCAM is a surface molecule and renders anchorage to the cell through cell-cell or cell-matrix interactions. PSA on the extracellular domain of the NCAM reduces cell adhesion and by acting as a spacer, allows dynamic changes in the membrane contacts making extension or outgrowth easier. PSA is a negatively charged polymer of α-2,8 sialic acid which is post-translationally added to NCAM by a specific PST enzyme. VPA treatment lead to downregulation of PST mRNA in ME-ARC region of the hypothalamus as compared to control proestrous rats. These results support our earlier report showing decrease in PSA-ir in external zone of ME-ARC in VPA treated rats (16).

Alterations in GnRH, GFAP, GAD and PST expression in the ME-ARC region along with decrease in serum estrogen levels in VPA treated rats suggests that VPA disrupts normal neuro-glial plasticity and GnRH pulse generator and causes disturbance in the reproductive function in adulthood through central mechanism. These results encourage further work to

study the central regulation of reproductive endocrine side effects of AEDs.

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