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Changes of Renal Peripheral Benzodiazepine Receptor in the Stress/ Anxiety Response

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Peripheral benzodiazepine receptor(PBR) has been indentified in various peripheral tissues including kidney. The physiological and pharmacological functions of PBR are still uncertain, althought it has been suggested that these are associated with the regulation of stress/anxiety response. Diazepam progeny, which were exposed to diazepam perinatally, was reported to be an animal model of chronic anxiety. However, PBR in the diazepam progenies are not known yet. In the present study, therefore, we examined the changes of PBR in the stress/anxiety response. Dams of rats were given injection of diazepam or vehicle during puerperium. Diazepam progenies showed increased level of anxiety on the performance of elevated plus maze, and increased Bmax of PBR. Saturation experiments followed by scatchard analysis of the results showed that the increase in the density of PBR and the affinity of the PBR remained unchanged. Forced swim stress increased anxiety on the plus maze in both groups of rats. In contrast to control, diazepam progenies did not show further upregulation of renal PBR immediately after swimming stress, but still higher than control. From the above results, it may be concluded that upregulation of renal PBR is associated with chronic anxiety as well as stress-induced response.

Key Words: Diazepam progeny, Swimming stress, Anxiety, Renal PBR, Upregulation

INTRODUCTION

Benzodiazepines are widely used clinically as sedative-hypnotics, anxiolytics, anticonvulsants and muscle relaxants. Their effects are mediated via binding to the central benzodiazepine receptors (CBR). CBR are coupled to γ-aminobutyric acid (GABA) receptors and to the chloride ion channel and are confined to the central nervous system (Skolnick & Paul, 1983; Skolnick & Paul, 1988). Surprisingly, [^{3}H]diazepam binds not only to CBR, but also to specific sites in various peripheral tissues. These sites are called peripheral benzodiazepine receptor (PBR) and are not coupled to the GABA receptor (Braestrup & Squires, 1977). It has been found that these PBR modulate cholesterol transport from the outer to the inner mitochondrial membrane, the rate-limiting step in steroidogenesis (Guarneri et al, 1992; Jung-Testas et al, 1989; Korneyev et al, 1992; Krueger et al, 1990). Results of several laboratories showed that acute stress produced upregulation of PBR in various peripheral tissues (Basile et al, 1985; Leon et al, 1989; Novas et al, 1987). Among these, renal PBR showed prominent and consistent changes (Novas et al, 1987). This finding has been explained that acute stress elicited massive release of adrenocorticohormone, which produced upregulation of renal PBR (Basile et al, 1985; Novas et al, 1987).

Perinatal exposure to diazepam produces enduring

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postnatal deficits of the CBR. This diazepam progeny was reported to be an animal model of chronic anxiety (Gai & Grimm, 1982; Kellogg et al, 1983a; Kellogg et al, 1983b, Livezey et al, 1985; Marczynski & Urbanic et al, 1988). It has been reported that PBR was downregulated in some anxiety disorders (Weizman et al, 1987), but changes of PBR in diazepam progenies are unclear as yet. In the present study, therefore, we examined the effects of acute stress and/or anxiety on renal PBR.

METHODS

Animals

Nulliparous female Sprague-Dawley rats were selected as dams, and were mated overnight with male rats of the same strain. The presence of a sperm-positive vaginal smear was used to designate gestational day 0. Pregnant animals were maintained on a 12-hr light/dark cycle (lights on at 06 : 00), housed individually, and given ad libitum access to food and water. Pregnant female rats received daily i.m. injections of either diazepam (donated from Korean Roche) or vehicle (40% propylene glycol, 10% ethyl alcohol) on days 18 of gestation through 28 of postpartum with an average dose of 5 mg/kg. The offspring were weaned at 28 days of age and placed in group housing according to litter and gender. Male rats of about 60 days old were analyzed separately in the following experiments.

Acute swimming stress

Male rats from vehicle and diazepam dams, weighing 200-250 g were forced to swim in a water basin as described by Trulla et al. Briefly, the stress was produced by forcing the rats to swim in a water basin ($50\times40\times25$ cm) at $20\pm1^{\circ}C$ for 5 min. Animals were sacrified by decapitation immediately after termination of forced swimming.

Elevated plus maze

To observe the level of anxiety, the elevated plus maze (Lister, 1987; Pellow et al, 1985; Pellow & File, 1986) was performed. The elevated plus maze was constructed entirely of black plexiglass, consisting of 2 open arms (30×5 cm) and 2 enclosed arms ($30\times5\times15$ cm) extending from a central platform (5×5 cm). The maze was elevated 31 cm above the floor. Behavioral evaluations were carried out in a quiet room with bright light. Animals were brought in the room an hour prior to the experiment. At the begining points, rats were placed in the middle platform of the plus maze facing an open arm. Performance in the plus maze was evaluated for 5 min. The time spent in the open arms, enclosed arms, and middle platform, and the number of entries into the arms were analyzed by plus maze monitoring program (Elevated plus maze, Vatican Production, Inc). Reduced time spent in the open arms and/or entries into the open arms were considered as an increase of anxiety.

Peripheral benzodiazepine receptor measurement

The kidney was removed immediately after decapitation. Tissues were disrupted in 50 volumes of 50 mM Tris-HCl buffer (pH=7.4) and centrifuged at $20,000\times g$ for 20 min, and the pellets were used. The binding of [^{3}H] Ro5-4864 (76.5 Ci/mmol, NEN), an agonist for PBR, to the membranes was assayed using a filtration technique. Each assay comprised triplicate samples containig 0.1 ~ 0.35 mg protein suspended in 0.5 ml of 50 mM Tris-citrate buffer (pH=7.4) incubated for 120 min at 4°C using a range of radiolabeled drugs between 0.2 and 10 nM. Nonspecific binding was determined in the presence of 100 mM PK 11195 and represented about 25% of total binding. The assays were terminated by filtration through GF/B filters (Whatman) and three washes with 5 ml of ice-cold bufffer using Harvesting apparatus (Brandel M-24R, Brandel Instruments, Gaithersberg, MD, USA). The radioactivity retained by the filters was measured in a liquid scintillation spectrometer (Wallac 1410, Turku, Finland) using 4 ml scintillation solution (Packard instruments B.V. Chemical Coporations, Groningen, Netherlands). Protein was determined by bichinchonic acid method (Pierce Chemicals, Rockford, IL, USA).

Statistics

Inter-group comparisions of behavior was made by ANOVA, followed by Neuman-Keuls multiple comparision analysis package (Systat Inc., Evanston, IL,

USA). Equilibrium binding constants for [^{3}H] Ro5-4864 binding to renal PBR were determined using non-linear regression analysis (Prism, Graphpad Software, San Diego, CA, USA). Inter-group comparisions of Kd and Bmax were made by ANOVA, followed by Neuman-Keuls multiple comparision analysis package (Systat Inc., Evanston, IL, USA).

RESULTS

Forced swim stress showed an anxiogenic effect on the performance of an elevated plus maze. In the stressed rats, percent open crosses and percent time in open arms were decreased compared to control rats. Percent open crosses was significantly ($p<0.05$) decreased from 35.9 ± 4.2 to 12.5 ± 4.2. Percent time in open arms was significantly ($p<0.05$) decreased from 25.2 ± 1.2 to 2.1 ± 1.9 (Table 1). For the stressed rats, saturation experiments followed by Scatchard analysis of the results showed that Bmax was significantly different compared to control rats. Bmax (fmol/mg protein) was significantly ($p<0.05$) increased from 4.3 ± 0.7 to 7.4 ± 0.7 (Table 2).

Diazepam progenies, which were exposed to diazepam in perinatal life, showed an increased level of anxiety on the plus maze. Percent time in open arms was significantly ($p<0.05$) decreased from 25.2 ± 1.2 to 9.0 ± 2.4 (Table 1). Diazepam progenies have been shown to increase the density of PBR and the affinity remained unchanged. Bmax (fmol/mg protein) was significantly ($p<0.05$) increased from 4.3 ± 0.7 to 6.2 ± 0.3 (Table 2).

Further anxious behavior was observed in the stressed diazepam progenies, which suffered from forced swim stress. The stressed diazepam progenies showed marked decrease in percent open crosses and percent time in open arms compared to diazepam progenies. Percent open crosses was significantly ($p<0.05$) decreased from 32.4 ± 5.7 to 7.0 ± 4.4. Percent time in open arms was significantly ($p<0.05$) decreased from 9.0 ± 2.4 to 1.9 ± 1.6 (Table 1). For the stressed diazepam progenies, Bmax of [^{3}H]Ro5-4864 binding sites of renal tissue did not show a significant difference compared to diazepam progeines, but still significantly ($p<0.05$) higher than control (Table 2).

Table 1. Effects of swimming stress on elevated plus-maze performance of rats

	Percent open crosses	Percet time in open arms
Control	35.9 ± 4.2	25.2 ± 1.2
Acutely stressed	12.5 ± 4.2*	2.1 ± 1.9*
Diazepam progenies	32.4 ± 5.7	9.0 ± 2.4*
Stressed diazepam progenies	7.0 ± 4.4*$^{\$}$	1.9 ± 1.6*$^{\$}$

Animals were evaluated for five minutes as described in the Method section.

Percent open crosses was expressed as (the number of entries into the open arms/total entries) $\times$ 100. Percent time in open arm was expressed as [the time in the open arms/(the time in the open arms + time in the enclosed arms)] $\times$ 100.

Values represent mean $\pm$ SE of 10 animals *$p<0.05$: Significantly different from control. $^{\$}p<0.05$: Significantly different from diazepam progenies.

DISCUSSION

CBR in rat brains is detectable 8 days before birth, their density reaching 35% of adults levels at birth, and near maximal levels by two weeks after birth. The ontogeny of receptor and enzymes that synthesize receptor ligands constitutes a delicate, genetically regulated homeostatic process. Thus, the expression of the genome responsible for synthesis of CBR should be sensitive to modulation by exposing the

Table 2. Alteration of the equilibrium binding constants of [^{3}H] Ro5-4864 binding to the renal PBR

	Bmax (pmol/mg protein)	Kd (nM)
Control	4.3 ± 0.7	3.0 ± 0.5
Acutely stressed	7.4 ± 0.7*	2.4 ± 0.6
Diazepam progenies	6.2 ± 0.3*	3.0 ± 0.7
Stressed diazepam progenies	8.2 ± 1.5*	2.6 ± 0.2

The Kd and Bmax values were determined using non-linear regression analysis. *$p<0.05$: Significantly different from control. Values represent mean $\pm$ SE of 7 experiments

developing animals to diazepam, a receptor agonist which should cause a suppression of CBR ontogeny. It was reported that perinatal exposure to diazepam produced enduring postnatal deficits of CBR (Livezey et al, 1986; Marczynski & Urbanic, 1988). Therefore, these animals have a reduced chance for endogenous benzodiazepine receptor agonists, which has been regarded as a physiologic regulator against stress/anxiety to CBR. These animals are regarded as an animal model of chronic anxiety (Gai & Grimm, 1982; Kellogg et al, 1983a; Kellogg et al, 1983b; Livezey et al, 1985; Livezey et al, 1986; Marczynski & Urbanic, 1988). In this experiment, diazepam progenies, which were exposed to diazepam perinatally, also showed increased anxiety in the performance of elevated plus maze.

PBR has been identified in various peripheral tissues such as the kidney, the liver and the lung. The physiological and pharmacological functions of PBR are not clear as yet, although it has been demonstrated that these "non-neuronal" binding sites are sensitive to endocrine changes and environmental stress (Basile et al, 1985; Leon et al, 1989; Novas et al, 1987). The subcellular localization of PBR in the mitochodrial fraction suggests that they have a possible role in intracellular metabolic events. In an attempt to elucidate the function of PBR, research has focused on the steroidogenic tissues. It has been found that these mitochondrial receptors modulate cholesterol transport from the outer to the inner mitochodrial membrane, the rate-limiting step in steroidogenesis (Guarneri et al, 1992; Jung-Testas et al, 1989; Korneyev et al, 1992; Krueger & Papadopoulos, 1990).

Biochemical analyses of rat tissue homogenates and whole-body autoradiographic studies have shown that the greatest densities of PBR occur in the adrenal gland. With high levels occurring in other steroidogenic tissues, including the testis and the ovary (Krueger & Papadopoulos, 1990; Garnier et al, 1994; Gravish, 1995; Papadopoulos et al, 1994). In addition, PBR are also localized in non-neuronal (glial) tissue of the brain (Weizman et al, 1987; Shoemaker et al, 1981). Glial cells in the brain are capable of de novo synthesis of cholesterol, progesterone, and pregnan-5-ene-3a-20-diol synthesis, and are further capable of conversion of progesterone into the metabolites pregnan-3, 20-dione and 3a-hydroxy-5a-pregnan-20-one. Biochemical and electrophysiological studies indicated that these metabolites could directly induce chloride flux at micromolar concentrations and at lower concentrations could act as an allosteric modulator of GABA-mediated chloride ion conductance (Bitran & Sciekh, 1995; Lan & Gee, 1994; Zimmerberg et al, 1994). It also has been reported that these neuroactive steroids showed anxiolytic and anticonvulsant activities (Bitran & Sciekh, 1995; Lan & Gee, 1994).

It appears that acute stress is associated with an upregulation of PBR, while chronic stress and some anxiety disorders are associated with a downregulation of PBR (Leon et al, 1989, Novas et al, 1987). In this study, forced swim stress increased the level of anxiety on the performance of an elevated plus maze. Forced swim stress has been shown to increase the maximal number of [^{3}H]Ro 5-4864 binding sites and the affinity of the PBR remained unchanged. This finding is consistent with studies by Leon et al and Novas et al.

Platelet PBR are diminished in some of the anxiety disorders, such as generalized anxiety disorder, panic disorder and posttraumatic stress disorder, but remain unaltered in another anxiety disorder, viz., obsessive-compulsive disorder (Weizman et al, 1987; Gilbert et al,1988). The present study demonstrates that anxious diazepam progenies, similar to stressed rats and in contrast to other anxiety disorders, is not associated with downregulation of PBR. This finding is not consistent with studies by Weizman et al and Gilbert et al.

When diazepam progenies were forced to swim in a water basin, they showed further increase of anxiety on the performance of an elevated plus maze. Diazepam progenies showed a higher value of Bmax of renal PBR after acute swimming stress, however, this value was proved to be not significantly different from diazepam progenies. For the diazepam progenies, renal PBR was already upregulated highly, therefore, it might be the possible reason why we could not get a statistical significance.

From the above results, it may be concluded that upregulation of renal PBR is associated with chronic anxiety as well as acute stress-induced response.

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