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Glycemic control, a worthwhile goal for people with diabetes, is limited by the barrier of hypoglycemia (Cryer, 2008; Cryer, 2009). Hypoglycaemia constitutes a unique metabolic brain insult. Hypoglycaemic brain injury is a common and serious complication of insulin therapy and occurs most frequently in patients attempting tight glucose control (Davis et al., 1998). Hypoglycemia causes brain fuel deprivation that, if unchecked, results in functional brain failure that is typically corrected after the plasma glucose concentration is raised (Cryer, 2007). Rarely, it causes sudden, presumably cardiac arrhythmic death or, if it is profound and prolonged, brain death (Cryer, 2007). Several experimental models have been described which provide information on the etiology of IDDM. Streptozotocin is a toxic agent selective to pancreatic β-cells that induces IDDM by causing the β-cell destruction (Like & Rossini, 1976; Paik et al., 1980). Increased blood glucose and decreased body weight during diabetes is similar with previous reports as a result of the marked destruction of insulin secreting pancreatic islet β-cells by streptozotocin (Junod et al., 1969). Hypoglycemia can cause recurrent morbidity in many people with type 1 diabetes and also in some with advanced type 2 diabetes (Zammit & Frier, 2005; Cryer, 2009). Hyperglycaemia occurs as a result of increased glycogenolysis, decreased glycogenesis, increased gluconeogenesis, impaired glucose transport across membranes and almost complete suppression of the conversion of glucose into fatty acids via acetyl-CoA. Hyperglycaemic state during diabetes is due to the increased gluconeogenic pathway, which is physiologically less sensitive to the inhibition by insulin (Burcelin et al., 1995). During diabetes there is decrease in body weight as a result of altered metabolic function.
Administration of 1.5 U/Kg of regular insulin produced a fall in glucose level below 50mg/dL after 1hour in C+IIH rats (Joseph et al., 2008). The minimum required dose to produce irreversible severe hypoglycaemia was 0.5 units/kg (Abdul-Ghani et al., 1989). In D+IIH rats, administration of 10U/Kg of insulin decreased the blood glucose level below 50 mg/dL after 3hours (Joseph et al., 2008). It is well recognized that the glucose level is the primary determinant of the hormonal and metabolic counter regulatory responses to insulin induced hypoglycaemia. Falling plasma glucose concentrations elicit a sequence of responses that normally prevent or rapidly correct hypoglycemia (Cryer, 2001; Cryer, 2009). A single episode of very mild hypoglycaemia (56 mg/dL) causes a reduction of neuroendocrine counter regulation that is readily discernible about 24 h later. A similar effect of a single hypoglycaemic episode has been shown in healthy (Hvidberg et al., 1996) and diabetic (Dagogo et al., 1993) humans. The risk increases with a history of hypoglycemia and an increased number of years of insulin treatment and age (Cryer et al., 2003; Donnelly et al., 2005). Hypoglycaemia is the most common metabolic complication occurring in older people with type 2 diabetes (Abdelhafiz & Sinclair, 2009). Studies determined the incidence and risk factors for developing severe hypoglycaemia among persons aged 80 yr or older, with diabetes mellitus were more (Greco & Angileri, 2004; Chelliah & Burge, 2004). The glycemic levels during antecedent hypoglycaemia in those studies were 46–50 mg/dL. The plasma glucose level during antecedent hypoglycaemia has been shown to be a major determinant of the effects on subsequent counter regulation (Davis et al., 1997). Heller and Cryer, (1991) reported a reduced counter regulatory response 18 h after one mild hypoglycaemic episode (plasma glucose, 54 mg/dL). In fully developed (i.e., C-peptide negative) type 1 diabetes, circulating insulin concentrations do not decrease as plasma glucose concentrations decline in response to therapeutic (exogenous)
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hyperinsulinemia (Dagogo et al., 1993; Cryer, 2009). Recurrent episodes of hypoglycaemia have been demonstrated to reduce subsequent endocrine counter regulation (Heller & Cryer, 1991; Davis & Shamoon, 1991; Davis et al., 1992; Widom & Simonson, 1992; Veneman et al., 1993; George et al., 1995; Davis et al., 1997). The prolonged effects of even mild hypoglycaemia on subsequent counter regulation underline the importance of scrupulously avoiding even mild hypoglycaemic episodes in patients with diabetes. The body weights of D+IIH and CI+IIH rats showed no significant change compared to control.

CENTRAL NERVOUS SYSTEM ALTERATIONS OF GLUTAMATE DURING HYPOGLYCAEMIA AND DIABETES

Diabetes mellitus is a metabolic disorder that not only causes a decrease in efficiency of the pancreatic β-cells to secrete insulin but also is accompanied by altered monoamine levels and their turnover rates in the CNS (Garris, 1990; Lackovic et al., 1990; Bhattacharya & Saraswathi, 1991). Glucose is the major source of brain energy and is essential for maintaining normal brain and neuronal function. Hypoglycaemia causes impaired synaptic transmission. Oxidative stress plays an important role in tissue damage caused by hypoglycemia and diabetes, which results in deterioration in glucose homeostasis caused by these metabolic disorders. Hypoglycaemia is associated with increased glutamate release (Sandberg et al., 1986) and conversely, glutamate toxicity in neurons is augmented by hypoglycaemia (Novelli et al., 1988). Hyperglycaemia is reported to be a major factor that damages the CNS monoaminergic activity as a result of neuronal degeneration in different regions of the brain. Onset of diabetes has been reported to inhibit the firing of dopaminergic neurons (Saller, 1984) with alteration in its metabolism. Our previous studies reported increased monoamine content in the plasma and platelet of diabetic
patients (Jackson *et al.*, 1997). We reported increased NE in the brainstem of young diabetic rats while the NE content decreased in old diabetic rats (Abraham & Paulose, 1999). EPI content increased in adult diabetic rats without any change in NE. NE to EPI turn over showed a significant increase during diabetes (Pius & Paulose, 1999). There is a significant reduction in the cortical and brainstem 5-HT content of diabetic rats (Jackson & Paulose, 2000). Alterations of central neurotransmission and environmental factors change the relative contribution of sympathetic outflow to the pancreas, liver, adrenal medulla and adipose tissues, leading to the modulation of glucose and fat metabolism (Nonogaki, 2000). Studies revealed that during severe energy deprivation following hypoglycemia and diabetes, mitochondrial free radicals scavenger system is down regulated, which leads to reactive oxygen species (ROS) generation (Singh *et al.*, 2004). Neurotransmitter alterations in the brain of insulin induced hypoglycaemic rats are poorly studied.

Glucose is known to serve as the major substrate for cerebral energy under normal conditions (Siesjo, 1978). Recent evidence suggests a direct correlation between glucose utilization and cognitive function (McNay *et al.*, 2000). Considerable evidence suggests that oxidative stress plays an important role in tissue damage associated with hypoglycemia and other metabolic disorders. The altered brain neurotransmitters metabolism, cerebral electrolyte contents and impaired blood-brain barrier function may contribute to CNS dysfunction in hypoglycemia (Bhardwaj *et al.*, 1999). Reports suggest that glucose deprivation induces damage by enhancing the formation of energy-yielding products and extracellular load of glutamate (Geng *et al.*, 1997). Age-related cognitive impairments are associated with structural and functional changes in the cerebral cortex. It was previously demonstrated in the rat that excitatory and inhibitory pre- and postsynaptic changes occur with respect to age
and cognitive status; however, in aged cognitively impaired animals (Majdi et al., 2007).

In the CNS, glutamate is an important factor for maintaining calcium homeostasis; it is the most abundant excitatory neurotransmitter and it is widely distributed. Glutamate is associated with various brain functions, such as synaptic plasticity, learning and long-term potentiation (Collingridge & Singer, 1990). Attention has been focussed on glutamate as a potential mediator of hypoglycaemic brain injury (Aral et al., 1998; Cavaliere et al., 2001; Marinelli et al., 2001). We observed an increase in the glutamate content in the cerebral cortex, cerebellum, hippocampus and pancreas of both adult and old diabetic and hypoglycaemic rats. The increase in the glutamate content was more prominent in the adult and old hypoglycaemic group. Glutamate is essential for synaptic communication in the CNS, but inadequate regulation of extracellular glutamate and glutamate receptor agonists cause toxicity in the nervous system (Olney, 1989; Choi, 1992; Coyle & Puttfarcken, 1993; Greene & Greenamyre, 1996; Doble, 1999) leading to neurodegenerative disorders. Prolonged insulin-induced hypoglycemia (IIH) causes widespread loss of neurons and permanent brain damage with irreversible coma (Kleihues et al., 1986).

The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA kainate (Choi, 1988). Acute hypoglycemia was found to enhance the excitotoxic effects of glutamate in the newborn (McGowan et al., 1995). Our previous studies also reported that GDH enzyme activity enhanced during diabetes and did not completely reverse even after insulin administration (Preetha et al., 1996; Aswathy et al., 1998). Studies using young and old diabetic rats clearly revealed that GDH activity regulation is essential to avoid diabetic associated brain glutamate toxicity (Biju & Paulose 1998). Studies from our laboratory have shown that an increased glutamate dehydrogenase activity
producing increased glutamate content in the cerebellum and thereby leading to glutamate toxicity (Joseph et al., 2007). Recent studies have shown that the abnormal accumulation of glutamate and NO plays a key mechanism of axonal degeneration in disorders such as multiple sclerosis (Ouardouz et al., 2009). It was shown by Larsen et al., (2006) that glucose deprivation caused 77% of the neurons are lost due to glutamate excitotoxicity. It was also shown that the main process implied in the neuronal cell death responsible for aging and the related neurodegenerative diseases are started by neurotrophic factors, hypoxia, hypoglycemia, excitotoxicity and oxygen and nitrogen free radicals (Peinado et al., 2000). Hypoglycemic brain damage associated with high levels of the excitatory amino acids aspartate and glutamate in the newborn piglets and adult pigs were demonstrated by Darling et al., (2001). Our studies on the cerebral cortex showed that the increased glutamate content increased brain damage during hypoglycemia compared with hyperglycemia which is suggested to contribute to cognitive and memory function (Joseph et al., 2008).

GLUTAMATE RECEPTOR ALTERATIONS AND FUNCTIONAL REGULATION IN CONTROL AND EXPERIMENTAL RATS

Cerebral Cortex

The cerebral cortex is the seat of our highest forms of intelligence. It plays a central role in many complex brain functions including memory, attention, perceptual awareness, thought, language and consciousness. One study has found positive association between the cortical thickness and intelligence (Katherine et al., 2007). Another study has found that the somatosensory cortex is thicker in migraine sufferers (Alexandre et al., 2007). All three ionotropic glutamate receptors exhibit a ubiquitous distribution in the brain, the NMDA receptors being particularly abundant in the
forebrain (Ozawa et al., 1998). Although all receptors have pivotal roles in brain functions, the NMDA receptors have received special attention in development and aging. They are involved in cell migration, growth and differentiation in the developing brain (Vallano, 1998). Furthermore, important roles have been assigned to them in cognitive functions, learning and memory (Kito et al., 1990). They also execute neuronal cell death (Szatkowski & Attwell, 1994), this possibly being relevant to aging of the brain and degenerative neurological diseases. Immunohistochemical studies have previously identified positive staining for presynaptic mGlu5 receptors in the rat cerebral cortex (Romano et al., 1995). The mGluR5 is reported to mediate a G-protein-dependent release of intracellular calcium stores (Valenti et al., 2002). Moreover, NMDA receptor function is inhibited by a rise in intracellular calcium (Rosenmund et al., 1995). Yu et al., (1997) pointed out that mGlu5 mediated direct inhibition via G-proteins also leads to NMDA receptor inhibition. NR2B is expressed selectively in the forebrain, with high levels in the cerebral cortex, hippocampal formation, septum, caudate-putamen, olfactory bulbs and thalamus. An increased NMDA2B mRNA level was found in the postmortem brain of Huntington’s disease patients showing neuronal degeneration due to glutamate excitotoxicity (Arzberger et al., 1997). Metabotropic glutamate receptors (mGluRs) have various functions on neuronal excitability in the CNS (Pin & Duvoisin, 1995). Group I mGluRs are positively coupled to phosphoinositide hydrolysis and the mobilization of intracellular Ca\(^{2+}\) leading to excitotoxic cell death. Metabotropic glutamate regulates synaptic glutamate release both in in vitro (Herrero et al., 1994) rat brain slices (Croucher et al., 1997; Thomas et al., 2000) and in vivo (Patel & Croucher, 1997). The role of NMDA2B and mGluR5 receptors in hypoglycemic brain damage is not reported before.
Our findings report an increase in total glutamate and NMDA receptors function in the cerebral cortex with no significant change in $K_d$. This increased $B_{\text{max}}$ observed shows the increased number of receptors with no change in the affinity of the receptors which was shown from the $K_d$. The increased receptor activity observed from the Scatchard plot was supported by the gene expression studies of NMDAR1, NMDA2B and mGluR5 glutamate receptor subtypes. Severe hypoglycemia with brain dysfunction limits intensified therapy in patients with insulin dependent diabetes mellitus, despite evidence that such therapy reduces the risk of chronic complications of the disease (Maran et al., 1994). Severe hypoglycemia causes neuronal death and cognitive impairment. Deficits in long-term potentiation during chronic diabetes arise from dysfunction of the NMDA subtype of glutamate receptors in early stages of the disease (Trudeau et al., 2004). The NMDA receptor is expressed in the cerebral cortex and hippocampus and is important in learning and memory. Recent studies reported that abnormal expression of NMDA receptor is involved in the development of diabetic neuropathy (Tomiyama et al., 2005). Among them, the neurotransmitter receptor NMDA shows strong age-related reduction of expression (Lu et al., 2004). Acute hypoglycemia was found to enhance the excitotoxic effects of glutamate in the newborn (McGowan et al., 1995). Previous studies reported that changes in the protein expression of the NMDA receptor subunits occur during the ageing process and it was greater than the changes seen in mRNA expression (Magnusson et al., 2002). Ageing does not affect all brain regions equally. Some regions seem to be more sensitive to ageing than others (Horiuchi & Saitoe, 2005; Lu et al., 2004). The brain regions - cerebral cortex and hippocampus of diabetic rats is suggested to be more vulnerable to glutamate toxicity via NMDA receptor activation. An age-related increase in mGlu1 receptor mRNA levels was found in thalamic nuclei, hippocampal CA3 with parallel increases in mGlu1a receptor protein expression (Simonyi et al.,...
2005). Exposure to acute hypoglycemia in newborn piglets showed increased glutamate binding sites of cerebral NMDA receptors (McGowan et al., 2002). It has been shown from previous studies that NMDA receptors, as well as mGluRs, play important roles in the cascade of biochemical reactions resulting in death of neuronal cells in vivo (Tsintsadze et al., 2001). Studies have reported that rats subjected to severe hypoglycemia showed deficits in the Morris water maze test, a standard measure of learning and spatial memory (Sang et al., 2005). We studied the glutamate content, the total glutamate and NMDA receptor kinetics and the gene expression of NMDA2B and mGluR5 glutamate receptor subunits in the cerebral cortex of hypoglycemic and hyperglycemic rats. Also, the immunohistochemistry studies using confocal microscope for the expression of NMDAR1, NMDA2B, mGluR5 and IP3 receptors confirmed the Scatchard analysis and real time PCR results. Our results showed an increased glutamate content and glutamate receptor gene expression in the cerebral cortex of both hypoglycemic and diabetic rats. Our study is focused on the hypoglycemic shock usually happening in an insulin or antihyperglycemic therapy to diabetic patients. This frequent hypoglycemic shock is going to reduce the supply of glucose to the brain which will have deleterious effect to the functioning of brain cells. Our results suggest that glutamate receptor alterations found in the brain regions contributes to cognitive and memory deficits during diabetes and hypoglycaemia as a function of age. It is observed that there is occurrence of seizures in hypoglycemic state which is due to the decreased glucose (energy) for the brain cells to function (Yoshikawa et al., 2003; Gordon, 2006). Evidence suggests that hypoglycemic neuronal death involves excitotoxicity and DNA damage (Sang et al., 2007). It is widely accepted that energy deprivation causes a neuronal death that is mainly determined by an increase in the extracellular level of glutamate (Marinelli et al., 2001). Our studies on the cerebral cortex showed that the increased glutamate content
increased brain damage during hypoglycemia compared with hyperglycemia which is suggested to contribute to cognitive and memory function (Joseph et al., 2008).

The extracellular concentration of the excitatory neurotransmitter L-glutamate in the CNS must be kept low to ensure a high signal to noise ratio during synaptic activation (Katagiri et al., 2001) and to prevent excitotoxicity due to excessive activation of glutamate receptors (Mangano & Schwarz, 1983; Rosenberg & Aizenman, 1989; Rosenberg et al., 1992; Rothstein et al., 1996; Tanaka et al., 1997; Wang et al., 1998) and this function is served by glutamate transporter proteins. Glutamate uptake into neurons and glia cells is important for termination of glutamatergic transmission. Glutamate transporters are essential for the maintenance of low extracellular levels of glutamate. Studies in brain autopsy specimens of HIV-1-infected patients have shown that the expression of EAAT-2 by activated microglia exert a compensatory effect that protects neurons from glutamate neurotoxicity (Xing et al., 2009). We observed a reduced expression of GLAST glutamate transporter which shows a reduced uptake of the extracellular glutamate which activated the glutamate receptor subtypes-NMDAR1, NMDA2B and mGluR5.

The present study showed that the second messengers- IP3, cGMP and cAMP were up regulated in the cerebral cortex of the entire experimental group of rats. All of glutamate receptors couple positively to phospholipase C via guanine nucleotide binding proteins (G-proteins) whereby they stimulate phosphoinositide hydrolysis generating a second messenger cascade consisting of diacylglycerol and inositol 1,4,5 trisphosphate (Berridge, 1987). Ng et al., (2004) reported that up regulation of glutamate receptors and calcium-binding proteins in the diabetic retina. Jo et al., (2008) demonstrated that NMDA and mGluR receptors mediate calcium release by stimulating IP3 and PKC. It was also reported that activation of the first-group mGluR (including mGluR1 and mGluR5) results in stimulation of metabolism of
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inositol phosphates and results in mobilization of the intracellular calcium (Schoepp & Conn, 1993). Excessive stimulation of glutamate receptor/ion channel complexes triggers calcium flooding and a cascade of intracellular events that results in apoptosis and/or necrosis (Johnston, 2005). Prolonged stimulation of glutamate receptor subtypes, followed by intracellular Ca$^{2+}$ overload and activation of specific genes, results in synthesis of enzymes involved in cell stress response (Caccamo et al., 2004). Excessive Ca$^{2+}$ overload in cells have been reported to cause apoptosis. Boehning et al., (2003) demonstrated a small amount of cytochrome C released from mitochondria binds to and promote Ca$^{2+}$ conductance through IP3 in the endoplasmic reticulum membrane. The released Ca$^{2+}$ further triggers mass exodus of cytochrome C from all mitochondria in the cell and thus activating the caspase and nuclease enzyme that finalize the apoptotic process. Elevation of intracellular calcium can lead to cell death (Choi, 1994). Studies of Abdul-Ghani et al., (1996) revealed that mGluR-mediated the production of IP3 and the mobilization of intracellular Ca$^{2+}$. Increase in intranuclear Ca$^{2+}$ that leads to altered transcription of apoptotic genes and activation of nuclear endonucleases resulting in hypoxia-induced programmed neuronal death (Mishra & Delivoria-Papadopoulos, 2004).

cGMP mediates physiological effects in the cardiovascular, endocrinological, and immunological systems as well as in CNS. In the CNS, activation of the NMDA receptor induces Ca$^{2+}$-dependent NOS and NO release, which then activates soluble guanylate cyclase for the synthesis of cGMP. Both compounds appear to be important mediators in long-term potentiation and long-term depression and thus play an important role in the mechanisms of learning and memory. Altered modulation of cGMP levels in brain seems to be responsible for the impairment of cognitive function (Erceg et al., 2005). cGMP has been implicated in the regulation of many essential functions in the brain, such as synaptic plasticity, phototransduction, olfaction and
behavioural state. Studies have reported that activation of N-methyl-d-aspartate receptors causes increase in cGMP in hyperammonemic rats (Cauli et al., 2008). Calcium flux through the NMDA receptor activates neuronal nitric oxide synthase (nNOS), which produces NO. In neurons activated by nitric oxide produces cGMP (Serulie et al., 2008). In the present work we observed an increase in cGMP in the cerebral cortex of hypoglycaemic and diabetic group with more prominent up regulation in the hypoglycaemic group. The NO/cGMP/PKG-mediates pathological mechanism that leads to hyperexcitability and sensitizes neurons to excitotoxic damage in neurodegenerative disorders (González-Forero et al., 2007). Our study showed the increased cAMP content in the cerebral cortex of hypoglycaemic and diabetic rats. Up regulation of cAMP activates cAMP dependent protein kinase resulting in phosphorylation.

The receptor analysis, gene expression and immunohistochemistry studies implicate a role for glutamate receptor subtypes in the manifestation of the cognitive and memory deficits associated with hypoglycaemia and diabetes in adult and old rats. The decreased glutamate transporter GLAST expression reduces the reuptake of the extracellular glutamate which was confirmed from the glutamate content analysis. The increased glutamate content and the receptor activity enhanced the second messengers-IP3, cGMP and cAMP which lead to the calcium influx and neurodegeneration. The enhanced glutamate receptors were more prominent in hypoglycemic group which is of significance in this study This increased brain damage observed during hypoglycemia compared with hyperglycemia is suggested to contribute to cognitive and memory function.
Cerebellum

Experimental evidence indicate the involvement of the cerebellum in variety of human mental activities including language (Fiez et al., 1996), attention (Allen et al., 1997), cognitive affective syndromes (Schmahmann & Sherman, 1998), fear and anxiety caused by threats of pain (Ploghause et al., 1999), thirst sensation and fear for air hunger (Parsons et al., 2001) and motor relearning (Imazumi et al., 2004; Hermann et al., 2004; Jiao et al., 2008). The cerebellum is known to be resistant to hypoglycaemia. Studies from our laboratory have demonstrated that cerebellum is susceptible to hypoglycaemia (Joseph et al., 2007). Some of the most frequent signs of cerebellar hypoplasia include poor fine motor skills, hypotonia and autistic features (Wassmer et al., 2003). The cerebellar vermis integrates and processes the inputs from the vestibular, visual and proprioceptive systems to coordinate muscle timing as a result of which the centre of gravity stays within the limits of stable upright standing (Diener et al., 1989). Damage to the cerebellum, in particular the vermis (Balogh et al., 1998) results in more postural sway than in control subjects (Ho et al., 2004, Marvel et al., 2004). Decreased postural stability would correspond with abnormalities of the vermis observed in autistic subjects (Gowen & Miall, 2005). Unlike explicit memory such as recognition memory and spatial memory, motor learning is characterized by slow development, without the requirement of conscious recall, and in general being lifetime-lasting (Llinas & Welsh, 1993; Tulving & Markowitsch, 1998; Eichenbaum, 2000). Based on the role of the cerebellum in motor activities such as fine motor movement and motor coordination as well as the computational network within the neural circuitries, cerebellar motor learning was first postulated by Marr (1969) and Albus (1971).

Recent studies have shown the involvement of NMDA receptor subunits-NMDAR1, NMDA2B in the cerebellum in motor learning in mouse (Jiao et al.,
2008). Our investigation revealed an increase in the glutamate content and glutamate receptor number. The Scatchard analysis of NMDA receptors also showed an increased NMDA receptor number with no significant change in the affinity of the receptors. Studies of Yan and Rivkees, (2006) reported that hypoglycaemia inhibits oligodendrocyte development and myelination and that hypoglycemia triggers apoptotic cell death in oligodendrocyte precursor cells in the cerebellum. It is thought that the combination of extracellular glutamate accumulation and mitochondrial damage is involved in neuronal death associated with brain ischemia and hypoglycemia and some neurodegenerative diseases such as Huntington's disease (Bittigau & Ikonomidou, 1997). Reports have suggested that the accumulation of endogenous extracellular glutamate after inhibition of its transporters induces a stimulation of mitochondrial respiratory chain activity, which leads to ROS production and GSH deficiency in a manner dependent on NMDA receptor activation (García et al., 2005). Earlier studies have conclude that during severe energy deprivation following hypoglycemia and diabetes, mitochondrial free radicals scavenger system is down regulated, which leads to reactive oxygen species (ROS) generation. High levels of ROS in turn activate the processes leading to DNA damage (Singh et al., 2004). Metabotropic glutamate (mGlu) regulate synaptic glutamate release both in in vitro (Herrero et al., 1994) rat brain slices (Croucher et al., 1997) and in vivo (Patel & Croucher, 1997). We also observed an increase in the gene expression of NMDAR1, NMDA2B, mGluR5 receptor sutypes. Activation of mGluRs modulates NMDA receptor activity and is implicated in synaptic transmission and activity-dependent synaptic plasticity (Pin & Duvoisin, 1995; Conn & Pin, 1997; Nicoletti et al., 1999).

The cerebellum is known to be resistant to hypoglycaemia, and selective cerebellar dysfunction caused by hypoglycaemia has not been reported. In a case of
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episodic bilateral cerebellar dysfunction caused by hypoglycaemia, quantitative dynamic PET study demonstrated decreased glucose uptake-to-utilization ratio and increased leak of glucose in the cerebellum indicating that cerebellum is not invariably resistant to hypoglycaemia (Auer 2004; Kim et al., 2005). It is widely accepted that energy deprivation causes a neuronal death that is mainly determined by an increase in the extracellular level of glutamate (Marinelli et al., 2001). Glutamate which causes excitotoxic neuronal damage, increases calcium influx through NMDA receptors in post synaptic neurons, leading to phospholipase $A_2$ mediated arachidonic acid release (Miriam et al., 1996). Our previous studies on cerebellum also reported that GDH enzyme activity enhanced during diabetes and did not completely reverse after insulin administration (Preetha et al., 1996; Aswathy et al., 1998). Studies using young and old diabetic rats clearly revealed that in cerebellum GDH activity regulation is essential to avoid diabetic associated brain glutamate toxicity (Biju & Paulose, 1998). Increased number of glutamate receptor activity leading to glutamate excitotoxicity and neuronal degeneration were reported from our lab (Joseph et al., 2007).

One of the major causes of neuronal death in neurodegenerative disease is excitotoxicity from the neurotransmitter glutamate. This form of cell death arises from either excess levels of glutamate due to decreased astrocyte clearance or due to increased susceptibility. Several glutamate transporters have been characterized, the Na$^+$–dependent glutamate/aspartate transporter, GLAST being the major uptake system within the cerebellum (Danbolt, 2001). We also observed a decrease in the GLAST glutamate transporter expression in both adult and old diabetic and hypoglycaemic rats. Previous studies from our lab showed the increased glutamate production via increased glutamate dehydrogenase enzyme activity (Joseph et al., 2007). The present study showed the increased glutamate content and NMDA receptor
number and gene expression in the cerebellum. The protective role of GLAST glutamate transporter in the multiple sclerotic cerebellum was reported by Mitosek-Szewczyk et al., (2008).

It has been shown from previous studies that NMDA receptors, as well as metabotropic glutamate receptors, play important roles in the cascade of biochemical reactions resulting in death of neuronal cells in vivo (Tsintsadze et al., 2001). Pharmacological tools now allow for the examination of the role of metabotropic glutamate receptors (mGluRs) in the development of sensitization (Spooren et al., 2000). mGluRs regulate synaptic transmission by modulating calcium and potassium channels and the activity of ionotropic glutamate receptors. mGluR5 receptors modulate NMDA receptor function because both receptors have been linked as signaling partners (O’Leary et al., 2000; Movsesyan et al., 2001; Kotecha et al., 2003). The activation of mGluR5 receptors leads to the potentiation of NMDA currents (Bleakman et al., 1992; Cerne & Randic, 1992), possibly through the activation of protein kinase C and the subsequent increase in intracellular Ca$^{2+}$, thereby acting as an indirect agonist of NMDA receptors (Benquet et al., 2002; Fujii et al., 2004). NMDA receptor activation in the cerebellum leads to an increase in the Ca$^{2+}$ also via IP3 receptors. Our results also show an increase in the IP3, cGMP and cAMP content in the cerebellum of hypoglycaemic and diabetic adult and old rats. The immunohistochemistry study done using confocal microscope also showed an increased expression of NMDAR1, NMDA2B, mGluR5 and IP3 receptors. Suvarna and O’Donnel (2002) reported the NMDA mediated increase in the cGMP in the neuronal culture studies. Baltrons et al., (1997) and Oh et al., (1997) reported an NMDA induce cGMP formation in the cultured cerebellar granule cells. Increased IP3 activation leads to Ca$^{2+}$ influx which in turn activates neuronal nitric oxide synthase (nNOS) to produce NO (Garthwaite, 2005). NO activates soluble guanylyl cyclase
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(sGC) to generate increased levels of cGMP which in turn activates protein kinase G (PKG) (Garthwaite, 2005) in the cerebellum. cGMP modulates phosphorylation in cerebellum by changing the relationship between cGMP-dependent protein kinase and type 2 inhibitor content (Biggio et al., 1977). The ability of rats to learn a Y-maze conditional discrimination task depends on the function of the glutamate–nitric oxide–cGMP pathway in brain (Piedrafita et al., 2007). Infusion of D-serine (1 mM) enhanced (150-200%) extracellular cGMP in the cerebellum with no age-related differences (Vallebuona & Raiteri, 1995). cGMP-dependent signal transduction in hippocampus and cerebellum is insufficient in senescent brain and have functional consequences in disturbances of learning and memory processes (Chalimoniuk & Strosznajder, 1998). Group I mGluRs couple positively to phospholipase C, the activation of which leads to stimulation of protein kinase C and release of intracellular Ca\(^{2+}\) (Conn & Pin, 1997), or to adenylyl cyclase, activation of which stimulates cAMP formation (Aramori & Nakanishi, 1992; Joly et al., 1995).

Rotarod test has been previously used to examine motor in-coordination (Cendelin et al., 2008). The rotarod experiment demonstrated the impairment in the motor function and coordination in the hypoglycaemic and diabetic adult and old rats. All the experimental rats showed lower fall off time from the rotating rod when compared to control suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture and at the same time adjust their limb movements on the metallic rod and is indicative of cerebellar dysfunction. Many other brain regions have been associated with timing tasks including the dorsal lateral premotor cortex, inferior parietal lobe, supplementary motor area, superior temporal gyrus, caudal putamen, ventrolateral thalamus and inferior frontal gyrus (Rao et al., 1997; Jancke et al., 2000; Lewis & Miall, 2003). However, increased timing variance has been observed in patients with cerebellar disorders (Ivry et al.,
1988). Loss of coordination of motor movement, inability to judge distance and timing, incapacity to perform rapid alternating movements and hypotonia has been reported during cerebellar damage (Gowen & Miall, 2005). Poor limb-eye coordination in patients with cerebellar dysfunction has been earlier report (Van Donkelaar & Lee, 1994). Studies conducted in subjects with cerebellum lesion, showed deficits in learning associated with component movement (motor learning) from deficits in performing compound movement (such as motor coordination) (Mussa-Ivaldi & Bizzi, 2000; Krakauer & Shadmehr, 2006). The lower fall of time shown by our experimental rats confirmed the cerebellar dysfunction. Thus the upregulation of glutamate receptor activity in the cerebellum causing the increase in second messengers which mediates the Ca\(^{2+}\) overload in the cells, leads to neurodegeneration. The enhanced NMDA receptors were more prominent in hypoglycaemic group which is of significance in this study suggesting that hypoglycaemia is causing more damage to the brain at the molecular level than the hyperglycaemic condition.

To summarize, our findings suggest dysfunction of the diabetic and hypoglycaemic cerebellum in both adult and old rats that is a reflection of cerebellar glutamatergic abnormality. The receptor analysis and gene expression studies along with the behavioural data implicate a role for glutamate, NMDA and mGlu5 receptors in the modulation of neuronal network excitability via changes in IP3, cGMP and cAMP. These neurofunctional deficits are one of the key contributors to motor deficits and stress associated with insulin induced hypoglycaemia and diabetes. The enhanced neurodegeneration in hypoglycaemia is suggested to have more impairment of the motor learning and memory ability which has clinical significance in the diabetes treatment.
**Hippocampus**

The effect of hypoglycaemic episodes is visible in brain regions associated with memory, especially the hippocampus. The hippocampal formation contains a rich glutamatergic and GABA-ergic input, GABA-ergic interneurones containing peptide co-transmitters and the glutamatergic perforant pathway interconnects with entorhinal cortex, subiculum, CA1, CA3 fields and dentate gyrus (Ottersen & Storm-Mathisen, 1984). Potentiation, defined as an increase in synaptic efficacy, is readily induced by high frequency stimulation (HFS) of the synapses between the Schaffer collaterals and the pyramidal cells in the hippocampus CA1 area (Collingridge & Bliss, 1995; Malenka & Nicoll, 1999). The excitatory synapse in the stratum radiatum of the CA1 area of the hippocampus has a number of features that have been attributed to various aspects of memory encoding (Martin et al., 2000). In this study, we focused on the glutamate receptor, which is abundantly expressed throughout the hippocampal formation. Our results showed increased glutamate content in the hippocampus of diabetic, D+IIH and C+IIH rats compared to control. The hippocampus contains a high concentration of NMDA receptors. Our experiments revealed an increased glutamate and NMDA receptor number in adult and old hypoglycaemic and diabetic rats. The expression studies also showed an increased expression of NMDAR1, NMDA2B and mGluR5 receptors in the hippocampus of experimental rats. These particular receptors are vulnerable to hypoglycaemic episodes. Studies of Ennis et al., (2008) suggested that hippocampus is vulnerable to hypoglycemia-induced neuronal death. Also, studies suggest that children with type I diabetes who experience hypoglycaemia exhibit impairment of hippocampal-dependent memory (Hershey et al., 1999). When hippocampal cultures were deprived of glucose, massive release of lactate dehydrogenase (LDH), an indicator of neuronal death, occurred via NMDA receptor activation (Geng et al., 1997). Neurons impaired of energy metabolism are
highly sensitive to excitotoxicity (Simon et al., 1984; Wieloch, 1985; Monyer et al., 1992; Cebers et al., 1998). Hippocampal region of the brain is particularly vulnerable to the adverse effects of hypoglycaemia (Abdelmalik et al., 2007). Pathological studies in humans and animals have shown that hypoglycaemia-induced neuronal death occurs preferentially in the hippocampus, superficial layers of the cortex and striatum (Wieloch et al., 1985; Auer 2004; Camacho & Massieu, 2006). It is reported that profound hypoglycaemia selectively damages CA1 and the dentate gyrus of the hippocampus (Tasker et al., 1992). The selectively greater reduction in hippocampal cerebral blood flow (CBF) indicate severe impairment in glucose metabolism at moderate levels of hypoglycaemia in these structures as compared with the remainder of the brain (Denise et al., 2004). Hippocampal neurons receive a rich glutamergic innervation and evidence suggests that hypoglycaemic injury in these neurons is precipitated almost entirely by sustained glutamate receptor activation (Auer et al., 1985). Tanaka et al., (2008) reported that absence of glucose, insulin accelerated the neuronal cell death both in the CA1 and DG. They also concluded that insulin has a double-edged effect on the neuronal cell death dependent on glucose concentration and that the CA1 and the DG have a different sensitivity to insulin in terms of cell survival. Recent reports suggest that both hypoglycaemia and hyperglycaemia have adverse effects on the brain neuronal structural changes and impaired long-term spatial memory (Malone et al., 2008). Long-term potentiation of neuronal activity in the hippocampus is thought to be a substrate for learning and memory. Gasparova et al., (2008) revealed that prolonged exposure to hypoglycaemic state influenced induction of LTP in the hippocampus and that it had deleterious effects on learning and memory. Ageing process affects NMDA receptors more in the intermediate hippocampus than the dorsal hippocampus (Magnusson et al., 2006). The dysfunction in hippocampal LTP, an electrophysiological model of synaptic plasticity thought to
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Subserve learning and memory processes is associated with diabetic conditions (Di Mario et al., 1995; Biessels et al., 1996). Abdelmalik et al., (2007) suggested that suppressing seizures during hypoglycemia using NMDA antagonist, decrease subsequent neuronal damage and dysfunction in hippocampus.

Based on extensive supportive experimental data, the release of high levels of glutamate by neurons is thought to be the underlying mechanism for the initiation of hypoglycaemic neurodegeneration. Quintana et al., (2006) reported that transient anoxia/hypoglycaemia is associated with a marked enhancement of excitatory transmission with an increased synthesis of excitatory receptor subunits in organotypic hippocampal slice cultures. Our experimental results support the earlier reports. The immunohistochemistry experiments in the present work supported the gene expression studies of NMDAR1, NMDA2B and mGluR5 receptors. This up regulation will increase the glutamate receptor activity and molecular cascades inside the cells. Our experiments also demonstrated decreased expression of GLAST glutamate transporter in the hippocampus of experimental rats compared to control. This decreased expression of glutamate transporter will lead to the decreased clearance of glutamate from the extracellular space and we report in our present study that glutamate content is high in the hippocampus of experimental group compared to control. Up regulation of NMDA receptor and down regulation of glutamate transporter expression suggests a response to altered synaptic glutamate levels (Lyon et al., 2008). It was found that GLAST glutamate transporter down regulation is involved in cell swelling in hippocampus during hypoglycaemia (Han et al., 2004).

Our experimental results also showed an increase in the IP3, cGMP and cAMP content in the hippocampus of experimental adult and old rats compared to control. Inositol 1, 4, 5-trisphosphate receptors (IP3Rs) mediate calcium release and they are involved in many biological processes and IP3R activity regulators will
contribute to neuronal functions (Kawaai et al., 2009). Ambient glutamate release from astrocytes occurs in a Ca$^{2+}$-independent manner (Cavelier & Attwell, 2005; Malarkey & Parpura, 2008). Astrocytes in the hippocampus release calcium from intracellular stores intrinsically and in response to activation of G(q)-linked G-protein-coupled receptors through the binding of inositol 1,4,5-trisphosphate to its receptor (Foskett et al., 2007). Our data showed an increased expression of IP3 receptors in all the experimental conditions which will lead to increased secretion of glutamate. IP3 induced Ca$^{2+}$ overload will cause neurodegeneration (Zhu et al., 2006). Increase in intranuclear Ca$^{2+}$ that leads to altered transcription of apoptotic genes and activation of nuclear endonucleases, result in hypoxia-induced programmed neuronal death (Mishra & Delivoria-Papadopoulos, 2004).

To summarize our results in hippocampus we observed an increased glutamate and NMDA receptor activity. A decreased glutamate transporter expression and increased glutamate content was observed. Enhanced NMDA receptor functions activated the second messenger cascade and our study showed increased IP3, cGMP and cAMP content. Glutamatergic neurotransmission is critically involved in many aspects of complex behaviour and cognition. Up regulated glutamatergic activity mediates neurodegeneration in the hippocampus. Thus our study showed impairment in the hippocampal glutamate system during hypoglycaemia and diabetes. This glutamatergic dysfunction in the hippocampus was intense in hypoglycaemia compared to diabetes which contribute towards cognitive and memory deficits.

Pancreas

Insulin secretion from the pancreatic islets is controlled by the central nervous system through sympathetic and parasympathetic nerves (Burr et al., 1976; Campfield & Smith, 1980; Ahren, 2000). Recent studies from our laboratory described the
regulatory role of the sympathetic and parasympathetic systems in pancreatic regeneration (Renuka et al., 2004, 2005; Mohanan et al., 2005a, b). Pancreatic islets receive innervations from both divisions of the autonomic nervous system, and pancreatic endocrine secretion is partly controlled by the autonomic nervous system (Liu et al., 2001). Anatomical studies suggest that the vagal efferent fibers originating from the nucleus ambiguus and dorsal motor nucleus of the brainstem directly innervate the pancreas (Bereiter et al., 1981) and have a role in neurally mediated insulin release (Azmitia & Gannon, 1986). Our laboratory has reported that dopamine differentially regulates glucose induced insulin secretion in the pancreatic islets, an effect mediated by pancreatic DA D2 receptors (Eswar et al., 2006). Studies from our laboratory suggests that the down-regulation of DA D2 receptors could influence the regulation of insulin secretion by releasing epinephrine and norepinephrine from the adrenal medulla, which leads to the inhibition of insulin secretion in the pancreas (Eswar et al., 2007).

The present study showed an up regulation in the glutamate content, glutamate and NMDA receptor activity in the experimental groups. It was shown in previous study that enhanced GDH produced glutamate, a second messenger of insulin secretion (Anno et al., 2004). Glutamate receptor agonists induce various cellular responses outside the CNS, such as a rise in intracellular calcium concentration in rat pituitary cells and stimulation of growth hormone secretion (Lindstrom & Ohlsson, 1992), stimulation of insulin and glucagons secretion from rat endocrine pancreas (Bertrand et al., 1992; Bertrand et al., 1993) and contractions of the myenteric plexus-longitudinal muscle of guinea pig ileum (Shannon & Sawyer, 1989). These pharmacological studies suggest the presence of glutamate receptors in peripheral tissues, including endocrine tissues. Two important findings were reported regarding a relationship between glutamate metabolism and insulin secretion. A new
form of persistent hyperinsulinemia with hypoglycemia of the infant (PHHI) was demonstrated to be caused by an excessive activity of glutamate dehydrogenase, which produces glutamate (Stanley et al., 1998; Stanley et al., 2000; Macmullen et al., 2001). Second, glutamate produced via α-ketoglutarate from glucose was reported to enhance insulin secretion under conditions of clamped cytosolic Ca\(^{2+}\) and ATP at high levels (Macmullen et al., 2001). Although the direction of metabolic flux between glutamate and α-ketoglutarate upon stimulation with glucose has been controversial in β-cells (Gao et al., 1999; MacDonald & Fahien, 2000), these results raised a novel postulation that glutamate play a role in transducing secretory signals from glucose metabolism to secretory vesicles and that this pathway involve in modulation of secretory vesicle pH, the acidity of which is thought to be generated mainly by vacuolar-type H\(^{-}\)-ATPase (Hutton & Peshavaria, 1982; Hutton, 1989; Bode et al., 1996; Nelson & Harvey, 1999). An increase in β-cell glutamate is an important messenger in the amplification of insulin secretion by glucose (Bertrand et al., 2002). We observed an increase in the gene expression of NMDAR1, NMDA2B and mGluR5 receptors in the pancreas of adult and old experimental rats compared to their control rats. Molnár et al., (1995) and Inagaki et al., (1995) reported the presence of NMDA receptor subunits in the pancreatic islets and also that the glutamate receptor ligands and NMDA increased insulin secretion. Studies have reported that glutamate, transmitted from α-cells and neurons, stimulates insulin secretion through activation of ionotropic glutamate receptors in β-cells (Inagaki et al., 1995). Glutamate receptors classified into the ionotropic glutamate receptors, functioning as ion channels and the metabotropic glutamate receptors, coupled to intracellular second messenger systems (Nakanishi, 1992b). We also have found an increase in the second messenger- IP3, cGMP and cAMP content in the pancreas. It has been demonstrated by Cabrera et al., (2008) that glutamate acts on iGluRs, resulting in membrane depolarization, opening
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of voltage-gated Ca\(^{2+}\) channels, increase in cytoplasmic free Ca\(^{2+}\) concentration. Our studies also support this. The activation of second messengers enhanced the Ca\(^{2+}\) release from pancreatic islets which was demonstrated from our in vitro studies. IP\(_3\) mediates rapid mobilization of Ca\(^{2+}\) from the endoplasmic reticulum, whereas diacylglycerol stimulates protein kinase C (Berridge et al., 2003). Recent studies by Diederichs, (2008) demonstrate that Ca\(^{2+}\) release stimulated by IP3 increased insulin secretion. Activation of the GLP-1-R on β- cells initiates a complex series of signalling events that include cAMP production, membrane depolarization, an increase of intracellular calcium concentration and exocytosis (Thorens, 1992; Holz et al., 1993, 1995, 1999; Gromada et al., 1995, 1998a, b; Bode et al., 1999; Nakazaki et al., 2002; Eliasson et al., 2003). Kang et al., (2005) demonstrated that cAMP production, promote Ca\(^{2+}\) mobilization in pancreatic β- cells and thereby increase insulin release.

Glutamate acts as an intracellular messenger that couples glucose metabolism to insulin secretion (Maechler & Wollheim, 1999). Glutamate produced via α-ketoglutarate from glucose was reported to enhance insulin secretion under conditions of clamped cytosolic Ca\(^{2+}\) and ATP at high levels (Macmullen et al., 2001). Our experiments showed that the glutamate content and the NMDA receptor activity were increased in both hypoglycaemic and diabetic condition. The GLAST glutamate transporter expression was decreased in both hypoglycaemic and diabetic condition. Previous studies reported that an increased islet content of L-glutamate is necessary, but not sufficient, to allow its net conversion into 2-oxoglutarate and its further metabolism in the Krebs cycle or the GABA shunt. This and the subsequent stimulation of insulin secretion, requires activation of GDH by L-leucine (Li et al., 2006). The insulin secretion stimulated by glutamate was blocked by an inhibitor of vacuolar type H\(^+\)-ATPase or by an inhibitor of vesicular glutamate transporter (Gao et
Decreased GLAST activity during diabetes could account for the inhibition of insulin secretion. It was also reported that the enhanced glutamate activity during insulin induced hypoglycaemia in pancreas also enhanced glucagon release (Cabrera et al., 2008).

**Effect of dopamine on Ca^{2+} release from pancreatic islets in vitro**

The control of insulin secretion by the pancreatic β-cell is achieved through a complex metabolic cascade converting glucose and other nutrients into signals leading to appropriate insulin release (Wollheim, 2000). Neurotransmitters especially catecholamines play an important role in insulin secretion. Dopamine is reported to modulate insulin secretion in the pancreatic islets (Nogueira et al., 1994; Eswar et al., 2006). Dopamine in the islets is essential for maintaining the equilibrium of insulin secretion.

In hypoglycaemic condition, dopamine significantly inhibited insulin secretion by pancreatic islets. Dopamine at high concentration is reported to inhibit insulin secretion from the islets (Nogueira et al., 1994). Our results also showed inhibition of Ca^{2+} release from pancreatic islets at 10^{-5} M concentration of dopamine in hypoglycaemic condition. In hyperglycemic condition, we observed a significant stimulation of Ca^{2+} release from pancreatic islets at 10^{-5} M concentration of dopamine. Previous studies from our laboratory also showed a maximum inhibition of insulin secretion at high concentration of dopamine in hypoglycaemic condition. In hyperglycemic condition a significant stimulation of insulin secretion at low concentration of dopamine and inhibition at high concentration was observed (Robinson, 2007). Thus the present study also supports that the concentration of dopamine is very critical for glucose homeostasis.
Modulation of insulin secretion by dopamine depends on specific receptor-receptor interactions. Our study showed that dopamine D2 receptor antagonist sulpiride significantly blocked the inhibitory action of dopamine on Ca$^{2+}$ release from pancreatic islets at hypoglycaemic condition and stimulatory action of dopamine during hyperglycaemic condition. Studies have reported that dopamine D$_2$ receptors are expressed in pancreatic cells and inhibit glucose induced insulin secretion (Blanca et al., 2005, Eswar et al., 2006; 2007). The role and the peripheral mechanism of action of central dopamine on basal pancreatic exocrine secretion in conscious rats revealed that central dopamine inhibited pancreatic exocrine secretion via dopamine D$_1$ like receptors and that the inhibitory effect is mediated via sympathetic nerves, especially $\alpha$-adrenoceptors (Blanca et al., 2005). Thus our studies suggest that dopamine D2 receptors are involved in the dopamine regulation of insulin secretion via Ca$^{2+}$ release from pancreatic islets.

Previous studies from our laboratory reported that addition of forskolin an activator of cAMP resulted in overcoming the effect of dopamine on insulin secretion (Abraham, 1998). The agonists of dopamine by acting through the neuroendocrine system improve peripheral energy metabolism and impaired islet function. 7-OH DPAT showed an inhibitory effect on glucose induced insulin secretion. Previous reports suggest that 7-OH DPAT induced hyperglycaemia decreased insulin secretion (Uvnäs-Moberg et al., 1996).

It was found that dopamine D2 receptor antagonists effectively blocked the stimulatory and inhibitory effect of dopamine on Ca$^{2+}$ release from pancreatic islets. Dopamine through dopamine D2 receptors differentially regulate the Ca$^{2+}$ release from pancreatic islets and thereby insulin secretion during hypoglycaemia and diabetes. Thus our results suggest that dopamine acting through dopamine D$_2$ receptors regulate the glucose homeostasis. This has immense clinical significance.
Effect of glutamate on Ca\(^{2+}\) release from pancreatic islets *in vitro*

Glutamate acts as an intracellular messenger that couples glucose metabolism to insulin secretion (Maechler & Wollheim, 1999). Recent studies reported that insulin secretion is under the control of mGlu5 receptors (Storto *et al*., 2006). The role of NMDA receptor subunit of glutamate on glucose induced insulin secretion by pancreatic islets is poorly studied. In our *in vitro* Ca\(^{2+}\) release studies, glutamate significantly increased Ca\(^{2+}\) release from pancreatic islets in both hypoglycaemic and hyperglycaemic condition. Elevation of ATP is necessary for the membrane-dependant increase in cytosolic Ca\(^{2+}\), the main trigger of insulin exocytosis (Maechler & Wollheim, 2000). It has been demonstrated by Cabrera *et al*., (2008) that glutamate acts on iGluRs, resulting in membrane depolarization, opening of voltage-gated Ca\(^{2+}\) channels, increase cytoplasmic free Ca\(^{2+}\) concentration. Intracellular Ca\(^{2+}\) measurements and electrophysiological recordings studies indicated that kainate, AMPA and NMDA all elicit increases of Ca\(^{2+}\) in single pancreatic β-cells and depolarize them (Inagaki *et al*., 1995). NMDA receptor antagonist, MK-801, blocked the stimulatory action of glutamate in hypoglycaemic and hyperglycaemic conditions. Previous studies reported that glutamate produced via α-ketoglutarate from glucose enhanced insulin secretion under conditions of clamped cytosolic Ca\(^{2+}\) and ATP at high levels (Macmullen *et al*., 2001). Our results suggest that glutamate regulation of insulin secretion is mediated through NMDA receptor which has therapeutic applications.

Our molecular and behavioural results showed that hypoglycaemic condition has more functional damage in brain than diabetes. The receptor mediated functional studies and *in vitro* studies using antagonists for the receptor subtypes confirmed the specific receptor mediated dopaminergic and glutamatergic brain damage in hypoglycaemia and diabetes. Thus it is suggested that the corrective measures for the
brain functional damage caused during diabetes and anti-diabetic treatment, through glutamatergic receptors, have clinical significance in the therapeutic management of hypoglycaemia and diabetes.