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ORIGINAL INVESTIGATION

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Peripheral-type benzodiazepine receptors on platelets are correlated with the degrees of anxiety in normal human subjects

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Abstract *Rationale:* Anxiety is the one of the main symptoms of psychiatric disorders. Psychosocial stressors have been shown to be related to the onset of anxious episodes. Peripheral-type benzodiazepine receptors (PBR) are involved in regulating stress responses. The sensitivity of PBR to acute or chronic stress has been demonstrated in various situations. The State-Trait Anxiety Inventory (STAI) is one of the longest standing and most frequently used measures of anxiety. The development, evaluation, and use of biological markers with anxious conditions in psychiatry are extremely important. *Objectives:* The aims of this survey are to see whether PBR can be used in screening the degrees of anxiety which occur when normal persons are placed in the stressful conditions. *Methods:* Twenty-four healthy volunteers (14 men, 10 women; mean age 46 years) participated in this study. We administered the STAI to all the volunteers. The binding of the radioactive PBR antagonist [^{3}H]PK 11195 to platelet membranes was determined for these volunteers. *Results:* The mean STAI scores were 40.3±8.0 for trait anxiety and 39.0±8.9 for state anxiety. B_{max} of the platelet PBR binding was 2845±2109 fmol/mg protein. Pearson correlational analyses revealed that B_{max} values were significantly and positively correlated with scores for trait anxiety but not significantly correlated with scores for state anxiety. *Conclusions:* PBR on platelets are correlated with trait anxiety scales of the STAI in healthy normal subjects. It is therefore suggested that the density of platelet PBR is highly associated with these personality traits for anxiety tolerance. PBR density in platelet could also be used as a promising biological marker of stressful conditions.

Keywords Peripheral-type benzodiazepine receptor · Platelet · State-Trait Anxiety Inventory · PK 11195 · Normal subject · Screening

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Introduction

Anxiety is one of the main symptoms of psychiatric disorders. Stress has also been implicated in the onset and maintenance of psychiatric disabilities, and psychosocial stressors have been shown to be related to the onset of anxious episodes. The biological system that has been most closely linked to the stress response in mammals is the limbic-hypothalamic-pituitary-adrenal axis through which a variety of stress-associated steroid hormones are produced. Chronic uncontrollable stress might be associated with overactivity of the hypothalamic-pituitary-adrenal axis.

Peripheral-type benzodiazepine receptors (PBR) are membrane proteins pharmacologically and structurally distinct from central-type benzodiazepin receptors, which are associated with the $GABA_A$ receptor-chloride channel complex. PBR have been localized in various peripheral organs and are even found in the brain and spinal cord. PBR appear to play a role in cholesterol translocation from the outer to the inner mitochondrial membrane, which is the rate-limiting step of steroidogenesis (Krueger and Papadopoulos 1990). PBR functions are known to be regulated by steroid hormones. Acute stress is associated with an increase in PBR in several tissues, whereas chronic stress is associated with a decrease in PBR binding capacity (Drugan 1996; Gavish et al. 1992).

A decrease in PBR has also been demonstrated in several psychiatric disorders, such as generalized anxiety disorder (Ferrarese et al.1990; Rocca et al. 1991; Weizman et al. 1987), generalized social phobia (Johnson et al. 1998), panic disorder (Marazziti et al. 1994), and posttraumatic stress disorder (Gavish et al. 1996). The main symptom of these disorders is anxiety.

The State-Trait Anxiety Inventory (STAI) is one of the longest standing and most frequently used measures of anxiety, and STAI has been used in over 3000 studies (Spielberger 1989). The scale has been translated into numerous languages, and its overall factor structure has been examined for a range of samples. STAI has been successfully applied to high school and college students, adults, military personnel, prison inmates, and a wide variety of psychiatric and medical patients. STAI is an instrument that can measure both state (A-state) and trait (A-trait) anxiety. Fear of death may be particularly high in situations in which specific childhood traumas are paired with elevated scores on the A-trait scale (Daugherty 1998). Higher scores on both the A-trait and A-state scales of the STAI indicate hypersensitivity to threat (Tripp et al. 1995).

The development, evaluation, and use of biological markers with anxious conditions in psychiatry are extremely important. However, with certain exceptions, truly sensitive and specific markers have not yet emerged in this field. Biological markers for anxiety tolerance have not yet been reported. The aim of this study is to see whether PBR can be used in determining the degree of anxiety in normal healthy persons.

Materials and methods

Twenty-four volunteers (14 men and 10 women) with a mean age of 46 years (range 28–60 years) participated in this study. All subjects were fully informed about the nature of the study and gave their written consent. We administered the STAI to all the volunteers.

None of the subjects had taken any medications, including psychotropic drugs, for 3 months, nor did they have any psychiatric histories. All subjects underwent physical examinations, including chest radiography, blood and urine samples, and upper gastrointestinal examinations, and no physical diseases were found. Moreover, there had not been any major stressful events as indicated in the stress ratings developed by Holmes and Rahe. Any psychiatric symptoms were those found in the *Diagnostic and Statistical Manual of Mental Disorders* IV psychiatric interviews by psychiatrists. Thus neither the psychiatric and nor the physical examinations indicated abnormal findings in any of the volunteers who participated in this study.

Preparation of platelet membranes

Platelet membranes were prepared using a modification of the method of Gavish et al. (1996). In brief, blood samples (20 ml) were obtained from the subjects in the morning between 9:00 a.m. and 10:00 a.m. They were collected in plastic-walled, evacuated blood collection tubes (Terumo Venoject II) and spun twice at 180 *g* for 15 min at 4°C. Platelet-rich plasma was collected and spun at 1,500 *g* for 15 min at 4°C. The platelet-containing pellet was frozen at –80°C. Prior to binding assay, the samples were thawed, and each pellet was homogenized in 10 ml ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a Polytron (PT-10). The homogenate was then centrifuged at 49,000 *g* for 15 min at 4°C, and the pellet was suspended in 100 vol Tris-HCl buffer. The platelet membranes were finally adjusted to 0.05 mg protein/ml with an assay buffer (50 mM Tris-HCl, pH 7.4). The protein content was determined by the Lowry technique.

Binding assay

The binding of [^{3}H]PK 11195 to platelet membranes was performed based on the method of Nakamoto et al. (1996). Platelet membranes (40 µg protein) were incubated with [^{3}H]PK 11195 (86.0 Ci/mmol; Daiichi Pure Chemical, Tokyo, Japan) at concentrations ranging between 0.08 nM and 10 nM, in an incubation volume of 1 ml. The reaction was carried out for 60 min at approximately 4°C, and terminated by rapid filtration over Whatman GF/B strips (FP-100) using a Brandel Cell Harvester (M-24), with five 5-ml washes of ice-cold buffer. Before filtration, the strips were coated with poly-L-lysine (Sigma, St. Louis, Mo., USA). Nonspecific binding was determined in the presence of 10 µM unlabeled PK 11195 (Research Biochemicals International). The radioactivity retained by the filters placed in PicoPlate 24 was measured with a Top Count microplate scintillation counter (Packard), using 500 µl Microscint-20 (Packard) as a scintillant.

The maximal number of binding sites (B_{max}, fmol/mg protein) and their affinity to [^{3}H]PK 11195 as expressed by the dissociation constant K_d, nM) were determined by least squares regression fitting using scientific graphing software (SigmaPlot, Jandel Scientific, USA). Pearson product-moment correlation and analysis of variance were used to look for associations among B_{max} values, scores of the STAI, and sociodemographic variables. All differences were considered significant at $P<0.05$. Statistica software (Japanese version) for Macintosh was used in the statistical analysis. Unless otherwise stated, the statistical data are presented as the mean and standard deviation.

Results

The mean scores of STAI were 40.3±8.0 (n=24) for trait anxiety and 39.0±8.9 (n=24) for state anxiety. The mean values of B_{max} and K_d were 2845±2109 fmol/mg protein (n=24) and 18.6±14.9 nM (n=24), respectively. Pearson correlational analyses revealed that B_{max} values were significantly and positively correlated with the STAI scores for trait anxiety with a correlation coefficient (r) of 0.433 (95% confidence limit of r: 0.036–0.712, P=0.03; Fig. 1) but not significantly correlated with the STAI scores for state anxiety (r=0.328 with a 95% confidence limit of –0.087 to 0.646, P=0.12; Fig. 2). On the other hand, K_d values were not significantly correlated with STAI scores. From the sociodemographic point of view, there was no significant correlation between B_{max} values and age nor was there a significant difference between the B_{max} values of men (3166±2282 fmol/mg protein, n=14) and that of women (2397±1860 fmol/mg protein, n=10)

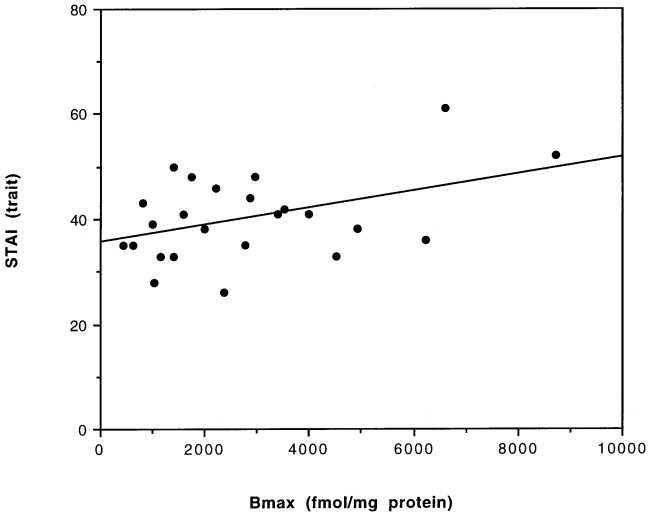

Fig. 1 B_{max} values were significantly and positively correlated with STAI scores for trait anxiety (r=0.433, P=0.03)

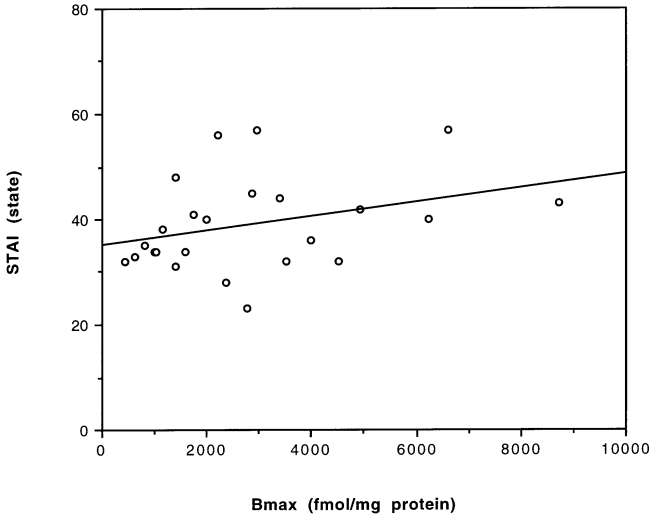

Fig. 2 B_{max} values were not correlated with STAI scores for state anxiety (r=0.328, P=0.12)

by analysis of variance. There was also no significant correlation between STAI scores and age, nor was there a significant difference between the STAI scores of men (39.9±7.1 for trait anxiety; 39.9±6.7 for state anxiety) and those of women (40.8±9.4 for trait anxiety; 37.6± 11.6 for state anxiety).

Discussion

In the present study we found that platelet PBR densities are significantly correlated with the degrees of anxiety, as determined by STAI. The subjects were healthy volunteers without previous psychiatric illness. Although the limited number of the subjects (14 men and 10 women) may affect the reliability of the findings, a small number of "outliers" with fairly high B_{max} and/or STAI were enough to make the correlation significant (Fig. 1). To our knowledge, this is the first report about the definite correlation between B_{max} of any receptors and STAI.

There are many reports in which groups of psychiatric disorders and groups of stressful conditions have been compared with their control groups for PBR densities. This was the first time that the B_{max} of platelet PBR was examined strictly in normal subjects. The subjects were selected from a normal population by examining their past histories and clinical symptoms. The subjects had not taken any medications, nor did they have past psychiatric histories. They were not experiencing any extraordinarily stressful circumstances.

The range of PBR density in platelet differs widely. According to previous studies, PBR densities vary considerably even in control groups, for example, the B_{max} values for control groups were reported 4327± 1850 fmol/mg protein (Johnson et al. 1998), 3106± 1986 fmol/mg protein (Weizman et al. 1993), or 2557± 937 fmol/mg protein (Weizman et al. 1995). We do not know whether this is due to differences in experimental methods or individual differences. Our results suggest that this variation reflects individual differences rather than differences in method. We found that the B_{max} of platelet PBR was not correlated with A-state anxiety but was correlated with A-trait. It is therefore suggested that the density of platelet PBR is highly associated with these personality traits for anxiety tolerance.

An increase in platelet PBR appears in the person who does not feel stressful, suggesting that this increase represents a precaution before symptoms appear. We could screen the person with stressful conditions from healthy people. PBR density in platelet could also be used as a promising biological marker of stressful conditions.

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