Depression
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Effects of chronic unpredictable mild stress on Brain-Pancreas Relative Protein in rat brain and pancreas

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**Abstract**  Brain-Pancreas Relative Protein (BPRP) is a novel protein whose biological functions remain unknown. Here we reported a possible role of BPRP in male rats exposed to chronic unpredictable mild stress (CUMS) to mimic depression for 3 weeks. Compared to unstressed rats, those exposed to CUMS showed significantly less weight gain with age, decreased consumption of (and preference for) sucrose without a change in total fluid consumption. Exposure to CUMS significantly reduced open-field exploration, rearing, and grooming indicative of lethargy, apathy, and bodily neglect, respectively. Brain MAO-A and MAO-B activities were both significantly increased in the stressed rats. These results verified induction of depressive symptoms by CUMS. The stressed animals showed a significant reduction in pancreatic BPRP, which was accompanied by an increase in levels of blood sugar and a decrease in insulin. But they showed no apparent alteration in levels or distribution of BPRP in the hippocampal formation, which nevertheless displayed a thinner dentate granule cell layer perhaps related to elevated MAO-B activity. These findings suggest that stress-induced reduction of pancreatic BPRP may cause diabetic symptoms. Nevertheless, whether those symptoms in turn contribute to the onset of depression requires further study.

**Keywords**  Brain-Pancreas Relative Protein, chronic unpredictable mild stress, depression, diabetes
Introduction

Using SDS–PAGE analysis, we discovered a dramatic decrease of a novel 260 kDa protein in ischemic brain tissue following experimental occlusion of the middle cerebral artery in rats (Yao et al, 2003). Using a polyclonal antibody, we found the protein primarily in hippocampal neurons of the brain and islets cells of the pancreas, with lesser amounts found in podocytes of the renal glomeruli and endothelial cells of venous sinuses in the spleen. For that reason, we named the 260kDa protein Brain-Pancreas Relative Protein (BPRP). While the localization of BPRP in vivo strongly implies its functions in these tissues, its physiological and pathophysiologic significances need to be clarified.

Given its location in both pancreatic islets and the hippocampus, we considered whether BPRP plays a role in depression, which is a common mental disorder with considerable morbidity and mortality (Schulz et al, 2002). Nevertheless, the factors associated with the development of depression have not been clearly identified. A high prevalence of depression has been found in diabetic patients. Recent studies demonstrated that depression and its associated symptoms constituted a major risk factor in the development of diabetes and may accelerate the onset of diabetes complications (Musselman et al, 2003). Onset of depression may result in decreased self-care measures such as exercise. Also, people with depression are more likely to abuse alcohol and smoke cigarettes compared with individuals without depression (Brown et al, 2005). These behaviors can potentially increase the risk of developing type-2 diabetes. Several prospective observational studies (Arroyo et al, 2004; Everson-Rose et al, 2004; Kumari et al, 2004; Palinkas et al, 2004; van der Akker et al, 2004) suggest that depression and depressive symptoms may be a risk factor for the development of type 2 diabetes, with relative risk estimates ranging from 1.3 to 3.0. Other studies indicate an effect of depression on the hippocampal structure and function (Shelinea et al, 2002). The hippocampus is vulnerable to damage by stroke, head trauma, and repeated stress (Sapolsky, 1992). It is not surprising, then, that hippocampal atrophy has been reported in a number of psychiatric disorders, including depression (Shelinea et al, 2002; McEwen, 2000).

Several behavioral paradigms have been used to create animal models of depression, among the more common of which is the chronic stress model (Katz, 1982). Willner and his colleagues introduced chronic unpredictable mild stress (CUMS) as a more realistic paradigm for inducing depression in animals. In that paradigm, animals were subject to chronic, low-grade stressors analogous to those associated with depression in humans (Willner et al, 1987). CUMS caused reduced sensitivity to reward, termed anhedonia, which is a major symptom of most human depressive states and a core feature of the DSM-IV subtype known as melancholia (APA, 1994). Anhedonia is a
“sharp, pervasive impairment of the capacity to experience pleasure or to respond affectively to the anticipation of pleasure” (Klein, 1974).

To determine whether or not BPRP is related to the onset and development of depression, we used CUMS to induce features of depression in rats. At weekly intervals, we compared control and stressed animals for indices of depression (weight loss, loss of preference for sucrose, agitated open-field behaviors, and brain levels of monoamine oxidases). These measures of depression were then correlated with brain and pancreatic BPRP levels observed at the same weekly intervals. We found that CUMS can induce features of diabetic symptoms and that those symptoms may be associated with a decrease in pancreatic BPRP.

Materials and Methods

Animals

Adult, male Sprague-Dawley (SD) rats, weighing between 200-220 g at the start of the experiment, were obtained from the Animal Center of Peking University Health Science Center. The rats were assigned randomly to 3 groups (including two stressed group, one group for open-field test and fluid consumption test, the other group for biochemical analysis) matched for weight, fluid consumption, and locomotor behavior in an open-field test before onset of CUMS. The stressed and control rats were kept in different rooms to allow independent manipulation of their environments. Control rats were housed together, while the stressed rats were housed singly. All rats were housed in Perspex cages at a room temperature of 22±2°C, and maintained on a 12-h light/dark cycle, with food and water freely available.

Fluid consumption tests

Rats had access to both tap water and a 1% sucrose solution in their home cages for a 48h prior to the start of the experiment. Over the next 23 h, the animals were food- and water-deprived. Fluid consumption was recorded by reweighing preweighed bottles of test solution. Bottles were counterbalanced across left and right sides of the cages throughout the experiment. Tests for fluid consumption were carried out weekly between 1400 and 1500 hours on the same day each week (Friday) throughout the experiment (Willner et al, 1987). A percent preference (PP) for sucrose was calculated by determining the percentage of total fluid consumption accounted for by ingestion of the 1% sucrose solution.
Open-field tests

Open-field test was conducted between 700 and 1100 hours on the same day each week (Thursday) in a quiet room. The open-field apparatus consisted of a square arena 100×100 cm, with a white, opaque wall 40 cm high. The floor was marked with a grid dividing it into 25 equal-size squares. The apparatus was illuminated with a low intensity diffuse light (45 W) situated 45 cm above the base. Each animal was tested in the apparatus once. It was placed in the central square and observed for 5 min. A record was kept of the time each rat stayed in the central square, the amount of time it spent grooming and rearing (defined as standing upright on its hind legs), and the number of grid lines it crossed with at least three paws. Between animal tests the apparatus was cleaned (Hallam et al, 2004; Kennett et al, 1985).

CUMS regimen

The stressed rats were subjected to the following conditions used by Willner et al (Willner et al, 1987) and Reid et al (Forbes et al, 1996; Matthews et al, 1995) with minor modification: 3 h and 5 h periods of white noise (85 dB), one 17 h period of group housing (5 rats to a cage), one 17 h period of exposure to a novel odor (household air freshener), one 17 h period of soiled bedding (150 ml water in a cage), one 7 h period of exposure to a foreign object (for example, a piece of plastic), 19, 22 and 24 h periods of food deprivation, 17, 19, 20 and 21 h periods of water deprivation, twice the normal period of overnight illumination (lights on for a total of 36 h), 7h and 17h periods of cage tilt (45°C), and two 10 minutes periods of a 160 Hz rocking bed (Table 1). At the beginning of first week and at the end of first, second, third week of CUMS, rats in one stressed group were sacrificed for determinations of BPRP and MAO-A/B in brain tissues and blood glucose and insulin.

Immunohistochemistry

Rats were anesthetized by i.p. injection of 3.5% chloral hydrate in normal saline (1 ml/100 g body wt.), and the brains and pancreas were removed. Tissue blocks 3 mm thick were cut and fixed with 4% formaldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 h. Fixed blocks from the pancreas and the brain posterior to the infundibular stalk were embedded in paraffin. Coronal tissue sections 6 μm thick were cut on a rotary microtome and mounted onto APES-coated slides. Paraffin sections were dewaxed with xylene and rehydrated in a graded series of ethanol. Slides were submerged in 3% hydrogen peroxide to quench any endogenous peroxidase activity, washed with distilled water and heated at 95-98°C in 1 mM EDTA (pH 8.0) for 15 min, then cooled at room
temperature for 40 min and washed with PBS. An aliquot of 10% non-immune goat serum was applied to eliminate nonspecific staining. Sections were incubated overnight at 4°C with 1:1000 diluted rabbit anti-rat 260 kDa protein antibody. The sections were washed with PBS and incubated with biotinylated goat anti-rabbit IgG antibodies for 40 min, rewashed with PBS, and incubated with peroxidase-conjugated streptavidin for 40 min. The peroxidase activity was visualized by incubating the sections with a peroxidase substrate solution, after sufficient washing. The sections were counterstained with hematoxylin and mounted. A 1:1000 diluted solution of non-immune rabbit serum was used as controls in the immunohistochemical localization of the BPRP protein.

Table 1. Chronic unpredictable mild stress regimen

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friday</td>
<td>10:00</td>
<td>clean cages, dry cages</td>
</tr>
<tr>
<td></td>
<td>13:00</td>
<td>make up test solution; weigh all rats and solutions</td>
</tr>
<tr>
<td></td>
<td>14:00</td>
<td>testing period</td>
</tr>
<tr>
<td></td>
<td>15:00</td>
<td>weigh bottles; restore food; switch on white noise for 3 h; switch lights to on overnight</td>
</tr>
<tr>
<td></td>
<td>18:00</td>
<td>switch off white noise</td>
</tr>
<tr>
<td>Saturday</td>
<td>10:00</td>
<td>commence 10 min rocking bed, room lights off</td>
</tr>
<tr>
<td></td>
<td>11:00</td>
<td>restore water</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>remove water</td>
</tr>
<tr>
<td>Sunday</td>
<td>10:00</td>
<td>restore water; remove food; tilt cages for 7 h</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>untilt cages; group housing overnight</td>
</tr>
<tr>
<td>Monday</td>
<td>10:00</td>
<td>rehouse singly;</td>
</tr>
<tr>
<td></td>
<td>12:00</td>
<td>restore food; switch on white noise for 5 h</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>switch off white noise; exposure to novel odour</td>
</tr>
<tr>
<td>Tuesday</td>
<td>10:00</td>
<td>remove novel odour; Place a foreign object in a cage</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>remove food and water; remove foreign object; switch lights to on overnight</td>
</tr>
<tr>
<td>Wednesday</td>
<td>12:00</td>
<td>weigh all animals; restore food and water; tilt cages</td>
</tr>
<tr>
<td>Thursday</td>
<td>07:00</td>
<td>open-field test</td>
</tr>
<tr>
<td></td>
<td>10:00</td>
<td>commence 10 min rocking bed, room lights off, unilt cages</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>commence food and water deprivation, soil bedding</td>
</tr>
</tbody>
</table>

**Western blotting**

Rats were anesthetized by i.p. injection of 3.5% chloral hydrate in normal saline (1 ml/100 g body wt.), and the hippocampus and pancreas were removed and stored at -80°C. The tissues were suspended in a solution containing 0.32M sucrose, 0.3 mM phenylmethylsulfonyl fluoride, and 20 mM Tris-HCl (pH 7.4), and homogenized in a Teflon-glass homogenizer. The homogenates were centrifuged at 4°C for 25 min at 800g. The supernatant was collected and centrifuged again at 4°C for 1.5 h at 16,000g. Protein concentrations of each sample were measured by the Bradford method. Aliquots were
stored at -80 °C prior to use. Separating (7.5%) and stacking (4%) polyacrylamide gels containing 0.1% SDS were used. Tissue proteins were suspended in a sample buffer containing 2% SDS, 0.1 M Tris-HCl buffer (pH 6.8), 10% glycerol (v/v), and 0.1 M dithiothreitol (DTT), and heated at 100°C for 5 min in a water bath. 50µg protein was loaded in each lane of the polyacrylamide gels, which were electrophoresed at 150 volts constant power for 1h. Proteins were transferred electrophoretically at 50 mA to a polyvinylidene difluoride (PVDF) immobil - P membrane (0.45-µm pore size: Millipore Corp, Bedford, MA) in a transfer buffer (pH 8.3) composed of 25 mM Tris-HCl, 192 mM glycine, and 20% methanol at 4°C overnight. The membranes were incubated in Tris-buffered saline (TBS, 100 mM Tris-HCl and 0.9% NaCl, pH 7.5) containing 5% skim milk for 1 h at 20-22°C and subsequently incubated with the primary antibody at 1:2000 dilution and shaken on a rotator at 20-22°C for 1.5 h. The membranes were washed three times with TBS containing 0.1% Tween-20 (TBS-T) and incubated with the 1:3000 streptavidin AP-conjugated antibody (Santa Cruz) as the second antibody in TBS containing 2.5% skim milk for 1 h at 20-22°C. The membranes were washed three times with TBS-T. The blots were developed using BCIP/NBT (nitro blue trazolium/5-Bromo-4-chlor-3indolyl phosphate salt) system. Densitometric analysis was used by Gel Doc 2000 (Bio-Rad).

Analysis of MAO-A and MAO-B activity in rat brain

For monoamine oxidase (MAO) enzyme preparation, a crude mitochondrial fraction was isolated from whole rat brains by the method of Thull and Testa (Thull and Testa, 1994) and was stored at -80ºC until use. Protein concentration was determined using bovine serum albumin as a standard, and MAO activity with kynuramine (Sigma) as a substrate (Kraml, 1965). The 380 nm fluorescence intensity of 4-hydroxyquinoline formed from kynuramine by MAO was measured in a fluorophotometer (PELS-50B) using a 315 nm excitatory wavelength. The activities of MAO-A and MAO-B in rat brain were measured after a 15 min preincubation in 1 M l-deprenyl (type B inhibitor) or clorgyline (type A inhibitor). The test solutions were dissolved in dimethylsulfoxide, which was confirmed to have no effect on MAO activity below 2.8% concentration.

Blood assay for glucose and insulin levels

Rats were anesthetized by i.p. injection of 3.5% chloral hydrate in normal saline (1 ml/100 g body wt.), and blood was collected. Blood was stored at room temperature for 30 min, then centrifuged for 10 min at 1000 rpm, after which aliquots were collected. Serum blood sugar levels were determined using a diagnostic kit (Nanjing Jiancheng
Bioengineering Institute), plasma insulin was assayed with a radioimmunoassay kit (China Atomic Energy Research Institute).

Statistical analysis

All data were presented as means ± SD. Two-way analysis of variance followed by Dunnett’s test was used for statistical evaluation. $P<0.05$ was considered statistically significant.

Results

CUMS decreased body weight

The mean body weight of rats in the two experimental groups did not differ significantly initially. Over the three weeks of the experiment (Fig. 1), the mean body weight increased in both groups, but the weight gain was significantly less in the group exposed to CUMS by the end of the first week ($P<0.05$) and continued to be significantly reduced by the end of the second and third week ($P<0.01$). At the end of third week, the control rats had gained 97 g, while the stressed rats had gained only 38 g ($P<0.01$).

![Figure 1](image.png)

**Figure 1.** Time course of weight gain in control (●) and stressed (○) rats. Data are represented as the means ± SD. *$P<0.05$, **$P<0.01$, n = 15 animals in each group.

CUMS reduced consumption of and preference for sucrose solution

No difference was found between control and stressed animals in total fluid consumption (tap water plus 1% sucrose solution) over the three weeks of the study (Fig. 2).
In contrast, the stressed animals consumed significantly less sucrose solution by the end of the first week and continued to do so at the end of the second and third weeks (P<0.01, Fig. 2B). As that suggests that ingestion of the sucrose solution accounted for a smaller percentage of total fluid consumption in the stressed than in the control animals (P<0.01, Fig. 2C). The percentage preference for sucrose was thus significantly reduced in the stressed animals.

Figure 2. Time course of total (A), sucrose (B) fluid consumption, and sucrose percentage (C) in control (●) and stressed (○) rats. Prior to each test, rats were food- and water-deprived for 23 h. They were then exposed to both a 1% sucrose solution and tap water. Sucrose preference = amount of sucrose solution consumption / total fluid consumption × 100%. Data are represented as means ±SD. **P < 0.01, n = 15 animals in each group.
Effect of CUMS on exploratory behavior, rearing, and grooming

Open-field testing is used to assess locomotion, exploration, and anxiogenic-like behavior of rats or mice (Keeney and Hogg, 1999). Compared to the controls, stressed animals spent less time in the center of the field (where they initially placed) by the end of the second (P<0.01) and third weeks (P<0.001) of the experiment (Fig. 3A). As that implies, the control animals were more active in the open field: from the end of the first week onward they crossed more grid lines (P<0.01, Fig. 3B), reared more frequently (P<0.01, Fig. 3C), and groomed more frequently (P<0.05 [week 1]; P<0.01 [week 3], Fig. 3D).

Figure 3. Time course of exploratory behavior (A, B), rearing frequency (C), and grooming frequency (D) in control (●) and stressed (○) rats. Rats were placed in the central square of the open field and observed for 5 min. The amount of time they stayed in that square was recorded (A), as was the number of lines crossed on the floor grid (B), the frequency of rearing (C), and the frequency of grooming (D). Data are represented as the means ± SD. *P<0.05, **P<0.01, ***P<0.001, n = 15 animals in each group.
CUMS increased activity of both MAO-A and MAO-B

The MAO isoenzymes MAO-A and MAO-B are involved in catabolism of the neurotransmitters dopamine, norepinephrine, and serotonin. Elevated MAO activity thus affects brain levels of those transmitters, among which norepinephrine and serotonin are often elevated in depression (Elhwuegi, 2004). Compared to control animals, brain MAO-A and MAO-B activity was significantly elevated in the stressed animals after 3 weeks of CUMS (P<0.05, Fig. 4). Consistent with the behavioral findings detailed above, this supports the view that CUMS induced a depressive state in our animals.

![Figure 4](image_url). Effect of chronic unpredictable mild stress (CUMS) on brain MAO-A and MAO-B activity in control rats and those subjected to CUMS for three weeks. Data are represented as the means ± SD, *P< 0.05, n = 8 animals in each group.

Effect of CUMS on BPRP levels in hippocampus and pancreas

Western blotting demonstrated that the hippocampus had higher levels of BPRP than the pancreas (P<0.01, Fig. 5). Western blotting also showed that 3 weeks of CUMS led to a significant reduction of BPRP in the pancreas (P< 0.001), but not in the hippocampus (Fig. 5).
Figure 5. Western blotting analysis of BPRP in the hippocampus and pancreas of control rats and rats subjected to chronic unpredictable mild stress for three weeks. Intensity of Western bands was quantified with a densiometric scanner. Data in the bar graph are represented as the means ± SD from four independent experiments. **P< 0.01, ***P< 0.001 vs. normal pancreas.

CUMS reduced BPRP immunoreactivity in the pancreas, but not the hippocampus

While CUMS had no clear effect on BPRP immunoreactivity in the hippocampus, it led to an apparent absence of such immunoreactivity in pancreatic islets three weeks after stress onset (Fig. 6).
Figure 6. Immunohistochemical localization of BPRP (brown line in A and B, brunneus circle in C) in hippocampal field CA1 (A, B) and pancreas (C, D) in control rats (A, C) and rats subjected to three weeks of chronic unpredictable mild stress (C, D). Tissue was counterstained with hematoxylin and is shown at ×100. Note that stress appeared to have no effect on BPRP immunoreactivity in the hippocampus, but appeared to eliminate it in the pancreas.

Effect of CUMS on dentate gyrus

Examination of coronal sections stained with hematoxylin and eosin revealed an obvious reduction in the thickness of the granule cell layer of the dentate gyrus in the stressed rats compared to the control animals after 3 weeks of CUMS (Fig. 7).
Figure 7. The dentate gyrus of control rats (A) and rats subjected to three weeks of chronic unpredictable mild stress (B) as seen at ×100 in coronal sections stained with hematoxylin and eosin. Note the reduced thickness of the granule cell layer.

**Effect of CUMS on blood glucose and insulin levels**

Three weeks of CUMS had opposite effects on blood glucose and insulin levels (Fig. 8): the former was increased significantly \((P<0.05)\), while the latter was decreased significantly after 3 weeks \((P<0.05)\).

Figure 8. Blood glucose and insulin levels in control rats and those subjected to chronic unpredictable mild stress for three weeks. Data are represented as the means ± SD. *\(P< 0.05\), \(n = 8\) animals in each group.
Discussion

Since it is believed that long-term exposure to multiple, inescapable stressors can promote clinical depression in humans, chronic unpredictable mild stress is considered a realistic means of producing an animal model of depression (Willner, 1986). No single stressor in the CUMS regimen appears necessary or sufficient to induce depressive behaviors in rats. Such behaviors arise instead from the variety and frequent presentation of stressors in the regimen (Muscat and Willner, 1992). Animals subjected to the CUMS regimen display several features of clinically depressed humans (Marta et al, 1998; Willner, 1984). Among the most notable is anhedonia, an inability to experience pleasure from normally pleasurable sources. Anhedonia in rats exposed to CUMS is evident in decreased self-stimulation of reward pathways in the brain (Harro et al, 1999) and in decreased preference for a dilute sucrose solution (Forbes et al, 1996; Matthews et al, 1995; Willner et al, 1987) as confirmed in the present study. The reduced preference for sucrose we observed was not attributable to decreased thirst in general, because total fluid consumption was not decreased in the stressed rats. Nor was it secondary to the slower weight gain in the stressed animals, as demonstrated in previous research (Willner et al, 1996). A change in PP is clearly a valuable measure. Willner et al (Willner et al, 1987) reported a statistically significant decrement in preference for a 1% sucrose solution, from a baseline value of around 80% to a value of 65% in stressed animals. Despite the PP in our study was about 60% which seemed no preference, there was a significant reduction in PP to around 30%. Reid et al (Forbes et al, 1996; Matthews et al, 1995) speculated that reduction in total sucrose consumption may be contributed to the weight loss, therefore, they analyzed the mean sucrose consumption per gram of body weight. If so, why the total fluid didn’t decrease followed the weight loss? So we think it may be reasonable to analyze the PP to evaluate the effect of CUMS. Anhedonia may thus be reflected as well in the depressed appetite of the stressed rats.

Other symptoms of clinical depression in humans are also likely in animals subjected to CUMS (Willner, 1997; Willner and Mitchell, 1992). In our open field tests, for example, lethargy, apathy, and bodily neglect may be inferred from reduced exploration, rearing, and grooming behavior, respectively. High score of grooming is considered to reflect a higher level of emotionality (Pardon et al, 2000). So anhedonia resulting from CUMS was further confirmed by the reduced grooming behavior.

Further evidence that animals subjected to CUMS develop depressive states is the repeated observation that loss of preference for sucrose can be reversed by treatment with antidepressives (Papp et al, 1996; Willner, 1997). One such antidepressive is Moclobemide (Moreau et al, 1993), which is a reversible inhibitor of MAO-A (Yamada and Yasuhara, 2004). Our study provided a likely explanation for that observation, because we found that CUMS significantly increased the activity of MAO-A in the brain.
An increase in brain MAO-B activity was also observed in the stressed animals. Since inhibition of MAO-B is neuroprotective (Yamada and Yasuhara, 2004), increased activity of that enzyme in the brain may play a role in the atrophy and/or cell death of dentate gyrus granule cells we observed in brains of rats subjected to CUMS (Luca et al, 2003). Such cells are normally replaced by neurons deriving from stem cells located in the subgranular zone of the dentate gyrus (Seri et al, 2004). Given that environmental stress reduces proliferation of subgranular stem cells (Fuchs et al, 2004; Malberg, 2004). CUMS may impair replacement of dentate granule cells and thereby lead to the noticeably thinner dentate granule cell layer we observed. This may be directly related to the depressive state of the animals given increasing evidence that antidepressants promote proliferation of subgranular stem cells with a lag time similar to that of the their clinical effectiveness (Malberg, 2004; Malberg and Schechter, 2005).

Within three weeks of CUMS onset, BPRP was unaffected in the hippocampal formation but was virtually undetectable in the pancreas by Western blotting or immunohistochemistry. The loss of pancreatic BPRP, which was normally found in islets (Yao et al, 2003), was accompanied by decreased blood levels of insulin and increased blood levels of glucose, consistent with a report of glucose intolerance in rats subjected to CUMS (Bhattacharya et al, 2000). Here hyperglycemia did not induce a decline or increase in hippocampal BPRP after CUMS, the reason why there was no effect on the hippocampal BPRP may be that the glucose level was not enough high to affect the hippocampal BPRP expression. In fact a researcher in our lab have found that hippocampal BPRP expression was decreased significantly in alloxan-induced diabetic rats in which glucose levels reached to 40mmol/L, while pancreatic BPRP was affected in the same way. Different response to the hyperglycemia and hypoinsulinemia suggests that the BPRP in pancreas is more sensitive to the changes in levels of glucose and insulin than the BPRP in hippocampus. With a full time course on BPRP levels and on measures of glucose tolerance and insulin sensitivity, it can be argued that the pancreas was affected after hyperglycemia developed, and we can determine that depressive symptoms in stressed animals are likely to be responsible for decreased pancreatic BPRP and that change is likely to play a role in onset of diabetic condition. A depressive state induced by CUMS can thus lead to relatively advanced diabetic conditions (hyperglycemia and hypoinsulinemia).

**Acknowledgement**

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