

32. Neurotransmitter-Hormonal Regulation of Diabetes and Liver Regeneration

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Introduction

Neurotransmitters like norepinephrine (NE), serotonin (5-HT), dopamine (DA), gamma-amino butyric acid (GABA), taurine, acetylcholine (ACh) as well as sphingolipids and polyamines are involved in the regulation of endocrine function through the mediation of hypothalamus and pituitary (Bilezikian, 1987; Dakshinamurti *et al.*, 1990). The release and receptor regulation of neurotransmitters are controlled by hormones secreted in response to various physiological states of the body (Halter *et al.*, 1980). The hypothalamus is the main neuroendocrine centre in the brain where the hormones of the target glands interact with the neurotransmitters (Bilezikian, 1987). The secretion by the anterior pituitary of ACTH, growth hormone, prolactin, TSH and gonadotropins (LH and FSH) is regulated by hypothalamic hormones. Regulation of the release of the stimulatory or inhibitory hormone involve complex neural circuitry in which serotonergic and catecholaminergic systems are components of the control mechanisms (Dakshinamurti *et al.*, 1986).

Neurotransmitters and Hypothyroidism

A direct relationship between hypothalamic serotonin turnover and TSH release has been reported by Smythe *et al.*, (1982). Dopaminergic neurons exert an inhibitory effect on the secretion of TSH. This effect is at the level of the pituitary, as bromocriptine blunts the stimulatory effect of thyrotropin releasing hormones (TRH) in euthyroid subjects (Brown *et al.*, 1979). The inhibitory effect of DA is abolished by DA receptor antagonists such as domperidone. The hypothalamus has high concentrations of NE, DA and 5-HT which are essentially antagonistic in their effects on pituitary hormone regulation (Krulich, 1979). The secretion of TSH is directly controlled by two factors, a negative feedback signal indicating serum thyroid status and a stimulatory factor, TRH released from the hypothalamus. The cold-induced secretion of TSH is mediated by NE. Studies using inhibitors of NE synthesis or α -adrenergic blockers have established a stimulatory role of NE in the control of TRH-mediated TSH secretion (Mannisto *et al.*, 1979). There is

a difference between the role of α -adrenergic receptor subtypes, α_1 - being inhibitory and α_2 - being stimulatory (Kruclich *et al.*, 1982). On balance, it appears that serotonergic system has a stimulatory effect on hypothalamic control of pituitary secretion of TSH in situations where the central response is natural, such as in timing of circadian rhythm and possibly in the pulsatile secretion of TSH (Mannisto, 1983). The classical formulation of hypothyroidism of hypothalamic origin is established in pyridoxine-deficient animal model. Hypothalamic hypothyroidism is due to deficient TRH secretion. Results suggest that hypothalamic type of hypothyroidism in pyridoxine-deficient rats is caused by the specific decrease in hypothalamic serotonin (Dakshinamurti *et al.*, 1986; 1990).

Neurotransmitters and Hypertension

Clinical reports (Bing *et al.*, 1980) indicate a direct correlation between hypothyroidism and hypertension. Saito *et al.* (1983) reported that the hypothyroid state accelerated the age-related increase in blood pressure and on thyroxine replacement, the blood pressure tended to normalize in the hypertensive hypothyroid patients. In view of the relationships of hypothyroidism and vitamin B6 deficiency, blood pressure regulation effect through neurotransmitter involvement using pyridoxine-deficient animal model was studied (Dakshinamurti *et al.*, 1990). Systolic blood pressure was recorded weekly by tail cuff plethysmography (Paulose *et al.*, 1988). At the end of eight-week experimental period, direct arterial blood pressure measurements were made. There was a significant increase in both systolic and diastolic blood pressure of these pyridoxine-deficient rats which was reversed by pyridoxine treatment within 24 h.

The reversible hypertension seen in the pyridoxine-deficient rat was also correlated with general sympathetic stimulation (Paulose *et al.*, 1988). Blood samples were withdrawn from conscious animal without trauma by implanting vascular access port with catheterization to the jugular vein (Paulose and Dakshinamurti, 1987). NE and epinephrine (EPI) levels in peripheral plasma of pyridoxine-deficient rats were almost three-fold higher compared with control values. Treatment of deficient rats with pyridoxine (10 mg/kg body weight, i.p.) resulted in a return of systolic blood pressure to control level. Correspondingly, the plasma catecholamine levels decreased to normal levels within 24 hrs of pyridoxine administration. The complete reversibility of the hypertension in such a short time would preclude permanent damage to the vessel wall of the deficient animal. The lesion is probably at the level of neurotransmitter regulation. The NE turnover in the heart in both deficient and control groups by estimating the decrease in the amine content due to α -methyl p-tyrosine was determined (Viswanathan *et al.*, 1990). There was no difference in myocardial NE content between the deficient and control groups. However, the NE turnover was increased significantly in the pyridoxine-deficient rats when compared to that of the control, thus supporting

the observation that peripheral sympathetic activity is increased in the deficient rats. Both clonidine and α -methyl DOPA reduced the systolic blood pressure of deficient rats to normal levels (Dakshinamurti *et al.*, 1990). Clonidine like drugs exert their cardiovascular depressive effects mainly through a centrally mediated sympathetic inhibition due to the stimulation of α -adrenoreceptors (Kobinger and Pichler, 1980).

The kinetics of ligand binding to α_2 -adrenoreceptors was examined using (^3H) p-aminoclonidine (PAC) binding to crude synaptosomal membrane preparation from the brain stem of pyridoxine-deficient and control rats (Viswanathan *et al.*, 1990). A reduced sympathetic outflow has been implicated in the mediation of the cardiovascular effects of GABA (Persson, 1980). The central mechanism of maintaining normal blood pressure in animals is regulated by the balance between sympathetic and para-sympathetic nervous system tonicities in the brain stem (De Jong *et al.*, 1975; Nomura *et al.*, 1985). The antihypertensive effect of clonidine results from its pharmacological reactivity for central α_2 -adrenoreceptors in the nucleus tractus solitarii (NTS). When α_2 -adrenoreceptors in NTS are stimulated, inhibitory neurons of the vasomotor centre are activated.

Neurotransmitters and Diabetes

Alterations in the neurotransmitter-hormonal regulation and sympathetic activity have been recognised as a major pathological symptom associated with diabetes mellitus. Streptozotocin (STZ)-induced diabetes is used as a model for insulin-dependent diabetes mellitus (IDDM). The major feature in this type of diabetes is a substantial reduction in insulin secreting β -cells (Gepts, 1965). Although the reason for such cellular destruction is not clearly known, genetic influences and both humoral and cell-mediated immunological phenomena are involved (Eisenbarth, 1986; Lefebvre, 1988). A role for environmental factors in the etiology of IDDM has recently been indicated by epidemiological studies which have demonstrated that there is a marked increase in newly diagnosed cases of IDDM, which can only be explained by changes in environmental influences (Krowlewski *et al.*, 1987). It has been suggested that people with diabetes have a greater risk of depression compared to the general population (Lloyd and Orchard, 1993). Hypothalamic serotonin is suggested to mediate the release of catecholamines which control the insulin release and function. Yehuda and Meyer (1984) found that depletion of brain 5-HT by intraventricular injection of 5,7-dihydroxytryptamine significantly attenuated corticosterone response to insulin. Similar effect was seen in 5-HT receptor blocker, methysergide treatment studies. An increased noradrenergic and decreased serotonergic activity in the hypothalamus in diabetic rats can be a control factor in the increased hyperglycemia (Fig. 1).

The drug 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT), a selective agonist for 5-HT_{1A}, was shown to trigger hyperglycemia and inhibition of

insulin release (Chaouloff and Jeanrenaud, 1987). Pharmacological analysis showed the involvement of both 5-HT_{1A} and α_2 -adrenoreceptors in the metabolic effects of this drug in elevating circulating adrenaline levels which mediated the 8-OHDPAT induced hyperglycemia (Chaouloff *et al.*, 1990). The common neuroendocrine alterations associated with diabetes mellitus include an increase in growth hormone response to exercise (Hansen, 1970), TRH and dopamine (Lorenzi *et al.*, 1980), and a decrease in GnRH release (Bowton *et al.*, 1980), TSH response to TRH and GH secretion. Most of these changes observed in diabetic state indicate an altered hypothalamic functioning in diabetes mellitus. The neuroendocrinological alterations in diabetes can be the result of either vascular insufficiency or secondary to a specific derangement in the neurotransmitter activity.

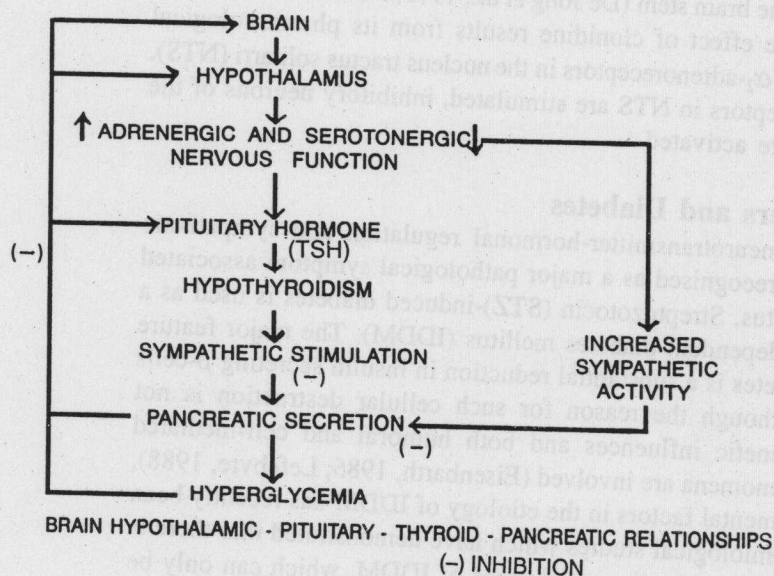


Fig. 1

During diabetes, the noradrenergic activity increased in cerebral cortex (CC), hypothalamus (Hypo) and brain stem (BS), while the cerebellum (CB) showed no change and the corpus striatum (CS) a decrease, as assessed by the monoamine content. EPI showed a significant increase in Hypo, BS and CS. Dopamine, showed a significant increase in CC, Hypo, CB, BS, and CS. The hypothalamic serotonin showed a significant decrease while that of BS and CS showed a significant increase (unpublished observation). The physiological significance of these changes is yet to be connected or delineated. The hypothalamic neurotransmitter changes are important as far as the hypothalamic endocrine regulation is considered. The increase in noradrenergic activity in the hypothalamus has been studied in detail in streptozotocin-diabetic rats by Bitar *et al.* (1987). The results of their analysis of 5-HT and 5-HIAA in hypothalamic nuclei showed difference between

diabetic and control rats. 5-HT in periventricular nucleus (PVN) showed a significant increase when compared to control rats, while the 5-HIAA level was significantly decreased in suprachiasmatic nucleus (SCN), supraoptic nucleus (SON) and ventromedial nucleus (VMN). The increased noradrenergic activity and a decreased serotonergic activity together can make important decisive change on the hypothalamic hormones. The regulation of hypothalamic noradrenergic and serotonergic activity on hypothalamic corticotrophic releasing factor (CRF) has been studied in stressed states (Maickel and Martel, 1983). The studies revealed that 5-HT is mediating the release of adrenal response to insulin stress. The noradrenergic neurons have a positive control of the corticotrophic response to stress. The noradrenergic activity is high in the hypothalamus and serotonergic activity is decreased in STZ diabetes. This change may be related to an increase in adrenal activity. As a consequence, the release of insulin may be inhibited causing an increased level of circulating glucose.

The most significant alteration in the diabetic state is the reduced level or absence of insulin or its function in the body. Although studies have shown about the direct cytotoxic action of the drug STZ to pancreatic β -cells, there is no study which clearly state about the extra pancreatic toxic effects of the drug. Autonomic neuropathy associated with STZ diabetes is reversed when insulin is supplemented (Sasaki and Bunag, 1983). In our study the insulin therapy was not found to fully bring back the altered brain neurotransmitter levels to normal values. These neurotransmitter changes in the brain regions may be a specific cause or associated with the direct behavioural or endocrine alteration, which have to be confirmed with receptor studies and second messenger studies at the DNA level. Also, the hormonal changes and reversal confirmatory studies have to be carried out.

Hormones and Liver Regeneration

The interaction of insulin, glucagon and epidermal growth factor (EGF) showed a significant effect on liver regeneration by way of increased DNA synthesis (Chung *et al.*, 1992). A direct hepatotrophic effect of glucagon in liver regeneration was recently observed in glucagon-treated hepatectomised rats (Huang *et al.*, 1993). Measurement of various hormone levels after various intervals of partial hepatectomy showed that ACTH and corticosterone increased significantly while TSH, T4 and testosterone decreased and T3 was unchanged. This indicated an increase in pituitary-adrenal function and a decrease in pituitary-thyroid activity during regeneration (Knopp *et al.*, 1991).

Tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), a specific target for insulin receptor β subunit kinase, was strikingly enhanced prior to hepatocyte DNA synthesis. There was a parallel increase in IRS-1 protein and mRNA expression as well as the tyrosine phosphorylation of β subunit. Phosphatidyl-inositol-3-kinase (PI-3 kinase) was associated with IRS-1

following tyrosine phosphorylation (Sasaki *et al.*, 1993). The PI-3 kinase has been implicated in transmembrane signal transduction by a number of growth factor receptors (Cantley *et al.*, 1991). 3,3',5'- triiodothyronine (T3) was implicated as a specific hepatomitogen (Short *et al.*, 1980). T3, aminoacids, glucagon and heparin were shown to stimulate DNA synthesis of unoperated rats (Short *et al.*, 1972). T3 injections stimulated the liver regeneration response that resembled in timing and magnitude, the DNA synthesis induced by 40% hepatectomy. T3 showed a dose-related response in presence of EGF in hepatocyte cultures (Francavilla *et al.*, 1994). Glucocorticoids were shown to cause an increase in EGF-induced proliferation of regenerating hepatocytes (De Juan *et al.*, 1992).

Neurotransmitters and Liver Regeneration

Neuronal stimulation of rat liver could result in changes in cellular metabolism and liver regeneration through a combination of direct innervation and intercellular communication via gap junction (Seske *et al.*, 1992). Vagotomy was shown to cause a marked depression of cell proliferation following hepatectomy (Maros, 1970; Ohtake *et al.*, 1993). Extirpation of the brain cortex was shown to increase the rate of cell proliferation implying that the cortex exerts a normal inhibitory function on liver cell division and growth. It was reported that transection of the spinal cord above the area innervating the liver resulted in depression of thymidine incorporation into hepatic DNA (Vaptzarova *et al.*, 1973). If transection was done at 16 hrs following partial hepatectomy, the already enhanced DNA synthesis was suppressed. Similar results were obtained with mid-thoracic transection of the cord implicating a suprasedgmental level of regulation of regeneration. These data suggest that an intact nervous system is an important component of the regulatory system for liver regeneration either through direct innervation of the liver or through indirect modulation of DNA synthesis resulting in cell division (Fig. 2).

The sympathetic nerve activity of the liver was reported to be suppressed at the early stage of regeneration after 1/4th hepatectomy (Iwai and Shimazu, 1992). Liver DNA synthesis following partial hepatectomy depends on extracellular and/or intracellular calcium levels and thus, one action of NE may be to function directly through modulating intracellular calcium levels (Rixon *et al.*, 1979). NE was shown to induce DNA synthesis in a dose-dependent manner in hepatocyte cultures, acting through the α_1 -adrenergic receptor (Cruise *et al.*, 1985). A strong synergistic interaction between NE and EGF, a complete hepatocyte mitogen, was observed in hepatocyte cultures incubated with both EGF and NE, or pretreated with NE and then exposed to EGF, and stimulated the hepatocytes to enter the DNA synthesis (Cruise and Michalopoulos, 1985). Low affinity EGF receptors are the only type present when mitogenic response is stimulated (Vintermyr and Doskeland, 1987). It was postulated that the low affinity receptors are the true mitogenic

EGF receptors (Wollerberg *et al.*, 1989). It is hypothesised that the EGF mitogenic signal is modulated by protein kinase C which causes the switching of the EGF receptor from high affinity to low affinity states in rat liver cells (Sharma *et al.*, 1994). It was shown that NE was able to decrease the dose-dependent inhibition of TGF- β , a potent inhibitor of hepatocyte primary cultures acting through the α_1 -adrenergic receptor (Houck *et al.*, 1988). Thus the results suggest that NE may trigger the initial proliferative response in liver regeneration by modulating the response of hepatocytes to growth factors such as EGF and growth inhibitors such as TGF- β .

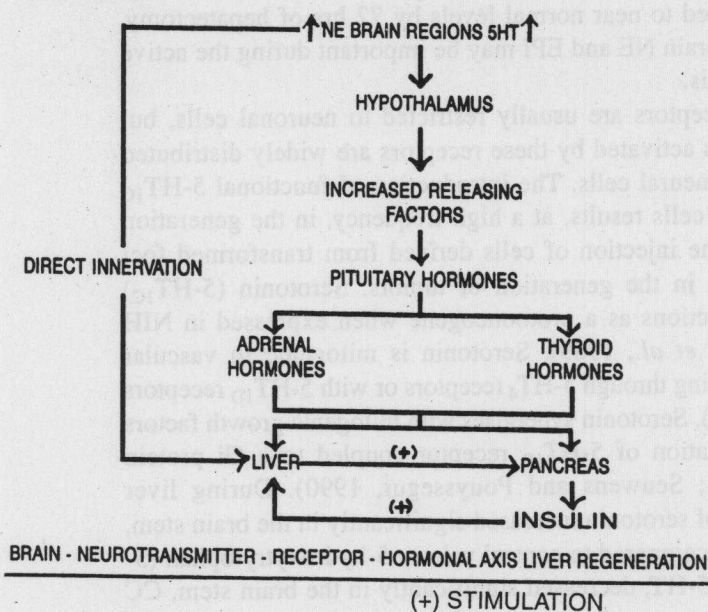


Fig. 2

Addition of NE to primary cultured adult rat hepatocytes stimulated the DNA synthesis in a dose-dependent manner, especially in the presence of insulin and EGF. The effect of EPI was also through the α_1 -adrenergic receptor. However, the α_1 -action of EPI was apparently not mediated by either activation of protein kinase C or Ca^{++} mobilisation (Takai *et al.*, 1988). The number of β -adrenergic receptors was found to increase with the maximal increase seen 48 hrs after partial hepatectomy. The α_1 -adrenergic receptors showed a transient decrease by about 35% at 18–24 hrs (Sandnes *et al.*, 1986). An increased β -adrenergic receptor responsiveness during liver regeneration was found to result from either an increase in the receptor number or from a more efficient receptor cyclase coupling (Riles *et al.*, 1984).

In vivo, plasma catecholamines or those delivered by the synaptic stimulus may contribute to the regenerative stimulus. As a prelude to study the *in*

vivo role of NE as a regulator of cell division, we have observed significant changes in the content of brain NE, especially in the cerebral cortex, brain stem and hypothalamus during 24 and 48 hrs of liver regeneration, compared to control values. Brain EPI was seen to increase significantly in the cerebral cortex during 24 and 48 hrs of liver regeneration compared to that of control suggesting that it can function synergistically with NE during the DNA replicative phase of liver regeneration. Circulating levels of NE increased significantly during 24 and 48 hrs of regeneration. We also noted that DNA synthesis was maximal during 24 and 48 hrs of the partial hepatectomy. Brain NE levels declined to near normal levels by 72 hrs of hepatectomy. This suggests that the brain NE and EPI may be important during the active phase of DNA synthesis.

Neurotransmitter receptors are usually restricted to neuronal cells, but the signalling pathways activated by these receptors are widely distributed in both neural and nonneural cells. The introduction of functional 5-HT_{1C} receptor into NIH 3T3 cells results, at a high frequency, in the generation of transformed foci. The injection of cells derived from transformed foci into nude mice results in the generation of tumors. Serotonin (5-HT_{1C}) receptor, therefore, functions as a protooncogene when expressed in NIH 3T3 fibroblasts (Julius *et al.*, 1989). Serotonin is mitogenic in vascular smooth muscle cells, acting through 5-HT₄ receptors or with 5-HT_{1D} receptors (Kavanaugh *et al.*, 1988). Serotonin synergises with mitogenic growth factors in fibroblasts via activation of 5-HT_{1B} receptor coupled to a Gi protein (Seuwens *et al.*, 1988; Seuwend and Pouyssegur, 1990). During liver regeneration, the level of serotonin increased significantly in the brain stem, CC and Hypo at 24 hrs compared to control values. 5-hydroxytryptophan (5-HTP), the precursor of 5-HT, decreased significantly in the brain stem, CC and Hypo at 24 hrs of regeneration compared to respective control values. 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolite, increased significantly in brain stem and Hypo and 24 hrs of hepatectomy compared to control values.

It is proposed that neurotransmitters directly and/or through the hormonal pathway regulate insulin function in diabetes and DNA synthesis and cell proliferation in liver regeneration after hepatectomy.

Acknowledgement

Supported by a research grant from DST. PSP and BS thank CSIR for SRF.

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