Effects of Soy Lecithin Phosphatidic Acid and Phosphatidylserine Complex (PAS) on the Endocrine and Psychological Responses to Mental Stress

J. HELLMANN*1, E. FRIES*2, C. BUSS3, V. ENGERT1, A. TUCH1, D. RUTENBURG*2 and D. HELLMANN1

*Neuropsychiatric Institute, Trier, Germany; †Department of Psychology, University of Trier, Germany; ‡Lipogen Ltd., Haifa, Israel

(REceived 7 February 2004; Revised 17 May 2004; In final form 28 May 2004)

Phosphatidylserine, derived from cow brains, has been shown previously to dampen the ACTH and cortisol response to physical stress. Further research investigated the influence of soy lecithin phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. In this study, we investigated the effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) supplementation on pituitary-adrenal reactivity (ACTH, cortisol) and on the psychological response (Spielberger State Anxiety Inventory stress subscale) to a mental and emotional stressor. Four groups of 20 subjects were treated for three weeks with daily dosages of either 400 mg PAS, 600 mg PAS, 800 mg PAS, or placebo before exposure to the Trier Social Stress Test (TSST). Treatment with 400 mg PAS resulted in a pronounced blunting of both serum ACTH and cortisol, and salivary cortisol responses to the TSST, but did not affect heart rate. The effect was not seen with larger doses of PAS. With regard to the psychological response, 400 mg PAS seemed to exert a specific, positive effect on emotional responses to the TSST. While the placebo group showed the expected increase in distress after the test, the group treated with 400 mg PAS showed decreased distress. These data provide initial evidence for a selective stress dampening effect of PAS on the pituitary–adrenal axis, suggesting the potential of PAS in the treatment of stress-related disorders.

Keywords: ACTH, Cortisol, Phosphatidic acid, Phosphatidylserine, STAI, Stress
the TSST, a stress protocol that has been developed in this laboratory. A recent meta-analysis of Dickerson and Kemeny (2004) compared 208 laboratory studies of acute psychological stressors. The analysis showed that the TSST (Kirschbaum et al., 1993) is the best standardised and most efficient psychological stress protocol for studies on HPA-reactivity in humans. Concerning psychological parameters, the TSST leads to a moderate increase in fear. The biological response comprises an increase in circulating ACTH, cortisol, prolactin, growth hormone, noradrenaline and adrenaline concentrations, and increased heart rate and blood pressure (e.g. Kirschbaum et al., 1993). Thus, we decided to use the TSST protocol to assess stress dampening effects of PAS. The study examined the effects of three dosages of PAS versus placebo.

METHODS

This was a double-blind, single centre study. The study duration was 4 weeks. Eighty panelists were invited to the laboratory for pre-tests and for the experiment. They were assigned to one of the four treatment groups (20 subjects per group; 10 males and 10 females) per day; the first group used placebo, the second group received 400mg/day PAS, the third group 600mg/day PAS and the fourth group 800mg/day PAS. Soy lecithin PAS complex capsules as well as placebo capsules were provided by Lipogen Ltd., Haifa, Israel.

PAS is a complex of phospholipids of which every '100mg' PAS capsule consists of 100mg phosphatidylserine (PS) and 125mg phosphatidic acid (PA), plus 270mg of other inert phospholipids (PC, PI, PE, Lyso Phospholipids) and 5mg silicon dioxide (anti-caking material). PAS is patent protected (US 6,410,522 published in June 25, 2002). The placebo was maize starch and the capsules looked identical to the PAS capsules.

SUBJECTS

Eighty subjects (adults age 20–45) were recruited for the study. All of the women were using oral contraceptives. Groups were matched for sex and socioeconomic status. As seen from Fig. 1, the mean age did not differ among the four groups (F(3, 75) = 0.11, p = 0.95). Further, the four treatment groups did not differ with respect to stress load and depression (Gindin et al., 1993, 1995) as measured by the Patient Health Questionnaire (PHQ; Spitzer et al., 1999; Loewer et al., 2002) (F(3, 174) = 0.80, p = 0.77) when entering the study.

Inclusion Criteria

Good medical health was verified by a clinical examination, the patient health questionnaire and a hemogram. The hemogram included assessments of glutamate–pyruvate transaminase, gamma-glutamyl transferase, creatinine, leukocytes, erythrocytes and reticulocytes (haemoglobin, haematocrit, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration), thrombocytes and leukocyte (lymphocyte, basophil, eosinophil, monocyte and neutrophil counts).

Exclusion Criteria

The following exclusion criteria were applied: subjects with a history of mental illness, subjects who used any systemic medication considered to affect the endocrine or behavioural measures, subjects who were pregnant or nursing, subjects participating in any other clinical study, subjects regarded by the investigator as not being able to complete the study, subjects deemed to be physically unhealthy. Subjects were recruited by e-mail and newspaper advertising. Pre-screening and introduction to the study were conducted by telephone and an appointment for the medical pre-examination was made. The medical pre-examination and the hemogram allowed exclusion of medically unhealthy subjects. Altogether, 85 subjects were pre-screened; 5 subjects were excluded according to the exclusion criteria or for personal reasons. As the great majority of the women were using oral contraceptives, we decided to keep the groups homogenous by including oral contraceptive use as a further inclusion criterion for women. The medical pre-examination was conducted by telephone and an appointment for the medical pre-examination was made. The medical pre-examination and the hemogram allowed exclusion of medically unhealthy subjects. Altogether, 85 subjects were pre-screened; 5 subjects were excluded according to the exclusion criteria or for personal reasons. As the great majority of the women were using oral contraceptives, we decided to keep the groups homogenous by including oral contraceptive use as a further inclusion criterion for women. The study finally included healthy male and female subjects.

As shown for a distress subscale, derived from the Spielberger State Anxiety Inventory. While the placebo group showed the expected increase in distress, the 400mg treatment group even showed a slight decrease in distress, suggesting a quicker habituation to a new stressor, which may then result in a dampened HPA response. The protocol of this study did not allow discrimination between effects of chronic and acute PAS treatment. Thus, we do not know yet if a bolus treatment alone can exert a similar stress dampening effect as the chronic treatment. There is currently strong evidence that an enhanced reactivity of the pituitary–adrenal axis is related to several mental and physical diseases, such as depression, some types of abdominal obesity and the metabolic syndrome (Chrousos, 2000; Pasquali et al., 2000). The striking effect of 400mg PAS daily in dampening the stress response may be promising with respect to possible clinical application in stress related disorders. This view is supported by the fact that no side effects were observed in this study.

Acknowledgements

This study was initiated and financed by Lipogen Ltd., Haifa, Israel.

References


the increase in serum cortisol (F(1, 34) = 0.14, p = 0.71), or the increase in salivary cortisol (F(1, 35) = 0.60, p = 0.44).

Also, no treatment effects were found for the heart rate response to the TSST, as well as for effects on total anxiety scores and mood under stress. Since no mood changes were observed in the MDBF, and knowing that the 20 STAI items assess a spectrum of mood and stress, not specific for the TSST, we expected that PAS may have exerted only specific effects on stress measures of the TSST. To test this hypothesis, we performed a factor analysis of STAI responses to the TSST of an independent sample of 113 subjects, matched for age and sex from the data bank of this laboratory. A principal component analysis (Promax rotation) was performed and three factors were extracted, assessing nervousness (F1), relaxation (F2) and distress (F3), respectively. The Eigenvalues and percentages of explained variance of the three factors were F1 = 6.14/30.7%, F2 = 5.11/7.6% and F3 = 1.42/7.1%. Commonalities ranged from 21 to 71. Reliabilities (Cronbach’s alpha) were for F1 = 0.76, F2 = 0.77 and F3 = 0.75, respectively.

Indeed, subjects treated with 400 mg PAS daily did not show the expected increase on the distress subscale (F3), as the control group did. Thus, 400 mg PAS resulted in a significant reduction of the psychological stress response to the TSST, when compared to placebo (Fig. 6; two-tailed t-test for the increase; t(34) = 2.026, p = 0.05), and there was a similar tendency in the 800 mg group. When all three treatment groups were analysed together and compared to placebo, PAS significantly prevented the expected increase in stress after the TSST (Mean increase value in the placebo group with n = 20: 1.05, mean increase value in the PAS group with n = 53: −0.755; two tailed t-test of the increase: n(71) = 1.941; p = 0.056).

PROCEDURES

Before entry into the study, subjects were pre-screened by the investigator for the criteria indicated above in the subject selection section. A medical history was also taken from each subject.

One day before initiation of treatment, salivary cortisol levels were assessed in all subjects (at 4 p.m.) in order to establish a pre-treatment salivary cortisol baseline level, to exclude hyper- or hypocortisolism and to familiarise the subjects with the saliva sampling procedure.

Groups received their respective test product dosage three weeks before the TSST exposure. Each test product consisted of 21 daily containers with 8 identical capsules each containing in sum, either 400, 600, 800 mg PAS (as “100 mg” PAS per capsule), or placebo (i.e. 0–4 placebo capsules per day for PAS-treated subjects, or 8 placebo capsules for the controls). Subjects were instructed to take any three capsules at breakfast, any three capsules at lunch and the last two capsules at dinner in the evening, every day. For compliance inspection, each subject was instructed to bring all the empty containers of the treatment capsules on the last day of treatment (the TSST exposure date) and to use daily a salivette before bedtime. Subjects expected that product levels would be assessed in these saliva samples. On the last day of treatment (day 21) subjects took the three capsules in the morning as usual. In the early afternoon they attended the TSST. Immediately before the introduction to the TSST (90 min before TSST exposure) the last three capsules were taken in the presence of the investigator.

FIGURE 4 Effects of PAS on the salivary cortisol response to the TSST. Baseline values were 11.58 nmol/l (placebo), 10.54 nmol/l (400 mg), 8.49 nmol/l (600 mg) and 12.30 nmol/l (800 mg), respectively.

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DISCUSSION

The data obtained from this study demonstrate the first evidence of a pronounced dampening effect of 400 mg PAS daily on the reactivity of the pituitary–adrenal axis to stress. For the other two treatment groups (600 and 800 mg PAS daily) these effects became weaker with increasing dosages, and did not reach a sufficient level of significance. This study was not designed to study PAS effects with respect to body weight and gender, and sample sizes did not allow more detailed statistical post hoc analyses. An explorative data analysis, however, revealed that women in this study had significantly lower body weight, so they received relatively more PAS per kg than men. Indeed, it seemed that PAS effects were even more pronounced in men when compared to women, as well as in individuals with lower PAS/body weight ratios (data not shown). The fact that the effects of PAS were not seen with increasing dosage still needs to be clarified.

In the TSST, women routinely show blunted ACTH and cortisol responses, when compared to men. Many studies to each group, carefully matched for socioeconomic status. Subjects were provided with extensive information on the study and read and signed a written informed consent form. Subjects received 100 Euro for their participation in the study. The protocol was approved by the Landesärztekammer Rheinland-Pfalz (ethical commission of the state’s Chamber of Medicine).

Every subject spent about 165 min in the laboratory for an introduction to the TSST, a pre-experimental resting period (90 min), the TSST itself (15 min) and a post-experimental resting period (60 min). After a first instruction the subject was led to experimental room #1, which served as the rest and preparatory area.

To gain spontaneous subjective responses about side effects of the test products, subjects were asked upon arrival in the laboratory if they experienced any psychological or physical changes during drug intake.

Forty-five minutes after arrival, subjects received an indwelling catheter in a forearm vein for the collection of blood samples. This first resting phase was necessary to exclude potential activation of the hypothalamic–pituitary–adrenal axis (HPA), possibly confounding later responsivity to the TSST. At the end of the resting period the first saliva and blood samples were collected.

A detailed protocol of the TSST has been described elsewhere by Kirschbaum et al. (1993). For a detailed description of our study protocol on TSST-day, see Fig. 2.

Before the TSST, each proband was introduced to the testing room (#2) and instructed to stand behind a microphone in front of a two-man committee. The subject was informed that the whole session would be video- and tape-recorded and that the committee was trained in behavioural observation. The experimenter instructed the subject to deliver a 5-min speech as if for a job application, for which he/she had 3 min to prepare, and that a second task would follow. After the free speech, the subject had to solve a mental arithmetic task (counting backwards from 2083 to 0 in steps of 17) as quickly and correctly as possible for 5 min.

Before and after the TSST subjects filled out two questionnaires, the “MDBF Mehrdimensionaler Befindlichkeitsfragebogen” (Steyert et al., 1987) aiming at assessing psychological well-being, and the German version of the State scale of the Spielberger State/Trait Anxiety Inventory (Spielberger et al., 1970) from Luks et al. (1981). The MDBF consists of 24 items (each with a five-level response scale) measuring three bipolar dimensions

FIGURE 2 Experimental procedure.
of acute psychological well-being: “good–bad disposi-
tion” (e.g. content, unhappy), “alertness–fatigue” (e.g. 
tired, rested) and “calmness–agitation” (e.g. tense, 
composed). A high MDBF-score indicates psychological 
well-being, and low scores indicate low mood. After the 
TSST procedure subjects stayed for another hour during 
which six more blood and saliva samples were collected.

Post-experimental Resting

The subject returned to experimental room #1, where the 
pilot-test assessments and debriefing took place. Saliva and 
blood samples were collected directly after the stress test 
and after a further 10 min and later at 15 min intervals. The 
STAI and MDBF were once again administered 
immunostaining was performed using a 
commercial readout system (Auto-CliniLumat LB 952, 
Berthold, Bad Wildbad, Germany). This assay has a lower 
Dampening effect on HPA axis stress responses, as can be 
seen from a significantly blunted ACTH response (Effect of 
groups: $F(1, 32) = 6.58, p = 0.008$) to the TSST (Fig. 3).

Subjects treated with 400 mg PAS showed a strong 
reduction in the increase in (total) serum cortisol 
concentration (Main Effect Time $F(2, 8, 84.3) = 24.03, 
p < 0.001$ and Main Effect Group $F(1, 30) = 2.84, p = 
0.05; $F(4)$, which was even more pronounced for the 
biologically active, free steroid fraction, as assessed in 
the Polar Precision Performance SW program (Version 

**RESULTS**

As shown in Figs. 3–5, treatment with 400 mg of PAS daily 
resulted in a significant blunting of the HPA response to 
psychological stress. Evidently, PAS exerts a central 
dampening effect on HPA axis stress responses, as can be 
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**STATISTICS**

Data were compared by analysis of variance (ANOVA). 
Since all women were taking oral contraceptives, serum 
cortisol concentrations were higher, while ACTH and 
salivary cortisol concentrations were lower than in the 
men. To adjust for such gender differences, we compared 
et net increases from baseline between treatment groups and 
controls. In the case of serum and salivary cortisol 
measurements, the data were analysed by ANOVA with 
repeated measures comparing the increase for each time 
point after the TSST to baseline. The data set was cleaned 
for extreme values ranging more than 2 s.d. from the 
mean. Since the study objective predicted dampening 
effects of PAS on plasma ACTH and serum cortisol, and 
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