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## Decreased [<sup>3</sup>H] YM-09151-2 binding to dopamine D<sub>2</sub> receptors in the hypothalamus, brainstem and pancreatic islets of streptozotocin-induced diabetic rats

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### Abstract

In the present study dopamine was measured in the hypothalamus, brainstem, pancreatic islets and plasma, using HPLC. Dopamine D<sub>2</sub> receptor changes in the hypothalamus, brainstem and pancreatic islets were studied using [<sup>3</sup>H] YM-09151-2 in streptozotocin-induced diabetic and insulin-treated diabetic rats. There was a significant decrease in dopamine content in the hypothalamus ( $P < 0.001$ ), brainstem ( $P < 0.001$ ), pancreatic islets ( $P < 0.001$ ) and plasma ( $P < 0.001$ ) in diabetic rats when compared to control. Scatchard analysis of [<sup>3</sup>H] YM-09151-2 in the hypothalamus of diabetic rats showed a significant decrease in  $B_{\max}$  ( $P < 0.001$ ) and  $K_d$ , showing an increased affinity of D<sub>2</sub> receptors when compared to control. Insulin treatment did not completely reverse the changes that occurred during diabetes. There was a significant decrease in  $B_{\max}$  ( $P < 0.01$ ) with decreased affinity in the brainstem of diabetic rats. The islet membrane preparation of diabetic rats showed a significant decrease ( $P < 0.001$ ) in the binding of [<sup>3</sup>H] YM-09151-2 with decreased  $K_d$  ( $P < 0.001$ ) compared to control. The increase in affinity of D<sub>2</sub> receptors in hypothalamus and pancreatic islets and the decreased affinity in brainstem were confirmed by competition analysis. Thus our results suggest that the decreased dopamine D<sub>2</sub> receptor function in the hypothalamus, brainstem and pancreas affects insulin secretion in diabetic rats, which has immense clinical relevance to the management of diabetes.

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**Keywords:** Dopamine D<sub>2</sub> receptor; Streptozotocin; Diabetes

### 1. Introduction

Dopamine, or *b*-(3, 4-dihydroxyphenyl) ethylamine, is an endogenous catecholamine that was first recognized as a neurotransmitter in the central nervous system (Carlsson, 1959). Dopamine in the central nervous system is involved in the control of both motor and emotional behavior (Vallone et al., 2000) and peripherally modulates insulin secretion in the pancreatic islets (Nogueira et al., 1994). Dopamine is stored in the secretory

granules of the pancreatic islets along with serotonin and calcium and could also be generated in pancreatic islets from its precursor L-dopa (Blanca et al., 2005). Hyperglycemia during diabetes is reported to damage dopaminergic functions, as shown by changes in dopamine metabolism in the human brain and the brains of animals with experimentally induced diabetes (Lozovsky et al., 1981; Trulson and Himmel, 1983; Serri et al., 1985; Laokovic et al., 1990). An increased D<sub>2</sub> receptor density has been observed in some brain regions (Lozovsky et al., 1981; Trulson and Himmel, 1983; Serri et al., 1985). Diabetes mellitus is often accompanied by emotional, behavioral, and mood disturbances or some centrally mediated neurological complications (Salkovic and Lackovic, 1987), the pathophysiology of which is still unclear, but might be related to dysfunction of the brain dopaminergic system, as described by several laboratories (Lozovsky et al., 1981; Trulson and Himmel, 1983; Serri et al., 1985; Laokovic et al., 1990).

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Insulin secretion from the pancreatic islets is controlled by the central nervous system through sympathetic and parasympathetic nerves (Ahren, 2000; Burr et al., 1976; Campfield and Smith, 1980). 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 and Gannon, 1986). The hypothalamus also has an important role in pancreatic secretion (Ying et al., 2003). The central vagal connection with dopaminergic innervations is reported to reach the pancreatic islets through the parhypothalamic ventricular nucleus (PVN), while adrenergic and serotonergic innervations reach the pancreas through the brainstem (Smith and Davis, 1985). The brainstem together with the hypothalamus serves as the key center of the central nervous system for regulating body homeostasis. In this respect, investigations of central dopamine receptors during diabetes mellitus is important, as recent reports suggest that pancreatic beta islets express dopamine receptors, and the role played by dopamine in modulating insulin secretion is mediated through its receptors (Blanca et al., 2005). Our laboratory has recently reported that dopamine differentially regulates glucose-induced insulin secretion in the pancreatic islets, an effect mediated by pancreatic D<sub>2</sub> receptors (Eswar et al., 2006). In the present study, alterations in the dopamine content and dopamine D<sub>2</sub> receptors of the hypothalamus, brainstem and pancreatic islets of streptozotocin-induced diabetic rats were studied to detect the functional correlation of changes in dopamine content and dopamine D<sub>2</sub> receptors during diabetes. Our studies suggest that dopamine is affected by diabetes mellitus both centrally and peripherally and this has important clinical implications.

## 2. Materials and method

Biochemicals used in the present study were purchased from Sigma Chemical Co., USA. All other reagents were of analytical grade purchased locally. YM-09151-2, *cis-N*-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide, was a gift from Yamanouchi Pharmaceuticals Ltd, Tokyo, Japan. [<sup>3</sup>H]YM-09151-2 (*cis-N*-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide

Table 1  
Dopamine content (nmol/ml plasma) in the plasma of control, diabetic and insulin-treated rats

| Animal status            | Dopamine                  |
|--------------------------|---------------------------|
| Control                  | 0.80±0.036                |
| Diabetic                 | 0.30±0.027 <sup>a</sup>   |
| Insulin-treated diabetic | 0.51±0.010 <sup>a b</sup> |

Values are means±S.E.M. of 4–6 separate experiments. <sup>a</sup> ( $P<0.001$ ) when compared to control, <sup>b</sup> ( $P<0.001$ ) when compared to diabetic.

Table 2

Dopamine content (nmol/g wet wt. of tissue) in the hypothalamus, brainstem and pancreas of control, diabetic and insulin-treated rats

| Animal status            | Hypothalamus              | Brainstem               | Pancreas                  |
|--------------------------|---------------------------|-------------------------|---------------------------|
| Control                  | 0.99±0.041                | 0.54±0.017              | 2.07±0.048                |
| Diabetic                 | 0.32±0.026 <sup>a</sup>   | 0.29±0.010 <sup>a</sup> | 0.84±0.036 <sup>a</sup>   |
| Insulin-treated diabetic | 0.69±0.024 <sup>a b</sup> | 0.31±0.010 <sup>a</sup> | 1.02±0.035 <sup>a c</sup> |

Values are means±S.E.M. of 4–6 separate experiments. <sup>a</sup> ( $P<0.001$ ) when compared to control, <sup>b</sup> ( $P<0.001$ ) when compared to diabetic, <sup>c</sup> ( $P<0.05$ ) when compared to diabetic.

Sp. activity-85.0Ci/mmol) was purchased from NEN Life Sciences Products, Inc. Boston, USA.

Adult male Wistar rats of 200–240 g body weight were purchased from the Central Institute of Fisheries Technology, Cochin, and used for all experiments. They were housed in separate cages under 12-h light and 12-h dark schedule and were maintained on standard food pellets and water *ad libitum*. Diabetes was induced with a single intrafemoral dose (65 mg/kg body weight) of streptozotocin prepared in citrate buffer, pH 4.5 (Hohenegger and Rudas, 1971; Arison et al., 1967). Animals were divided into the following groups: i) control ii) diabetic iii) insulin-treated diabetic rats. Each group consisted of 4–6 animals. The insulin-treated diabetic group received a daily dose (1 Unit/kg body weight) of Lente and Plain insulin (Boots India). The dose was increased daily according to the blood glucose level (Sasaki and Bunag, 1983). Rats were killed by decapitation on the 14th day of the experiment. The brainstem, hypothalamus and pancreas were dissected out quickly over ice according to the procedure of Glowinski and Iversen (1966). The tissues were stored at  $-70^{\circ}\text{C}$  until assayed.

### 2.1. Quantification of dopamine

Dopamine content was assayed according to Paulose et al. (1988), using high-performance liquid chromatography (HPLC) integrated with an electrochemical detector (HPLC-ECD) (Shimadzu, Japan) fitted with C18-CLC-ODS reverse-phase column. The tissues from brain regions were homogenized in 0.4 N perchloric acid. In the case of pancreas, the tissues were homogenized in 0.1 N perchloric acid. The homogenate was

Table 3  
[<sup>3</sup>H]YM-09151-2 binding parameters in the hypothalamus of control, diabetic and insulin-treated diabetic rats

| Animal status            | $B_{\text{max}}$ (fmol/mg protein) | $K_d$ (nM)                 |
|--------------------------|------------------------------------|----------------------------|
| Control                  | 151.2±1.034                        | 1.48±0.0125                |
| Diabetic                 | 82.0±1.014 <sup>a</sup>            | 1.08±0.0122 <sup>a</sup>   |
| Insulin-treated diabetic | 112.2±1.163 <sup>a b</sup>         | 1.27±0.0200 <sup>c b</sup> |

<sup>a</sup> ( $P<0.01$ ) when compared to control, <sup>b</sup> ( $P<0.001$ ) when compared to control, <sup>c</sup> ( $P<0.001$ ) when compared to diabetic.  $B_{\text{max}}$  — Binding Maximum, (fmol/mg protein),  $K_d$  — Dissociation constant (nM). Values are means±S.E.M of 4–6 separate experiments. Scatchard analysis of [<sup>3</sup>H]YM-09151-2 against sulpiride in the hypothalamus of control, diabetic and insulin-treated diabetic rats. Incubation was done with 0.25 nM to 2.0 nM of [<sup>3</sup>H]YM-09151-2 in a total incubation volume of 300  $\mu\text{l}$ . Five micromolar sulpiride was used to determine the specific binding. The reaction was stopped by rapid filtration through GF/B (Whatman) filters which were then counted in a scintillation counter.

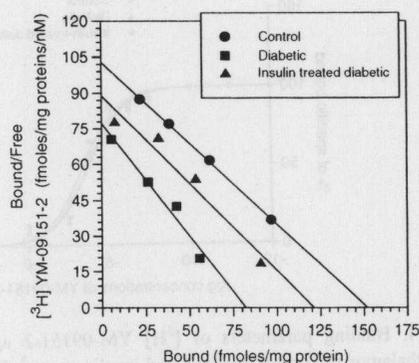


Fig. 1. Scatchard analysis of [ $^3\text{H}$ ] YM-09151-2 binding against sulpiride in the hypothalamus of control, diabetic and insulin-treated diabetic rats (A representative graph).

centrifuged at  $5000 \times g$  for 10 min at  $4^\circ\text{C}$  (Kubota refrigerated centrifuge) and the clear supernatant was filtered through  $0.22\text{-}\mu\text{m}$  HPLC-grade filters and used for HPLC analysis. The mobile phase consisted of 75 mM sodium dihydrogen orthophosphate, 1 mM sodium octyl sulfonate, 50 mM EDTA and 7% acetonitrile (pH 3.25), filtered through the  $0.22\text{-}\mu\text{m}$  filter (Millipore) and degassed. A Shimadzu pump (model 10 AS) was used to deliver the solvent at a rate of 1 ml/min. Dopamine was identified by using an electrochemical detector (Model 6A, Shimadzu, Japan) with a reduction potential of +0.8 V. The peaks were identified by their retention times compared with those of standards and quantitatively estimated using an integrator (Shimadzu, C-R6A-Chromatopac) interfaced with the detector.

Plasma monoamines were assayed as described by Jackson et al. (1997). Plasma (1.0 ml) was diluted with an equal volume of distilled water and  $50\ \mu\text{l}$  of 5 mM sodium bisulphite was added, followed by  $250\ \mu\text{l}$  of 1 mM Tris buffer pH 8.6. Acid alumina (20 mg) was then added and mixed for 20 min, the supernatant was aspirated off and the alumina was washed twice with 2.0 ml of 5 mM sodium bisulphite. To the final pellet of alumina 0.2 ml of 0.1 N perchloric acid was added. The supernatant was filtered through a  $0.22\text{-}\mu\text{m}$  HPLC-grade filter and used for HPLC determinations. Monoamine levels in the hypothalamus, brainstem, pancreas, and plasma of the experimental and control rats were statistically analyzed.

Table 4  
[ $^3\text{H}$ ] YM-09151-2 binding parameters in the brainstem of control, diabetic and insulin-treated diabetic rats

| Animal status            | $B_{\text{max}}$ (fmol/mg protein) | $K_d$ (nM)         |
|--------------------------|------------------------------------|--------------------|
| Control                  | $174.25 \pm 0.85$                  | $2.10 \pm 0.044$   |
| Diabetic                 | $160.40 \pm 4.16^c$                | $2.42 \pm 0.190^a$ |
| Insulin-treated diabetic | $168.25 \pm 0.85$                  | $1.31 \pm 0.011^b$ |

<sup>c</sup> ( $P < 0.01$ ) when compared to control, <sup>a</sup> ( $P < 0.001$ ) when compared to control, <sup>b</sup> ( $P < 0.001$ ) when compared to diabetic.  $B_{\text{max}}$  — Binding Maximum (fmol/mg protein),  $K_d$  — Dissociation constant (nM). Values are means  $\pm$  S.E.M. of 4–6 separate experiments. Scatchard analysis of [ $^3\text{H}$ ] YM-09151-2 against sulpiride in the brainstem of control, diabetic and insulin-treated diabetic rats. Incubation was done with 0.25 nM to 2.0 nM of [ $^3\text{H}$ ] YM-09151-2 in a total incubation volume of 300  $\mu\text{l}$ . Five micromolar sulpiride was used to determine the specific

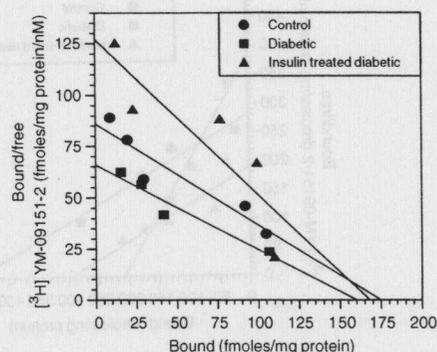


Fig. 2. Scatchard analysis of [ $^3\text{H}$ ] YM-09151-2 binding against sulpiride in the brainstem of control, diabetic and insulin-treated diabetic rats (A representative graph).

## 2.2. Dopamine $D_2$ receptor analysis

Dopamine  $D_2$  receptor binding assay was done according to the modified procedure of Unis et al. (1998) and Madras et al. (1988). The pancreatic islets were isolated and then used for membrane preparation. The isolated pancreatic islets, hypothalamus and brainstem were homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer, along with 1 mM EDTA, 5 mM  $\text{MgCl}_2$ , 1.5 mM  $\text{CaCl}_2$ , 120 mM NaCl, and 5 mM KCl pH.7.4. The homogenate was centrifuged at  $48,000 \times g$  for 30 min. The pellet was washed and recentrifuged with 50 volumes of the buffer at  $48,000 \times g$  for 30 min. The pellet was suspended in an appropriate volume of buffer.

The concentrations of [ $^3\text{H}$ ] YM-09151-2 used in the binding assay with pancreatic islets ranged from 0.1 nM to 2.0 nM. Different concentrations were used in the hypothalamus and brainstem binding assays i.e., 0.25 nM to 2.0 nM of [ $^3\text{H}$ ] YM-09151-2 in 50 mM Tris-HCl buffer, along with 1 mM EDTA, 5 mM  $\text{MgCl}_2$ , 1.5 mM  $\text{CaCl}_2$ , 120 mM NaCl, 5 mM KCl with 10  $\mu\text{M}$  pargyline and 0.1% ascorbic acid in a total incubation volume of 300  $\mu\text{l}$  containing 200 to 300  $\mu\text{g}$  of protein. Specific binding was determined using 5.0  $\mu\text{M}$  unlabeled sulpiride. Competition studies with hypothalamus and brainstem preparations were carried out with 0.25 nM [ $^3\text{H}$ ] YM-09151-2 in each tube with unlabeled ligand concentrations varying from  $10^{-12}$

Table 5  
[ $^3\text{H}$ ] YM-09151-2 binding parameters in the pancreatic islets of control, diabetic and insulin-treated diabetic rats

| Animal status            | $B_{\text{max}}$ (fmol/mg protein) | $K_d$ (nM)         |
|--------------------------|------------------------------------|--------------------|
| Control                  | $450 \pm 3.98$                     | $1.61 \pm 0.015$   |
| Diabetic                 | $207 \pm 5.52^a$                   | $0.55 \pm 0.040^a$ |
| Insulin-treated diabetic | $297 \pm 4.14^{a,b}$               | $1.57 \pm 0.045^b$ |

<sup>a</sup> ( $P < 0.001$ ) when compared to control, <sup>b</sup> ( $P < 0.001$ ) when compared to diabetic.  $B_{\text{max}}$  — Binding Maximum (fmol/mg protein),  $K_d$  — Dissociation constant (nM). Values are means  $\pm$  S.E.M. of 4–6 separate experiments. Scatchard analysis of [ $^3\text{H}$ ] YM-09151-2 against sulpiride in the pancreatic islets of control, diabetic and insulin-treated diabetic rats. Incubation was done with 0.25 nM to 2.0 nM of [ $^3\text{H}$ ] YM-09151-2 in a total incubation volume of 300  $\mu\text{l}$ . Five micromolar sulpiride was used to determine the specific binding. The reaction

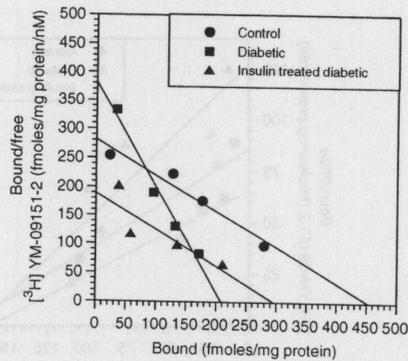


Fig. 3. Scatchard analysis of [<sup>3</sup>H] YM-09151-2 binding against sulpiride in the pancreatic islets of control, diabetic and insulin-treated diabetic rats. (A representative graph).

to  $10^{-4}$  M of YM-09151-2. Competition studies with pancreatic islets preparations were carried out with 0.5 nM [<sup>3</sup>H] YM-09151-2 in each tube with unlabeled ligand concentrations varying from  $10^{-12}$  to  $10^{-4}$  M YM-09151-2. Tubes were incubated at 25 °C for 60 min and their contents were filtered rapidly through GF/B filters (Whatman). The filters were washed quickly three times with 5.0 ml of ice-cold 50 mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

### 2.3. Protein determination

Protein was measured by the method of Lowry et al. (1951), using bovine serum albumin as standard.

### 2.4. Receptor data analysis

The linear regression data were analyzed according to Scatchard (1949) and the Scatchard plots were made using SIGMA PLOT (Ver 2.03). The non-linear regression displacement data were analyzed using GraphPad Prism software, GraphPad, Inc., USA. The concentration of the competing drug that competes for half the specific binding was defined as  $EC_{50}$ , which is same as  $IC_{50}$  (Unnerstall, 1990). The affinity of the receptor for the competing drug was designated  $K_i$  and is defined as the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors (Chen and Prusoff, 1973).

Table 6

Binding parameters of [<sup>3</sup>H] YM-09151-2 against YM-09151-2 in hypothalamus of control, diabetic and insulin-treated diabetic rats

| Animal status            | Best fit model | Log ( $EC_{50}$ ) | $K_i$                  | Hill slope |
|--------------------------|----------------|-------------------|------------------------|------------|
| Control                  | One site       | -7.672            | $1.664 \times 10^{-8}$ | -1.046     |
| Diabetic                 | One site       | -7.837            | $1.246 \times 10^{-8}$ | -1.000     |
| Insulin-treated diabetic | One site       | -7.767            | $1.371 \times 10^{-8}$ | -0.952     |

Data are from displacement curves as determined by non-linear regression analysis using the computer program PRISM. The affinity of the competing drug is designated  $K_i$ .  $EC_{50}$  is the concentration of the competitor that competes for half the specific binding and is the same as  $IC_{50}$ . The equation built into the program is defined in terms of the  $\log (EC_{50})$ .

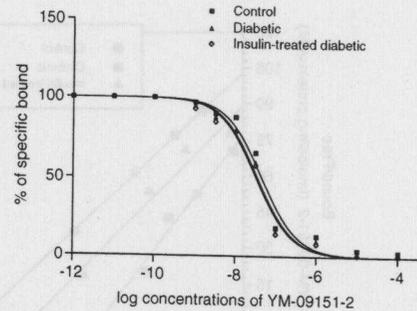


Fig. 4. Binding parameters of [<sup>3</sup>H] YM-09151-2 against YM-09151-2 in hypothalamus of control, diabetic and insulin-treated diabetic rats.

Statistical evaluations were done with ANOVA (analysis of variance), using InStat (Ver.2.04a).

## 3. Results

### 3.1. Dopamine content of hypothalamus, brainstem, pancreas and plasma was decreased in diabetic rats

The plasma dopamine concentration of diabetic rats was significantly lower than that of the control ( $P < 0.001$ ). Insulin treatment significantly reversed ( $P < 0.001$ ) the altered dopamine content when compared with that of the diabetic group (Table 1). The dopamine content of the hypothalamus was significantly ( $P < 0.001$ ) lower in 14-day diabetic rats. Insulin treatment reversed the decreased dopamine content to near control levels (Table 2). The dopamine content of the brainstem was significantly ( $P < 0.001$ ) lower in diabetic rats than in control rats. Insulin treatment did not reverse the decreased dopamine content ( $P < 0.05$ ) (Table 2). The dopamine content of the pancreas was significantly lower in the diabetic rats than in the control rats ( $P < 0.001$ ). Treatment with insulin did not completely reverse the decreased dopamine to control levels ( $P < 0.001$ ) but increased dopamine levels ( $P < 0.05$ ) relative to those of untreated diabetic rats (Table 2).

### 3.2. Dopamine $D_2$ receptors in hypothalamus, brainstem, pancreas were decreased in diabetic rats

Scatchard analysis of [<sup>3</sup>H] YM-09151-2 binding in the hypothalamus of diabetic rats showed a significant decrease in  $B_{max}$  ( $P < 0.001$ ) and  $K_d$  ( $P < 0.001$ ) when compared to control.

Table 7

Binding parameters of [<sup>3</sup>H] YM-09151-2 against YM-09151-2 in brainstem of control, diabetic and insulin-treated diabetic rats

| Animal status            | Best fit model | Log ( $EC_{50}$ ) | $K_i$                  | Hill slope |
|--------------------------|----------------|-------------------|------------------------|------------|
| Control                  | One site       | -7.514            | $2.735 \times 10^{-8}$ | -1.000     |
| Diabetic                 | One site       | -7.604            | $2.255 \times 10^{-7}$ | -0.970     |
| Insulin-treated diabetic | One site       | -7.319            | $4.030 \times 10^{-8}$ | -1.012     |

Data are from displacement curves as determined by non-linear regression analysis using the computer program PRISM. The affinity of the competing drug is designated  $K_i$ .  $EC_{50}$  is the concentration of the competitor that competes for half the specific binding and is the same as  $IC_{50}$ . The equation built into the

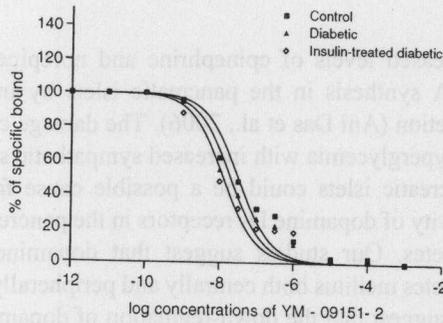


Fig. 5. Binding parameters of [<sup>3</sup>H] YM-09151-2 against YM-09151-2 in the brainstem of control, diabetic and insulin-treated diabetic rats.

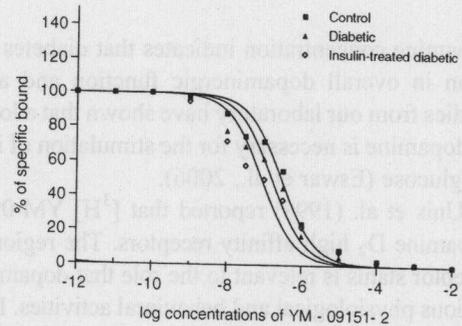


Fig. 6. Binding parameters of [<sup>3</sup>H] YM-09151-2 against YM-09151-2 in pancreatic islets of control, diabetic and insulin-treated diabetic rats.

Insulin treatment reversed the decreased kinetic parameters to near control (Table 3; Fig. 1). This decrease in  $K_d$  with a change in  $B_{max}$  reflected an increased affinity of  $D_2$  receptors for dopamine with a change in receptor number. In the brainstem of diabetic rats, Scatchard analysis showed a significant decrease in  $B_{max}$  ( $P < 0.01$ ) with a significant increase in  $K_d$  ( $P < 0.001$ ) compared to control, reflecting a decrease in affinity. The  $B_{max}$  was reversed to control values by insulin treatment. Insulin treatment lowered the  $K_d$  significantly when compared to that of untreated diabetic rats ( $P < 0.001$ ) (Table 4; Fig. 2). A significant decrease in the  $B_{max}$  ( $P < 0.001$ ) and  $K_d$  ( $P < 0.001$ ) of [<sup>3</sup>H] YM-09151-2 binding in the islet membrane preparation of diabetic rats was observed when compared to control. Insulin reversed the decrease in the  $K_d$  to control values ( $P < 0.001$ ). In comparison with the control,  $B_{max}$  in insulin-treated diabetic rats remained significantly decreased ( $P < 0.001$ ) (Table 5; Fig. 3).

The binding data were confirmed by competition binding assay with [<sup>3</sup>H] YM-09151-2 against YM-09151-2. Dopamine  $D_2$  receptor affinity increased significantly in the hypothalamus of diabetic rats in the displacement analysis, which fitted to a single site model. Treatment with insulin reversed the decreased  $K_i$  and  $\log(EC_{50})$  partially to control values. The Hill slope value was near unity ( $-0.952$ ) and fitted a one site model, as did the data for control rats (Table 6; Fig. 4). Dopamine  $D_2$  receptor affinity decreased significantly in the brainstem of diabetic rats in the displacement analysis and fitted a one site model best. Treatment of diabetic rats with insulin decreased the  $K_i$  value to lower than the control, shifting the affinity to a higher affinity state. The  $\log(EC_{50})$  value was decreased during diabetes with a decrease in affinity. In insulin-treated diabetic rats, the  $\log($

$EC_{50})$  increased as the affinity increased (Table 7; Fig. 5), showing a decreased  $K_i$  value compared with that of the control. Dopamine  $D_2$  receptor displacement analysis of control, diabetic and insulin-treated groups, using the islets preparation and [<sup>3</sup>H] YM-09151-2, fitted a single site model, with Hill slope values near unity. In diabetic rats both  $\log(EC_{50})$  and  $K_i$  decreased, showing an increase in affinity. Insulin treatment caused a partial reversal of both  $\log(EC_{50})$  and  $K_i$  values (Table 8; Fig. 6).

#### 4. Discussion

Diabetes mellitus is often accompanied by emotional, behavioral, mood disturbances and centrally mediated neurological complications (Salkovic and Lackovic, 1992). Most of the central disturbances in diabetes, the pathophysiology of which is related to striatal dopaminergic neurons (Shimomura et al., 1988), are thought to be mediated through dopamine  $D_2$  receptors. Insulin secretion from pancreatic islets is controlled by the central nervous system through sympathetic and parasympathetic nerves (Ahren, 2000; Burr et al., 1976; Campfield and Smith, 1980). Diabetes is reported to decrease the dopamine transporter, thus reducing dopaminergic signaling and thereby affecting dopamine-related functions (Galli et al., 2002). Our data are consistent with these findings, suggesting that the disturbances in the central dopamine receptors during streptozotocin-induced diabetes affect dopamine-related functions.

Neurotransmitters, especially catecholamines, play an important role in insulin secretion in the pancreatic islets and in glucose homeostasis. Alterations in the brain monoamine content in diabetic rats (Bitar et al., 1987) and the relationship between the enhanced monoamine content in the brain, a characteristic of hyperinsulinemic and insulin-resistant animals, and islet dysfunction, have been reported (Liang et al., 1999). Studies from our laboratory have shown that neurotransmitters, such as those in the pancreatic islet, can influence the synthesis and release of insulin (Ani Das et al., 2006; Mohanan et al., 2006; Renuka et al., 2004; Asha and Paulose, 1999; Jackson and Paulose, 2000). Endogenously synthesized dopamine has been suggested to modulate insulin secretion in the pancreatic islets (Nogueira et al., 1994). The plasma concentration of dopamine is used as an indicator of central nervous system

Table 8  
Binding parameters of [<sup>3</sup>H] YM-09151-2 against YM-09151-2 in pancreatic islets of control, diabetic and insulin-treated diabetic rats

| Animal status            | Best fit model | Log ( $EC_{50}$ ) | $K_i$                  | Hill slope |
|--------------------------|----------------|-------------------|------------------------|------------|
| Control                  | One site       | -6.727            | $1.430 \times 10^{-7}$ | -0.980     |
| Diabetic                 | One site       | -6.903            | $6.486 \times 10^{-8}$ | -1.012     |
| Insulin-treated diabetic | One site       | -6.552            | $2.130 \times 10^{-7}$ | -0.960     |

Data are from displacement curves as determined by non-linear regression analysis using the computer program PRISM. The affinity of the competing drug is designated  $K_i$ .  $EC_{50}$  is the concentration of the competitor that competes for half the specific binding and is the same as  $IC_{50}$ . The equation built into the program is defined in terms of the  $\log(FC_{50})$ .

dopamine concentration indicates that diabetes causes an alteration in overall dopaminergic function and activity. *In vitro* studies from our laboratory have shown that a low concentration of dopamine is necessary for the stimulation of insulin secretion by glucose (Eswar et al., 2006).

Unis et al. (1998) reported that [ $^3\text{H}$ ] YM-09151-2 binds to dopamine  $D_2$  high affinity receptors. The regional difference in receptor status is relevant to the role that dopamine plays during various physiological and behavioral activities. In the intralateral hypothalamic area, blockade of dopamine  $D_2$  receptors by a specific antagonist in tumor bearing and non-tumor bearing rats increased food intake, indicating the involvement of dopamine  $D_2$  receptors in feeding mechanisms (Zhang et al., 2001). Impairment of dopamine  $D_2$  receptors is an important factor that leads to hyperphagic and polydipsic conditions because dopamine participates in the regulation of meal size (Oler et al., 1997). Dopaminergic neurons are the direct targets for insulin action and participate in reward-seeking behavior (Figlewicz et al., 2003). Dopamine  $D_2$  receptor disruption is reported to impair body growth and the somatotroph population (Becu-Villalobos et al., 2002). Also, a decrease in dopamine receptors during diabetes may result in hyporesponsiveness (Kamei et al., 1994).

The hypothalamus and brainstem are two important parts of the brain for monitoring the glucose status and the regulation of feeding (Guillod et al., 2003). Dopaminergic action is important in the regulation of hypothalamic-pituitary hormone release. Also, dopamine and its receptors are implicated in satiety, hunger, and body weight maintenance. The identification of dopamine  $D_2$  receptors in the pancreatic islets of rodents suggests that these receptors play an important role in insulin secretion (Blanca et al., 2005). Studies with pancreatic islets have suggested that dopamine has a differential regulatory role on glucose-induced insulin secretion — an inhibitory effect is seen at increased concentrations and a stimulatory effect is seen at lower concentrations (Eswar et al., 2006). Dopamine  $D_2$  receptors are thought to function through G protein stimulation and inhibition.

When a neuroleptic drug is given, it increases dopamine receptor supersensitivity. Studies have shown that neuroleptic-treated diabetics have a significantly higher prevalence and severity of tardive dyskinesia (Casey et al., 1991). Earlier studies demonstrated antagonism of neuroleptic-induced dopamine receptor supersensitivity by the neuropeptide L-Prolyl-L-Leucyl-Glycinamide (Simon et al., 1981).

During diabetes, norepinephrine and epinephrine, the levels of which are increased due to the increased conversion of dopamine (Tassava et al., 1992; Jackson et al., 1997; Jackson and Paulose, 1999), could bind to  $\alpha_2$  adrenergic receptors, increasing sympathetic nerve discharge, which could inhibit insulin secretion from the pancreatic islets. Studies from our laboratory suggest that the increased norepinephrine level in diabetic conditions blocks the uptake of dopamine into pancreatic islets and inhibits the stimulatory effect of dopamine on insulin secretion (Eswar et al., 2006). It has been shown that infusions of epinephrine (McEvoy and Hegre, 1978) and nor-

decreased levels of epinephrine and norepinephrine facilitate DNA synthesis in the pancreatic islets by increasing insulin secretion (Ani Das et al., 2006). The damage caused as a result of hyperglycemia with increased sympathetic stimulation in the pancreatic islets could be a possible cause for the decreased activity of dopamine  $D_2$  receptors in the pancreatic islets during diabetes. Our studies suggest that dopamine is affected by diabetes mellitus both centrally and peripherally. From our data, we suggest that the down-regulation of dopamine  $D_2$  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. On the basis of our results, we suggest that the decrease in dopamine  $D_2$  receptor function in the hypothalamus, brainstem, and pancreas during streptozotocin-induced diabetes differentially regulates pancreatic islets insulin secretion, which is of clinical relevance to the management of diabetes.

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#### References

- Ahren, 2000. Autonomic regulation of islet hormone secretion—implications for health and disease. *Diabetologia* 43, 393–410.
- Ani Das, V., Remya, Robinson, Paulose, C.S., 2006. Enhanced  $\beta$  adrenergic receptors in the brain pancreas during pancreatic regeneration in weanling rats. *Mol. Cell. Biochem.* 289, 11–19.
- Arison, R.N., Ciaccio, E.I., Glitzer, M.S., Cassaro, A.V., Pruss, M., 1967. Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. *Diabetes* 16, 51–56.
- Asha, Abraham, Paulose, Cheramadathikudyil S., 1999. Age related functions in noradrenergic function in the brain stem of streptozotocin diabetic rats. *J. Biochem. Mol. Biol. Biophys.* 3, 171–176.
- Azmitia, E.C., Gannon, P., 1986. Anatomy of the serotonergic system in the primate and sub-primate brain. *J. Adv. Neurol.* 43, 407–468.
- Becu-Villalobos, D., Diaz-Torga, G., Feierstein, C., Libertun, C., Gelman, D., Kelly, M.A., Low, M.J., Rubinstein, M., 2002. Disruption of the  $D_2$  dopamine receptor alters GH and IGF-I secretion and causes dwarfism in male mice. *Endocrinology* 143, 1270–1279.
- Bereiter, D.A., Rohner-Jeanrenaud, F., Berthoud, H., Jeanrenaud, R., 1981. CNS modulation of pancreatic endocrine function: multiple mode of expression. *Diabetologia* 20, 417–425.
- Bitar, M.S., Koulu, M., Linnoila, M., 1987. Diabetes induced changes in monoamine concentrations of rat hypothalamic nuclei. *Brain Res.* 409, 236–242.
- Blanca, R., Ljubacic, S., Pournourmohammadi, S., Carobbio, S., Armanet, M., Bartely, C., Maechler, P., 2005. Dopamine  $D_2$  like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion. *J. Biol. Chem.* 280, 36824–36832.
- Burr, I.M., Slonim, A.E., Sharp, R., 1976. Interaction of acetylcholine and epinephrine on the dynamics of insulin release *in vitro*. *J. Clin. Invest.* 58, 230–239.
- Campfield, L.A., Smith, F.J., 1980. Modulation of insulin secretion by the autonomic nervous system. *Brain Res. Bull.* 4, 103–107.
- Carlsson, A., 1959. The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmacol. Rev.* 11, 490–493.
- Casev, D.E., Ganzini, L., Hejntz, R.T., Hoffman, W.F., 1991. The prevalence of

- Chen, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant ( $K_i$ ) and the concentration of an inhibitor that causes a 50% inhibition of an enzymatic reaction. *Biochem. Pharmacol.* 22, 3099–3108.
- Esler, M.D., Lambert, G.W., Eisenhofer, G., Cox, H.S., Home, M., Kalff, V., Kelly, M., Jennings, G.L., 1991. Direct determination of homovanillic acid release from the human brain, an indicator of central dopaminergic activity. *Life Sci.* 49, 1061–1072.
- Eswar, Shankar P.N., Santhosh, K.T., Paulose, C.S., 2006. Dopaminergic regulation of glucose-induced insulin secretion through dopamine D2 receptors in the pancreatic islets *in vitro*. *IUBMB Life* 58, 157–163.
- Figlewicz, D.P., Evans, S.B., Murphy, J., Hoen, M., Baskin, D.G., 2003. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res.* 964, 107–115.
- Galli, A., Carvelli, L., Moron, J.A., Kahlig, K.M., Ferrer, J.V., Sen, N., Lechleiter, J.D., Leeb-Lundberg, L.M., Merrill, G., Lafer, E.M., Ballou, L.M., Shippenberg, T.S., Javitch, J.A., Lin, R.Z., 2002. PI 3-kinase regulation of dopamine uptake. *J. Neurochem.* 81, 859–869.
- Glowinski, J., Iversen, L.L., 1966. Regional studies of catecholamines in the rat brain: the disposition of [ $^3$ H] norepinephrine, [ $^3$ H] dopa in various regions of brain. *J. Neurochem.* 13, 655–669.
- Guillod, E., Penicaud, L., Leloup, C., Lorsignol, A., Alquier, T., 2003. Brain glucose sensing mechanism and glucose homeostasis. *Curr. Opin. Clin. Nutr. Metab. Care.* 5, 539–543.
- Hohenegger, M., Rudas, 1971. Kidney functions in experimental diabetic ketosis. *Diabetologia* 17, 334–338.
- Jackson, J., Paulose, C.S., 1999. Enhancement of [*m*-methoxy [ $^3$ H] MDL 100907 binding to 5-HT<sub>2A</sub> receptors in the cerebral cortex and brainstem of streptozotocin induced diabetic rats. *Mol. Cell. Biochem.* 199, 81–85.
- Jackson, J., Paulose, C.S., Cheramadathikudiyil S., 2000. Brain 5HT<sub>2A</sub> receptor regulation by tryptophan supplementation in streptozotocin diabetic rats. *J. Biochem. Mol. Biol. Biophys.* 5, 1–7.
- Jackson, J., Padayatti, P.S., Paul, T., Paulose, C.S., 1997. Platelet monoamine changes in diabetic patients and streptozotocin-induced diabetic rats. *Curr. Sci.* 72, 137–139.
- Kamei, J., Saitoh, A., Iwamoto, Y., Funada, M., Suzuki, T., Misawa, M., Nagase, H., Kasuya, Y., 1994. Effects of diabetes on spontaneous locomotor activity in mice. *Neurosci. Lett.* 178, 69–72.
- Laokovic, Z., Salkovic, M., Kuci, Z., Relja, M., 1990. Effect of long-lasting diabetes mellitus on rat and human brain monoamines. *J. Neurochem.* 54, 143–147.
- Liang, Y., Luo, S., Cincotta, A.H., 1999. Long term infusion of norepinephrine plus serotonin into the ventromedial hypothalamus impairs pancreatic islet function. *Metabolism* 48, 1287–1289.
- Liu, Y.Q., Montanya, E., Leahy, J.L., 2001. Increased islet DNA synthesis and glucose derived lipid and amino acid production in association with beta cell hyperproliferation in normoglycaemic 60% pancreatectomy rats. *Diabetologia* 44, 1026–1033.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurements and folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Lozovsky, D., Saller, C.F., Kopin, I.J., 1981. Dopamine receptor binding is increased in diabetic rats. *Science* 214, 1031–1033.
- Madras, B.K., Michele, A., Fahey, D.R.C., Roger, D.S., 1988. D<sub>1</sub> and D<sub>2</sub> dopamine receptors in caudate-putamen of nonhuman primates (*Macaca fascicularis*). *J. Neurochem.* 51, 934–943.
- McEvoy, R.C., Hegre, O.D., 1978. Syngeneic transplantation of fetal rat pancreas. II. Effects of insulin treatment on the growth and differentiation of pancreatic implants 15 days after transplantation. *Diabetes* 27, 988–995.
- Mohan, V.V., Finla, C., Paulose, C.S., 2005a. Decreased 5-HT<sub>2c</sub> receptor binding in the cerebral cortex and brainstem during pancreatic regeneration in rats. *Mol. Cell. Biochem.* 272, 165–170.
- Mohan, V.V., Kaimal, B.S., Paulose, C.S., 2005b. Decreased 5-HT<sub>1A</sub> receptor gene expression and 5-HT<sub>1A</sub> receptor protein in the cerebral cortex and brainstem during pancreatic regeneration in rats. *Neurochem. Res.* 30, 25–32.
- Mohan, V.V., Reas, K., Paulose, C.S., 2006. Hypothalamic 5-HT functional regulation through 5-HT<sub>1A</sub> and 5-HT<sub>2c</sub> receptors during pancreatic regeneration. *Life Sci.* 78, 1603–1609.
- Nogueira, C.R., Machado, U.F., Curi, R., Carpinelli, A.R., 1994. Modulation of insulin secretion and 45Ca<sup>2+</sup> efflux by dopamine in glucose-stimulated pancreatic islets. *Gen. Pharmacol.* 25, 909–916.
- Oler, Yang, Z.J., Meguid, M.M., Chai, J.K., Chen, C., 1997. Bilateral hypothalamic dopamine infusion in male Zucker rat suppresses feeding due to reduced meal size. *Pharmacol. Biochem. Behav.* 58, 31–35.
- Paulose, C.S., Dakshinamurti, K., Packer, S., Stephens, N.L., 1988. Sympathetic stimulation and hypertension in pyridoxine deficient adult rat. *Hypertension* 11, 387–391.
- Porte, D.J., Graber, A.L., Kuzuya, T., Williams, R.H., 1966. The effect of epinephrine on immunoreactive insulin levels in man. *J. Clin. Invest.* 45, 228–236.
- Renuka, T.R., Ani Das, V., Paulose, C.S., 2004. Alterations in the Muscarinic M1 and M3 receptor gene expression in the brainstem during pancreatic regeneration and insulin secretion in weanling rats. *Life Sci.* 75, 2269–2280.
- Renuka, T.R., Savitha, B., Paulose, C.S., 2005. Muscarinic M1 and M3 receptor binding alterations in pancreatic regeneration of young rats. *Endocr. Res.* 31, 259–270.
- Salkovic, M., Lackovic, Z., 1987. Central nervous system in diabetes. *Diabetol. Croat.* 16, 5–17.
- Salkovic, M., Lackovic, Z., 1992. Brain D<sub>1</sub> dopamine receptor in alloxan-induced diabetes. *Diabetes* 41, 1119–1121.
- Sasaki, S., Bunag, R.D., 1983. Insulin reverses hypertension and hypothalamic depression in streptozotocin diabetic rats. *Hypertension* 5, 34–40.
- Scatchard, 1949. The attraction of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* 51, 660–672.
- Serri, O., Reiner, G., Somma, M., 1985. Effects of alloxan-induced diabetes on dopaminergic receptors in rat striatum and anterior pituitary. *Horm. Res.* 21, 95–101.
- Shimomura, Y., Shimizu, H., Takahashi, M., Sato, N., Uehara, Y., Suwa, K., Kobayashi, L., Tadokoro, S., Kobayashi, S., 1988. Changes in ambulatory activity and dopamine turnover in streptozotocin-induced diabetic rats. *Endocrinology* 123, 2621–2625.
- Simon, Chiu, Paulose, C.S., Ram Mishra, K., 1981. Neuroleptic drug induced dopamine receptor supersensitivity: antagonism by L-Prolyl-L-Leucyl-Glycinamide. *Science* 214, 1261–1262.
- Smith, P.H., Davis, B.J., 1985. Effect of substantia nigra lesions on the volume of A, B, and D cells and the content of insulin and glucagon in the rat pancreas. *Diabetologia* 28, 756–762.
- Tassava, T.M., Okuda, T., Romsos, D.R., 1992. Insulin secretion from ob/ob mouse pancreatic islets: effects of neurotransmitters. *Am. J. Physiol.* 262, E338–E343.
- Trulsson, M.E., Himmel, C.D., 1983. Decreased brain dopamine synthesis rate and increased [ $^3$ H] spiroperidol binding in streptozotocin-diabetic rats. *J. Neurochem.* 40, 1456–1459.
- Unis, A.S., Micheal, D.R., Renee, R., James, H.A., Daniel, M.D., 1998. Ontogeny of human brain dopamine receptors I. differential expression of [ $^3$ H]-SCH23390 and [ $^3$ H]-YM-09151-2 specific binding. *Dev. Brain Res.* 106, 109–117.
- Unnerstall, J.R., 1990. Computer analysis of binding data. In: Yamamura, H., Enna, S., Kuhar, M. (Eds.), *Methods in Neurotransmitter Receptor Analysis*. Raven Press, pp. 247–255.
- Vallone, Daniela, Picetti, Roberto, Borrelli, Emiliana, 2000. Structure and function of dopamine receptors. *Neurosci. Biobehav. Rev.* 24, 125–132.
- Ying, Li, Wu, Xiaoyin, Zhu, Jinxia, Yan, Jin, Chung, Owyang, 2003. Hypothalamic regulation of pancreatic secretion is mediated by central cholinergic pathways in the rat. *J. Physiol.* 552, 571–587.
- Zhang, L., Sato, T., Meguid, M.M., Fetissov, S.O., Chen, C., 2001. Hypothalamic dopaminergic receptor expressions in anorexia of tumor-bearing rats. *Am. J. Physiol., Regul. Integr. Comp. Physiol.* 281, R 1907–R 1916.