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Short Communication

Effects of PhD examination stress on allopregnanolone and cortisol plasma levels and peripheral benzodiazepine receptor density

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Received 8 September 2003; received in revised form 21 January 2004; accepted 17 February 2004

KEYWORDS

Allopregnanolone;
Peripheral
benzodiazepine
receptor; Cortisol;
Acute stress;
Neurosteroids; PhD
examination

Summary Peripheral benzodiazepine receptor (PBR) density in blood platelets and plasma allopregnanolone concentration in humans were determined following acute stress as represented by PhD examination. Fifteen healthy PhD students participated. Heart rate, blood pressure, plasma allopregnanolone, plasma cortisol, and PBR density were measured at different time points.

Allopregnanolone and cortisol concentration and PBR density were significantly increased during examination. A positive correlation between allopregnanolone and PBR density was found.

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1. Introduction

The peripheral benzodiazepine receptor (PBR), which is located in the central and peripheral nervous system, is involved in steroidogenesis and is considered to be an important potential therapeutic target in psychiatric disorders (Ansseau et al., 1991). The PBR, located at the outer mito-

chondrial membrane, plays a role in the translocation of cholesterol from the outer to the inner mitochondrial membrane (Krueger and Papadopoulos, 1990), which is the rate limiting step for the synthesis of (neuro)steroids (Krueger and Papadopoulos, 1992). Allopregnanolone is a metabolite of progesterone and a potent positive allosteric modulator of the gamma-aminobutyric acid receptor type A (GABA(A) receptor) (Majewska et al., 1986). It has anxiolytic, anti-convulsive, and hypnotic/sedative properties (Rupprecht and Holsboer, 1999). The anxiolytic effect appears to depend on the presence or absence of ovarian steroids (Laconi et al., 2001).

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As allopregnanolone can both accumulate in the brain irrespective of supply from peripheral endocrine organs and can be synthesised de novo from sterol precursors, it is called a neurosteroid (Baulieu et al., 1999). Neurosteroids are involved in many central nervous system disorders like depression, anxiety, learning and memory dysfunctions, and epilepsy (Majewska, 1992; Mellon, 1994; Bickova et al., 2000). In animals, brain and plasma allopregnanolone levels, and in animals and humans, platelet PBR density, both increase following acute stress (Purdy et al., 1991; Drugan, 1996). No conclusive data exist with respect to plasma allopregnanolone levels following acute stress in humans (Girdler et al., 2001; Altemus et al., 2001). Furthermore, the relation between PBR density and allopregnanolone plasma levels following acute stress has not been investigated. The mechanism by which PBR density rapidly increases following acute stress is not known. It could be that there exist PBR containing vesicles that are released by fusion of the vesicles with the cell membrane. If this is true and how acute stress causes this exocytosis remains to be elucidated.

In the present study, we tried to replicate previous findings of increased PBR density following acute stress as represented by an examination situation (Karp et al., 1989). Furthermore, we examined our hypothesis that in acute stress, plasma allopregnanolone concentration increases and that this increase is correlated with the increase in PBR density. Recently, it has been shown that allopregnanolone levels decrease during panic provocation in patients with panic disorder but not in controls (Ströhle et al., 2003). Baseline levels in these patients are increased (Ströhle et al., 2002), specifically in the early follicular phase of the menstrual cycle (Brambilla et al., 2003). These findings suggest that during an induced panic attack, patients with panic disorder fail to maintain compensatory increased allopregnanolone levels. Considering the anxiolytic effect of allopregnanolone and the important role of PBR agonists in the biosynthesis of allopregnanolone, a positive correlation between the two during acute stress may support the suggestion that synthetic PBR agonists may prove to be potent and effective new anxiolytic drugs.

2. Methods

Fifteen healthy PhD students (12 men and 3 women), with a mean age of 35 years (range 29–41 years), were recruited by means of advertisements. No abnormalities were found during physical and psychiatric examination. After complete

description of the study to the subjects, written informed consent was obtained.

In brief, PhD examination in the Netherlands constitutes the presentation and defense of one's scientific work in front of a board of professors. After the presentation and defense, is a break of 15 min in which the board retires and reaches a conclusion about the PhD student as having passed the examination or not. After the break, the board returns and informs the PhD student about its conclusion. PhD examination is considered to be a stressful event and was therefore used in the present study as a model of acute stress (van Rood et al., 1991). Stress was measured by changes in cortisol plasma concentration, blood pressure (BP), and heart rate (HR). Plasma levels of allopregnanolone, and the maximal number of binding sites in blood platelets (B_{max}) for the PBR specific ligand ^3H -PK11195 were measured. All measurements took place four weeks before the PhD examination (T1), 45 min before the examination (T2), during the examination (T3), and four weeks after the graduation (T4). Blood samples at T2 were drawn between 12:30 and 15:00 h; blood samples at T3 were drawn at 14:30 or 16:30 h.

The preparation of platelets was essentially as described earlier (Gavish et al., 1986b). The resulting blood platelet fractions were used for the PBR assay, the (plasma) supernatant was used for the hormone measurements. Both were kept at -80°C until analysis. The PBR binding assay was performed essentially as described earlier (Gavish et al., 1986a) with some modifications. Separation of bound from free ^3H -PK11195, was performed as described earlier (Benraad and Fockens, 1990). The inter assay coefficient of variation was 11.7%.

Plasma allopregnanolone concentration was measured by radioimmunoassay as described in detail earlier (Bixo et al., 1997). The cortisol levels were measured by an immunofluorometric assay (IFMA), the time-resolved fluoroimmunoassay technique (Delfia) using the reagent kit 1244-060 DELFIA® Cortisol Kit (Wallac OY, Turku, Finland) according to the manufacturer's instructions. Serum mixed from both women and men were used as controls in the Delfia assay. The intra assay coefficient of variation in the cortisol assay was 5% and inter assay 4.5%. The analytical sensitivity was 15 nmol/l serum. The inter assay coefficient of variation for the allopregnanolone assay was 8.5% and the detection limit 18 pg.

In the three women, all measurements took place in the follicular phase except for one measurement: baseline measurement (time point 1) in one woman took place in the luteal phase. As

a (physiologically) higher allopregnanolone value was found at this time point compared to the other time points (time points 2–4) in this woman, the difference in phase has not confounded the results of this study (high allopregnanolone levels were found during the PhD examination (time point 3) in most of the subjects).

Differences in means at the different time points and regression coefficients and their corresponding Pearson correlation coefficients were statistically tested using mixed model analyses of variance (ANOVA) with subjects as random factor and time points as fixed factor. Post-hoc comparisons between time points were tested with paired *t*-tests. In order to adjust for time of blood sampling at T_1 and T_4 , all samples from all time points (T_1 – T_4) were divided into two groups: samples drawn before or after 12:00 h.

3. Results

Results of B_{\max} of PBR binding, allopregnanolone and cortisol are presented in Fig. 1. No effect of time of blood sampling could explain the significant differences in allopregnanolone, cortisol concentrations and B_{\max} .

There was a statistically significant effect of time for HR ($F = 17.6$, $df = 3, 41$, $p < 0.001$) with $T_3 > T_1, T_4$; systolic BP ($F = 28.53$, $df = 3, 41$,

$p < 0.001$) with $T_3 > T_1, T_4$; and diastolic BP ($F = 12.38$, $df = 3, 41$, $p < 0.001$) with $T_3 > T_1, T_4$.

B_{\max} was significantly correlated with allopregnanolone plasma concentrations ($r = 0.35$; $B = 3.3 \times 10^{-5}$; $F = 5.92$; $df = 1, 44$; $p = 0.02$). Cortisol plasma concentrations were significantly correlated with allopregnanolone plasma concentrations ($r = 0.40$; $B = 598$; $F = 7.97$; $df = 1, 44$; $p = 0.007$), systolic ($r = 0.40$; $B = 11$; $F = 8.08$; $df = 1, 43$; $p = 0.007$) and diastolic BP ($r = 0.38$; $B = 18$; $F = 6.89$; $df = 1, 43$; $p = 0.012$).

4. Discussion

The results showed that allopregnanolone in humans is increased following acute stress. Furthermore, this is the first study to show that, in acute psychological stress, the increase in plasma allopregnanolone concentration is correlated with an increase in PBR density in blood platelets. The fact that during the PhD examination, plasma cortisol concentration, BP, and HR reached their highest scores, supported our assumption that PhD examination is a valid model for acute stress.

The increase in PBR density during the examination in the present study is in accordance with previous findings. Karp and colleagues have shown increased PBR densities in residents in psychiatry

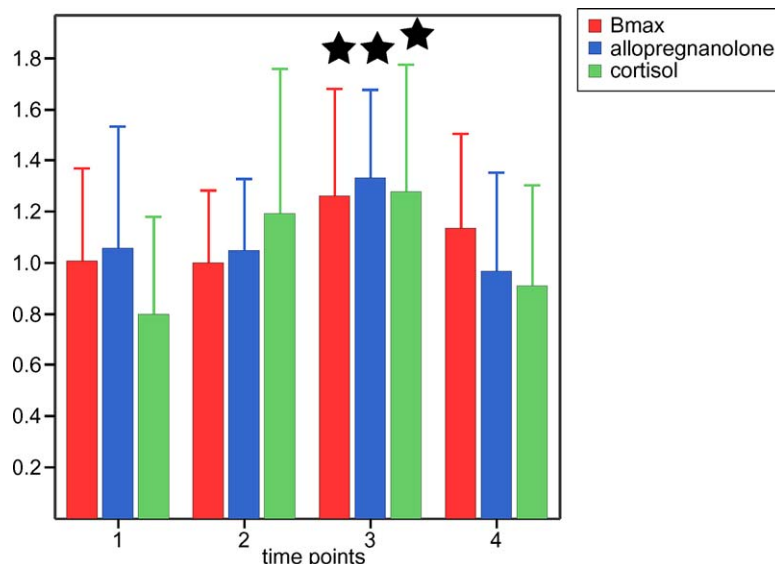


Fig. 1. PBR density in blood platelets and plasma allopregnanolone and cortisol levels. Bars indicate means and error bars one standard deviation. Time points on the x-axis (1–4) refer to: four weeks before PhD examination (1); 45 min before PhD examination (2); during PhD examination (3); and four weeks after PhD graduation (4). B_{\max} (maximal number of binding sites in blood platelets for the PBR specific ligand $^3\text{H-PK11195}$) expressed in 10^{-11} mol/mg protein, allopregnanolone in nmol/l, and cortisol in $\mu\text{mol/l}$. Significant effect of time (*) for PBR density ($F = 2.94$, $df = 3, 42$, $p < 0.05$) with $T_3 > T_1, T_2$; plasma allopregnanolone ($F = 6.38$, $df = 3, 42$, $p < 0.01$) with $T_3 > T_1, T_2, T_4$; and plasma cortisol ($F = 5.27$, $df = 3, 42$, $p < 0.01$) with $T_3 > T_1, T_4$.

immediately after the acute stress of board examination (Karp et al., 1989).

The increase in plasma cortisol during acute stress was not correlated with the increase in PBR density. This suggests that acute stress-evoked increases in cortisol concentrations do not depend on rapid biosynthesis.

Considering the correlational nature of this study, no causal relationship between the increase in PBR density and allopregnanolone concentration can be drawn from the results. Further research is needed to demonstrate if synthetic PBR agonists enhance the biosynthesis of allopregnanolone and, subsequently, if they may be of use in the treatment of anxiety disorders.

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