NEUROENDOCRINOLOGY

Saptarshi Bhattacharya and Nikhil Tandon

Introduction

This article reviews the developments in the field of both basic and clinical neuroendocrinology in India over the last two decades. The contributions in basic and experimental (mammalian) neuroendocrinology will be elaborated first, and will be followed by a short brief of some of the clinical data published from India. Kaur and her team of scientists have carried out in depth investigations at the department of Biotechnology, Guru Nanak Dev University, Amritsar, on hypoglycemic brain damage, opiodergic modulation of GnRH release and neuroendocrine plasticity and its role in GnRH secretion. At the department of Zoology, Vivekananda College, Kolkata, Kundu and his colleagues have studied thyroid hormone homeostasis in rat brain. Also the effect of HIV-1 clade C infection (cf. clade B infection in West) on hypothalamic-pituitary- adrenal axis has been defined. Important contributions have been made on various aspects of clinical neuroendocrinology from centers all across the country.

Hypoglycemia induced brain damage: In 1983, Kaur published their work on changes in hexokinase (HK) isoenzyme activity during insulin-induced hypoglycemia in rat brain. A significant increase was observed in HK type II isoenzyme in both soluble and particulate fractions in cerebral hemispheres, cerebellum and brain stem of rat brain 1 h after insulin administration (Kaur et al, 1983). In a later publication in 1988, they demonstrated significant decrease in the acetylcholinesterase (AChE) and Na^+-K^+ATPase activities in the soluble and total particulate fractions from cerebral hemispheres, cerebellum, brain stem, and diencephalon and basal ganglia after insulin-induced hypoglycemia (Kaur & Arora, 1994). Changes in free radical scavenger system with insulin-induced hypoglycemia and starvation were also elucidated (Bhardwaj et al., 1998). They further demonstrated selective down regulation of the neuronal plasticity marker proteins (GAP-43 and NCAM), and enhanced expression of GFAP and synaptophysin in acute hypoglycemia and postulated mechanisms other than energy failure may also contribute to neuronal cell damage in the hypoglycemia (Singh et al., 2003). During severe energy deprivation following hypoglycemia and diabetes, mitochondrial free radicals scavenger system down regulation leads to reactive oxygen species (ROS) generation. High levels of ROS in turn activate the processes leading to DNA damage in brain (Singh et al., 2004a). The analysis of such alterations is important in ultimately determining the basis of neuronal dysfunction during metabolic stress conditions, such as hypoglycemia, and further defining the nature of these changes may help to develop therapeutic means to cure metabolically stressed brain tissue.

Diabetes and GnRH secretion: Recent work shows a high prevalence of low testosterone and inappropriately low luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations in type 2 diabetes (Singh et al., 2004b) In a study published in 1994, Lakhman (Lakhman et al., 1994) reported that induction of alloxan induced diabetes in rats resulted in alteration of glucose metabolism enzymes in specific brain areas. Activity of HK, lactate dehydrogenase (LDH), and glucose-6-phosphate dehydrogenase (G6PDH) enzymes were analyzed from discrete brain regions. Enzyme activity was assayed from hypothalamic areas such as medial preoptic area and median eminence-arcuate region which have gonadotropin releasing hormone cell bodies and their terminals, respectively and other brain regions like septum, amygdala, hippocampus, and thalamus. In all the areas studied, induction of diabetes resulted in a significant decrease in particulate bound HK activity, whereas soluble HK, LDH and G6PDH activity showed increase. Insulin treatment of diabetic rats led to recovery in enzyme activity (Lakhman et al., 1994). The results suggested that altered cerebral glucose metabolism may also be responsible for reproductive failure observed in diabetic rats. The exact etiology of hypogonadotropic hypogonadism in diabetes is still not clearly defined and this observation can help in elucidating its mechanism.

Opiodergic modulation of GnRH release: Intra-cerebro-ventricular administration of morphine was found to markedly alter monoaminergic system activity in the median eminence-arcuate region, medial preoptic area and cerebral cortex in brain from estrogen- and progesterone-primed ovariectomized rats. Administration of naloxone followed by morphine resulted in recovery of these changes (Sharma et al., 1998). The authors also subsequently published their study on opioidergic mediated suppression of gonadotropin release through the down regulation of signal transduction cascade (Sharma et al., 2000). They also showed that opioidergic modulation of GnRH release is mediated through cholinergic and GABAergic neurotransmission besides monoaminergic control (Kaur, 2001). These studies provided insights into the precise opioidergic modulation of GnRH release mechanisms and contributed in demonstrating the basis of various neuronal dysfunctions in opioid addicts.

Neuroendocrine plasticity in GnRH release: Morphological changes in the GnRH neurons in the preoptic area and their terminals in the median eminence-arcuate region are reported to occur during ovarian cycle that may be involved in the GnRH release into the portal blood during preovulatory surge. However, the neuronal substrates participating in altered GnRH neuronal plasticity are poorly understood. Expression of neuronal plasticity markers in the GnRH neuron cell bodies in the preoptic area and their terminals in the median arcuate region of hypothalamus during pulsatile GnRH release in cycling female rats was analyzed by Kaur (Kaur et al., 2002). The plasticity markers studied were polysialylated neural cell adhesion molecule (PSA-NCAM), high molecular weight isoforms of NCAM, growth associated protein (GAP-43), glial fibrillary acidic protein (GFAP) and synaptophysin. Regularly cycling female rats were sacrificed at diestrous,

i.e., when GnRH release is low, and at proestrous, i.e., when preovulatory GnRH surge occurs. Median arcuate region showed more pronounced increase in the protein expression of these markers of neuronal plasticity as compared to the preoptic, whereas, hippocampal region did not show any significant change in the content of these markers showing specificity of the changes to the GnRH system. In another study, the expression of PSA-NCAM, a known marker of neuronal plasticity, correlated closely with dynamic structural changes in GnRH neuron terminals in the median arcuate region of the hypothalamus during the estrous cycle of rat (Parkash & Kaur, 2005). These results demonstrated that the involvement of synaptic proteins in the dynamic plasticity of the median arcuate region of hypothalamus, allowing GnRH nerve terminals to release the neurohormone into the pituitary portal blood on the day of proestrous.

Women with epilepsy using antiepileptic drug valproic acid (VPA) often suffer from reproductive endocrine disorders, menstrual disorders and polycystic ovaries. Kaur studied GnRH, GABA and PSA-NCAM expressions in median preoptic area and median arcuate eminence region of hypothalamus in valproate treated and control rats. GnRH and PSA-NCAM staining was much higher in control proestrous rats, whereas VPA treatment significantly enhanced GABA expression, and reduced both GnRH and PSA-NCAM expression. These findings suggested that treatment with VPA disrupts hypothalamo-hypophyseal-gonadal axis at the level of GnRH pulse generator in hypothalamus (Lakhanpal & Kaur, 2007). The investigators further demonstrated that phenoxybenzamine and GABA significantly reduced the expression of PSA-NCAM (Parkash & Kaur, 2007a) and that PSA-NCAM has a permissive role in the structural remodeling of GnRH neurons (Parkash & Kaur, 2007b).

Thyroid hormone homeostasis in brain Thyroid hormones are essential for normal functioning of adult mammalian brain. Kundu studied the time course of thyroid hormone homeostasis in adult rat brain. Animals were rendered hypothyroid by PTU injections and serum and synaptosomal T3/T4 content, synaptosomal AChE and Na+-K+-ATPase activities were determined on alternate days (Kundu et al., 2006). Cerebro-cortical synaptosomal T3 level increased on the 2nd day from the control, attained a peak on the 4th day, remained stable until the 18th day, and abruptly declined on the 20th day. Synaptosomal T4 content remained negligible or undetected throughout. Synaptosomal membrane Na+-K+-ATPase and AChE activity exhibited an inverse relationship during the experimental regime, being much more prominent on the 2nd, 18th and 20th day coinciding with the variations in brain T3 level. The study identified the onset of central homeostasis between the first and second day, its continuation for about 16-18 days and its termination between the 18th and 20th day. Kundu further analyzed the mechanism behind this homeostasis by injecting the protein synthesis blockers actinomycin D and cycloheximide along with propylthiouracil to adult male rats during the days of onset (day 2) and termination (day 20) of the homeostatic mechanism. The investigators found unchanged or lower levels of synaptosomal T3 on the 2nd and

on the 20th day, respectively. This was in parallel supported by reflections in cerebrocortical deiodinase II activity and cAMP levels. The activities of cerebrocortical synaptosomal Na+-K+-ATPase and AChE, which are the two important physiological parameters for neuronal function, was found to be supportive of the involvement of a neuronal protein-mediated factor in the 'on' and 'off' reactions in central homeostasis during peripheral hypothyroidism (Kundu et al., 2007).

In another study the authors analyzed the interplay between the central homeostasis mechanism and sympathetic nervous system activity and thyroid hormones. The alpha2 receptor agonist did not alter the onset of central homeostasis, but prolonged its duration. Similar prolongation was observed with alpha2 antagonist and beta agonist, but these compounds amplified the normal anti-thyroid drug-induced rise in cerebrocortical T3 content on the day of onset of central homeostasis. Beta antagonist did not cause any perturbations. All these observations were supported by parallel changes in cerebrocortical deiodinase II (DII) activity, cAMP and calcium content. The authors concluded that there was a close correlation between cerebral T3 content, DII activity, cAMP and calcium content that are regulated by the AR system (Kundu et al., 2008). Thus, thyroid hormone homeostasis in the adult mammalian brain is maintained primarily by the beta-adrenergic pathway along with an unexpected pharmacological involvement of the alpha adrenergic pathway.

Antifertility effects of fluphenazine The underlying mechanisms in human infertility associated with hyperprolactinemia have yet to be established. Hyperprolactinemia is a known side-effect of fluphenazine, a broad spectrum, long-acting phenothiazine known to be D2 dopamine receptor antagonist. Dose-related effects of fluphenazine decanoate on the fertility of 60-day treated, adult male rats were studied by Gill-Sharma (Gill-Sharma et al., 1992). Significant increase in the serum levels of prolactin and decrease in the levels of LH and FSH were seen at doses of 1-3 mg/kg/day. No effect was evident on the serum testosterone and estradiol. The tissue levels of inhibins were not affected. Mating occurred within 10 days of cohabitation in the control and 1-2 mg/kg/day drug treated groups but delayed in the 3 mg/kg/day drug treated group with a significant effect on potency. Implantation sites, litter size and fertility index were significantly reduced at 2-3 mg/kg/day doses of fluphenazine. No effects however were seen on sperm counts or motility whereas morphological changes were apparent in the acrosome. Hypothalamic tyrosine hydroxylase levels were increased in 1-3 mg/kg/day dose range. Hyperprolactinemic males sired fewer pups as compared to controls. Hypothalamic tyrosine hydroxylase was upregulated at all the doses. The antifertility effects of fluphenazine-induced hyperprolactinemia appeared to be unrelated to testosterone. In addition, FSH decrease might have affected the intrinsic sperm quality and thereby reduced litter size.

Hypothyroidism associated reproductive disorders Plasma tachykinin levels are known to be altered with sexual acyclicity and loss of reproductive

function. Ovulatory dysfunction, as seen in postmenopausal women, is also often encountered in hypothyroid patients. Tachykinin expression is up regulated in hypothyroidism as it does in the sexually acyclic females. Kundu performed DD-PCR with the pituitary RNA of control and hypothyroid rats to see the differentially expressed gene profile (Ghosh et al., 2007). The authors observed differential expression of tac2 along with other tachykinin genes and their receptors in rat pituitary and ovary, which suggests that hypothyroidism, affects the expression of these genes in these tissues. The experiments were repeated in ovarian tissue obtained at surgery from hypothyroid human patients, which showed similar expression pattern of TAC3 (equivalent to rat tac2) and their receptors as in rat ovary. Significant reduction of tac2 expression in reproductively less active rat ovary suggests the association of tac2 with reproductive senescence. These results suggest that decline in reproductive function in hypothyroidism is associated with altered expression level of tac2 and its receptors. Further investigation in this area could elucidate the possible mechanism of tachykinins' involvement in loss of sexual cyclicity and other reproductive disorders associated with hypothyroidism.

HPA axis activity and neuropathogenesis in HIV-1 clade C infection A disturbance in the activity of the hypothalamic-pituitary-adrenal (HPA) axis has been reported among individuals with HIV-1 infection. However, these studies have been carried out in the West where the infecting clade is clade B. In India, the HIV-1 infecting clade is largely clade C with many structural differences between clades B and C. Chittiprol (Chittiprol et al., 2008) investigated the HPA axis activity in HIV-1 infection clade C.and observed that HIV clade C infection interferes with the functions of the hippocampus and thereby affects the HPA axis. They studied asymptomatic HIV-1 seropositive individuals (n=117) and, age-matched, HIV-1 seronegative controls (n=29). Neuroendocrine function of the HPA axis was evaluated using plasma levels of cortisol, ACTH, and DHEA-S, both in the morning (0800-1000 hr) and evening (2000-2200 hr). A significant elevation of cortisol levels during A.M. and P.M. hours was observed in HIV-1 infected individuals when compared to the controls. Interestingly, no significant change in ACTH level was observed in HIV-1 seropositive subjects, either during A.M or P.M hours. Elevated levels of cortisol in HIV-1 seropositive subjects appear to be independent of ACTH and may be the result of a defective negative feedback mechanism. On the other hand, a significant decrease in the plasma levels of DHEA-S was observed during A.M. and P.M. hours in HIV-1 infected individuals, leading to an increased cortisol to DHEA-S ratio. Another follow up study over a 2-year period at 12 monthly intervals in clinically asymptomatic HIV-1 clade C seropositive patients demonstrated attenuated autonomic functions, a disconnection between response of ACTH and cortisol to the mirror star tracing challenge test, and an inverse relationship between plasma levels of catecholamines and cortisol (Chittiprol et al., 2007). Since plasma catecholamines and cortisol are the peripheral mediators of the autonomic and HPA axis function, the findings of this study reflected the overall adverse effect of HIV-1C infection on autonomic as well as HPA axis functions.

Clinical neuroendocrinology Acharya reported the clinical presentation, response to medical treatment, and long-term follow-up of 39 children and adolescents with prolactinoma (F:M; 30:9 : 30 macro and 9 microadenoma) diagnosed at the age of 9-20 years (Acharya et al., 2008). Mean duration of follow up was 56 months. All patients were treated with bromocriptine at doses ranging from 2.5 to 20 mg/day or by cabergoline at doses ranging from 0.5 to 2 mg/week orally. Two patients received external conventional radiotherapy after surgery. In 25 patients, bromocriptine normalized PRL levels and caused variable, but significant, tumor shrinkage. Cabergoline normalized PRL concentrations in 14 patients. Pregnancy occurred in 6 patients while on treatment. Pregnancies were uncomplicated, and the patients delivered normal newborns at term.

Chatterjee et al (Chatterjee et al., 2008) reported that apart from pituitary adenoma and Sheehan syndrome snake bite as a common etiology of hypopituitarism in India.. Bajpai et al (Bajpai et al., 2008) reported the profile of 59 children with central DI (40 boys, 19 girls). Diagnosis included post-operative central DI (13, 22%), central nervous system (CNS) malformations (5, 8.6% holoprosencephaly 4 and hydrocephalus 1), histiocytosis (11, 18.6%), CNS pathology (11, 18.6%; craniopharyngioma 3, empty sella 2, germinoma 2, neuro-tuberculosis 2, arachnoid cyst 1 and glioma 1) and idiopathic central DI (19, 32.2%) . Bhansali and colleagues (Bhansali et al., 2004) analyzed the profile and outcome of 6 patients with idiopathic giant cell granulomatous hypophysitis. Headache and visual disturbances were the most frequent presenting symptoms. All patients had hypogonadism, four had hypoadrenalism and three were hypothyroid at presentation. None of them had diabetes insipidus preoperatively. A sellar mass with suprasellar extension on MRI with loss of the posterior pituitary 'bright spot' was a consistent observation in all patients. All patients underwent surgical excision of the mass lesion with histopathological confirmation of giant cell granulomatous hypophysitis. Postoperatively, all patients became hypothyroid and hypogonad, five patients had adrenal insufficiency, while two developed permanent diabetes insipidus.

Garg et al. (2001) evaluated target gland functional status in non-Cushing pituitary macroadenoma undergoing transsphenoidal microsurgery.Hypothyroidism, hypoadrenalism and hypogonadism were present in 24%, 54% and 52% of patients. Preoperative hypopituitarism correlated with tumor size. Thyroid, adrenal and gonadal function improved in 87%, 50% and 31%; deteriorated in 4%, 29%, and 37%, respectively after trans-sphenoidal microsurgery. Pandey et al (Pandey et al., 2005) described the profile, management and outcome of 42 children with pituitary adenomas. The majority of the tumors were functioning adenomas (40/42, 95.2%). Amongst the functioning tumors, there were 20 patients (47.6%) with prolactinomas, 11 patients (26.2%) with Cushing's disease and nine patients (21.4%) with growth hormone (GH)-secreting adenomas.

Desai and colleagues (Desai et al., 1991) evaluated 430 children referred for the evaluation of short stature and 100 (23%) were confirmed to have growth hormone deficiency . The distinctive feature of this study was the marked predominance of the familial cases 31% and a high incidence of growth hormone resistant cases (11%). The author later analyzed the prevalence of GHRH-R E72X non-sense mutation in west Indian patients with idiopathic growth hormone deficiency and the mutation was found in 71% of this series, in 90% of familial idiopathic growth hormone deficiency, 36% of non-familial idiopathic growth hormone deficiency, and in 78% with phenotype isolated growth hormone deficiency type IB. The characteristic clinical phenotype of those with this mutation was sharp features, lean habitus and lack of frontal bossing or hypoglycemia (Desai et al., 2005).

Thus over the last two decades some important contributions have been made in the field of basic and experimental neuroendocrinology from India. But still precise epidemiological and clinical data regarding various neuroendocrine diseases in this country is lacking. Some of the publications in the field of basic research have indeed thrown light on pathophysiology and mechanism of neuroendocrine diseases.

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