Brain 5HT$_2A$ Receptor Regulation by Tryptophan Supplementation in Streptozotocin Diabetic Rats

JACKSON JAMES and CHERAMADATHIKAUDYIL S. PAULOSE*

Molecular Neurobiology and Cell Biology Unit, Department of Biotechnology, Cochin University of Science and Technology, Cochin – 682022, Kerala, India

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5-HT$_2A$ receptor binding parameters were studied in the cerebral cortex and brain stem of control, diabetic, insulin, insulin + tryptophan and tryptophan treated streptozotocin diabetic rats. Scatchard analysis using selective antagonist, $[^3]$H(±)2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol] ($[^3]$HMDL100907) in cerebral cortex of diabetic rats showed a significant decrease in dissociation constant ($K_d$) without any change in maximal binding ($B_{max}$). Competition binding studies in cerebral cortex using ketanserin against $[^3]$HMDL100907 showed the appearance of an additional site in the low affinity region during diabetes. In the brain stem, Scatchard analysis showed a significant increase in $B_{max}$ and $K_d$. Displacement studies showed a shift in the receptor affinity towards a low affinity state. All these altered parameters in diabetes were reversed to control level by insulin, insulin + tryptophan and tryptophan treatments. Tryptophan treatment is suggested to reverse the altered 5-HT$_2A$ binding and blood glucose level to control status by increasing the brain 5-HT content.

Keywords: Diabetes, Serotonin, 5-HT$_2A$ receptor, Tryptophan, Streptozotocin

INTRODUCTION

5-Hydroxytryptamine (5-HT) is a neurotransmitter known to play an important role in several physiological functions. The synthesis of 5-HT by monoaminergic neurons depends on the availability of its precursor tryptophan [1,2]. The uptake of tryptophan into the brain is determined by factors such as diet and the circulating insulin level [3]. Diet can play a major role in the availability of tryptophan, since a tryptophan deficient diet can lead to decreased circulating tryptophan. This in turn leads to a decrease in brain tryptophan and thereby a decreased 5-HT content [4–6]. During diabetes the uptake of tryptophan into the brain is decreased due to decreased circulating insulin [7,8]. This in turn increases the competition of tryptophan with other long chain amino acids for uptake into the brain and thereby depletes brain 5-HT turnover in streptozotocin (STZ) induced diabetic rats [9,10].

In the present study we investigated the kinetic parameters of 5-HT$_2A$ receptors in cerebral cortex and
brain stem of STZ induced diabetic rats and the role of tryptophan in regulating the 5-HT$_{2A}$ receptors and insulin regulation.

MATERIALS AND METHODS

Materials

$[^3]$HMDL100907 (82.0 Ci/mmol) was purchased from Amersham radiochemical U.K. ketanserin was a generous gift from Janssen Laboratories, Belgium. Streptozotocin was purchased from Sigma chemical Co., St. Louis, USA. All other biochemicals used were of analytical grade.

Animal Experiments

Adult male Wistar rats of 200–240g body weight were used for all experiments. They were housed in separate cages under 12 hour light and 12 hour dark periods and were maintained on standard food pellets and water ad libitum. The animals were randomly divided into control, diabetic, insulin treated diabetic (D+I), diabetic treated with insulin + tryptophan (D+I+T) and diabetic treated with tryptophan alone (D+T). Diabetes was induced by a single intrafemoral dose (65 mg/kg body weight) of STZ prepared in citrate buffer, pH 4.5 [11,12]. The cerebral cortex and brain stem were homogenised in 10 volumes of ice cold 0.32M sucrose in a Potter-Elvejhem homogeniser. The homogenate was centrifuged at 900xg for 10 min and the resulting supernatant was centrifuged at 17,000xg for 1 hour. The pellet was resuspended in 50 volumes of 50mM Tris HCl, pH 7.5, and incubated at 37°C for 10min to remove endogenous 5-HT and recentrifuged at 17,000xg for another 1 hour. The final pellet was resuspended in a minimum volume of Tris HCl, pH 7.7 containing 4mM CaCl$_2$ and was used for assay. The nonspecific binding determined showed 30–40% in all our experiments.

Binding assays were done using different concentrations i.e., 0.25nM–2.5nM of $[^3]$HMDL100907 in Tris buffer, pH 7.7 containing CaCl$_2$ (4mM), ascorbate (0.2%), and pargyline (10µM) in a total incubation volume of 25µl. Specific binding was determined using 100µM cold ketanserin. Competition studies were carried out with 0.5nM $[^3]$HMDL100907 in each tube with cold concentration varying from 10$^{-9}$–10$^{-4}$M of ketanserin.

Tubes were incubated at 37°C for 30 min. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 3.0 ml of ice cold Tris buffer, pH 7.7. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Protein Determination

Protein was measured by the method of Lowry et al. [17] using bovine serum albumin as standard.
TABLE I Blood glucose levels and body weight of experimental animals

<table>
<thead>
<tr>
<th>Animal Status</th>
<th>Blood glucose level (mg/dl)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>83.41 ± 13.39</td>
<td>210 ± 19</td>
</tr>
<tr>
<td>D</td>
<td>376.50 ± 27.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>190 ± 14</td>
</tr>
<tr>
<td>D+I</td>
<td>124.81 ± 15.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>200 ± 20</td>
</tr>
<tr>
<td>D+I+T</td>
<td>180.58 ± 20.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>200 ± 24</td>
</tr>
<tr>
<td>D+T</td>
<td>220.25 ± 18.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>160 ± 10</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M of 4-6 separate experiments.
C - control, D - diabetic, D+I - diabetic + insulin, D+I+T - diabetic + insulin + tryptophan, D+T - diabetic + tryptophan

<sup>a</sup> p<0.001 compared to control.
<sup>b</sup> p<0.001 compared to diabetic.

Receptor Data Analysis

The receptor data were analysed by non-linear regression using GraphPad Prism software, GraphPad Inc., USA. The concentration of the competing drug that competes for half the specific binding was defined as EC<sub>50</sub>, which is same as IC<sub>50</sub>[18]. The affinity of the receptor for the competing drug is designated as Ki 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 [19].

Statistics

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (Ver 2.03).

RESULTS

Streptozotocin administration to rats brought about a significant increase (p<0.001) in blood glucose level. Treatment with insulin, insulin + tryptophan and tryptophan significantly reduced (p<0.001) the blood glucose to near control value (Table I).

There was a significant decrease in 5-HT content of CC and BS (p<0.01 and p<0.05 respectively) in 14-day diabetic rats (Table II). This decreased 5-HT content was significantly reversed (p<0.01 and p<0.05 respectively) to control level by insulin, insulin + tryptophan and tryptophan treatment compared to diabetic group. There was a significant decrease (p<0.05) in 5-HTP content in CC. The turnover rate of 5-HIAA/5-HT in the CC was significantly increased in diabetic group when compared to control (C - 0.84 ± 0.07, D - 2.65 ± 0.07; p<0.01), but the turnover of 5-HTP/5-HT did not show any significant change. The turnover of 5-HTP/5-HT and 5-HIAA/5-HT in the BS was significantly increased in diabetic group compared to control (C - 0.15 ± 0.08, D - 2.76 ± 0.48; p<0.001 and C - 1.65 ± 0.19, D - 3.18 ± 0.28; p<0.05 respectively). The increased turnover of 5-HIAA/5-HT in the CC and BS was significantly reversed by insulin, insulin + tryptophan and tryptophan treatments.

Scatchard analysis in CC of diabetic rats did not show any significant change in B<sub>max</sub> when compared to controls, but the K<sub>d</sub> of diabetic rats showed a significant decrease (p<0.05). Treatment with insulin, insulin + tryptophan and tryptophan significantly (p<0.05) reversed the K<sub>d</sub> to near control level (Table III). Competition binding assay with [³H]MDL100907 against ketanserin showed a low affinity site fitting to a two-site model instead of the one-site model seen in control.

Scatchard analysis in BS of diabetic rats showed a significant increase in B<sub>max</sub> (p<0.05) and K<sub>d</sub> (p<0.05) when compared to control. The B<sub>max</sub> was significantly reversed (p<0.05) to control by insulin, insulin + tryptophan and tryptophan treatments. These results showed an up-regulation of 5-HT<sub>2A</sub> receptors accompanied by a decrease in its affinity during diabetic state. Competition binding studies showed a shift from one-site model to a two-site model in diabetic group. Insulin, insulin + tryptophan and tryptophan treatments reversed the two-site model to one-site model. In CC and BS of diabetic group the Hill slope is away from unity (0.66 and 0.54) confirming the two-site model. Insulin, insulin + tryptophan and tryptophan treatments effectively reversed the two-site model to one-site model having a Hill slope above unity (Table IV & V).
TABLE II Serotonin & metabolites in the cerebral cortex and brain stem of 14-day experimental rats (nanomoles/gram wet weight)

<table>
<thead>
<tr>
<th>Animal status</th>
<th>Cerebral cortex</th>
<th>Brain stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-HTP</td>
<td>5-HIAA</td>
</tr>
<tr>
<td>C</td>
<td>0.22 ± 0.01</td>
<td>1.14 ± 0.11</td>
</tr>
<tr>
<td>D</td>
<td>0.09 ± 0.03a</td>
<td>0.96 ± 0.10</td>
</tr>
<tr>
<td>D+I</td>
<td>0.56 ± 0.11c</td>
<td>1.17 ± 0.14</td>
</tr>
<tr>
<td>D+I+T</td>
<td>0.46 ± 0.07c</td>
<td>1.47 ± 0.05</td>
</tr>
<tr>
<td>D+T</td>
<td>0.35 ± 0.02c</td>
<td>1.59 ± 0.02</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. of 4-6 separate determinations.


a. p<0.05 when compared to control.
b. p<0.01 when compared to control.
c. p<0.05 when compared to diabetic.
d. p<0.01 when compared to diabetic.

TABLE III 5-Hydroxytryptamine2A (5-HT2A) receptor binding parameters in cerebral cortex and brain stem of experimental rats

<table>
<thead>
<tr>
<th>Animal Status</th>
<th>Cerebral cortex</th>
<th>Brain stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bmax (fmol/mg protein)</td>
<td>Kd (nM)</td>
</tr>
<tr>
<td>C</td>
<td>230.00 ± 45.70</td>
<td>1.08 ± 0.11</td>
</tr>
<tr>
<td>D</td>
<td>208.66 ± 59.84</td>
<td>0.60 ± 0.09a</td>
</tr>
<tr>
<td>D+I</td>
<td>212.00 ± 39.58</td>
<td>0.95 ± 0.15b</td>
</tr>
<tr>
<td>D+I+T</td>
<td>226.34 ± 43.21</td>
<td>1.13 ± 0.20b</td>
</tr>
<tr>
<td>D+T</td>
<td>246.26 ± 40.26</td>
<td>1.20 ± 0.18b</td>
</tr>
</tbody>
</table>

Bmax – Binding maximum (fmol/mg protein), Kd – Dissociation constant (nM)
Values are mean ± S.E.M. of 4-6 separate experiments.

a. p<0.05 compared to control.
b. p<0.05 compared to diabetic.

table IV Binding parameters of [3H]MDL100907 against Ketanserin in cerebral cortex of experimental animals

<table>
<thead>
<tr>
<th>Animal status</th>
<th>Cerebral cortex</th>
<th>Brain stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log(EC50)-1</td>
<td>log(EC50)-2</td>
</tr>
<tr>
<td>C</td>
<td>-7.45</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>-7.98</td>
<td>-5.986</td>
</tr>
<tr>
<td>D+I</td>
<td>-7.58</td>
<td>-</td>
</tr>
<tr>
<td>D+I+T</td>
<td>-7.91</td>
<td>-</td>
</tr>
<tr>
<td>D+T</td>
<td>-7.74</td>
<td>-</td>
</tr>
</tbody>
</table>


Data are from displacement curves as determined by non-linear regression analysis using the computer program PRISM and a one-site Vs two-site model. The affinity for the first and second site of the competing drug are designated as K(H) (for high affinity) and K(L)(for low affinity). EC50 is the concentration of the competitor that competes for half the specific binding and it is same as IC50. The equation built-into the programme is defined in terms of the log(EC50).
### DISCUSSION

The major findings of this study include an increased affinity of 5-HT₂A receptors in the CC and up-regulation of these receptors in the BS of STZ-induced diabetic rats. Tryptophan treatment reversed these changes to control state. In addition to this, tryptophan administration was also able to reverse the blood glucose level to near control.

In our experiments we have observed a significant reduction of 5-HT content in CC and BS of diabetic rats. These findings agree with the previous reports of decreased 5-HT in brain regions during diabetes [20-22]. In CC the decrease in 5-HT content is due to a reduction in the conversion of 5-HTP to 5-HT. This is because of a significant decrease in 5-HTP content. Another contributing factor for the decreased 5-HT is the significant increase in the breakdown of 5-HT to 5-HIAA that is catalysed by monoamine oxidase, which is known to regulate insulin secretion [23]. In case of BS the decrease in 5-HT content is brought about by a significant increase in the rate of synthesis of 5-HT and its breakdown to 5-HIAA. There is also no significant increase of 5-HTP during diabetes. This leads to a decreased accumulation of 5-HT in the serotonergic neurons.

Insulin, insulin + tryptophan and tryptophan treatments were able to significantly increase the 5-HT content in CC, and BS. This increase in the brain 5-HT content is due to the increase in tryptophan uptake through the BBB with other neutral amino acids. Jamnicky et al., [24] have reported that administration of tryptophan in combination with insulin to diabetic rats have reversed the levels of brain tryptophan, 5-HT, 5-HIAA and serum concentrations of valine, leucine and isoleucine towards control. Oral administration of 5-HTP to diabetic patients also showed an increased brain 5-HT content [25]. During diabetes there is a significant reduction of brain tryptophan, 5-HT and 5-HIAA content [20,26]. The decrease in 5-HT content is due to decreased uptake of tryptophan into the brain, which is determined by circulating insulin level. Trulson et al. [8] have reported that after 4 weeks of administration of streptozotocin, the brain tryptophan content was decreased by 27%. Insulin administration was able to reverse the brain tryptophan and 5-HIAA levels to control. Tryptophan uptake across the BBB is increased in the presence of insulin. Insulin enhances the uptake of branched chain amino acids into the muscles thereby decreasing their plasma concentration. Since these amino acids compete with tryptophan for transport into brain, an increased brain tryptophan is observed [1].

The decreased brain 5-HT content leads to an up-regulation of 5-HT₂A in brain stem and an increased affinity of these receptors in cerebral cortex [20-22]. This leads to an increased sympathetic stimulation by increased centrally mediated catecholamines and by epinephrine (EPI) release from adrenal glands, thereby decreasing insulin secretion from pancreatic islets [27]. An up-regulation of
5-HT$_{2A}$ receptors also increases the risk of diabetes induced major depression [28,29]. Administration of tryptophan to rats resulted in an increased uptake of tryptophan into the brain leading to an increased synthesis of 5-HT. The increase in brain 5-HT reverses the altered 5-HT$_{2A}$ receptor binding parameters in cerebral cortex and brain stem and reduces sympathetic nerve stimulation.

Diet can also influence the brain 5-HT content. Consumption of tryptophan deficient diet can also lead to reduced circulating tryptophan and brain 5-HT content [30]. DeMarte and Enesco [31] maintained a group of mice for 78 weeks on tryptophan restricted, protein restricted and control diet. They found that brain 5-HT levels were significantly reduced only in mice on the tryptophan-restricted diet, but not for mice on the protein restricted diet. It is not only tryptophan that is influenced by the diet but other amino acids such as tyrosine that is the precursor for dopamine and norepinephrine, also influenced by diet. The same process is applicable for the uptake of choline, which is the precursor for acetylcholine [30]. From this, it appears that diet also play an important role in the induction of diabetes through the serotoninergic system by reducing the brain 5-HT content.

Thus, from our study we conclude that STZ induced diabetes causes an increase in affinity of cerebral cortex 5-HT$_{2A}$ receptors without any change in their number. The brain stem 5-HT$_{2A}$ receptors are up-regulated accompanied by the appearance of a low affinity site which was reversed to control by insulin, insulin + tryptophan and tryptophan treatments. Administration of tryptophan along with insulin can bring about a better control of diabetes and reduce the risk of diabetes induced depression.

Acknowledgements

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References


5HT₂A RECEPTOR REGULATION IN DIABETES


