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

J. HELLHAMMER[a,b,*], E. FRIES[b], C. BUSS[b], V. ENGERT[b], A. TUCH[b], D. RUTENBERG[c] and D. HELLHAMMER[b]

[a]Neuropattern, Trier, Germany; [b]Department for Psychobiology, University of Trier, Germany; [c]Lipogen Ltd., Haifa, Israel

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

INTRODUCTION

In this study we investigated a possible stress dampening effect of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on endocrine, autonomic and psychological measures evoked by the trier social stress test (TSST).

Phospholipids have the very important biological function of constituting the basis of all biological cell membranes. Phosphatidylserine was first derived from cow brains (Bovine Cortex Phosphatidylserine—BCPS). Different studies showed that single intravenous treatment (50 and 75 mg, respectively) as well as repeated oral intake (800 mg per day for 10 days) of BCPS reduced ACTH- and cortisol responses to physical stress (Monteleone *et al.,* 1990, 1992). Since 1992, soy lecithin phosphatidylserine, the first 100% solvent free phosphatidylserine, has become available, and this has excellent bioavailability by the oral route (Shinitzky, 1999). In a first clinical trial, 72 subjects

aged 60–80 years, were randomly assigned to placebo and therapy groups and treated for three months with 300 mg phosphatidylserine and phosphatidic acid daily. The results indicated a strong and significant positive effect of treatment on memory and mood (Gindin *et al.*, 1993, 1995). Benton *et al.* (2001) demonstrated a positive effect of a one month treatment with 300 mg/day phosphatidylserine on perceived stress during a stressful mental arithmetic task. These data suggest a possible beneficial effect on hypothalamus–pituitary–adrenal axis (HPA) responsivity under psychological stress. Based on these data, we were interested to explore effects of phosphatidylserine and phosphatic acid (PAS) on subjects under mental stress conditions. As seen under physical stress, we expected to see a blunted ACTH and cortisol response in both men and women, associated with an attenuated heart rate and psychological stress response. We further expected a dose dependent effect of PAS, increasing from 400 to 800 mg. To study PAS effects, we used

*Corresponding author. Address: Department for Psychobiology, University of Trier–FB I, Johanniterufer 15, D - 54290 Trier, Germany. Tel.: +49-651-2013464. Fax: +49-651-2012934. E-mail: juliane.hellhammer@uni-trier.de

Group	Sex	Mean Age +Standard Error
Placebo	10 female 10 male	28.2 ± 2.1 28.2 ± 2.2
400 mg PAS	10 female 10 male	25.6 ± 1.1 30.1 ± 2.4
600 mg PAS	10 female 10 male	25.3 ± 1.5 29.1 ± 2.2
800 mg PAS	10 female 10 male *	25.3 ± 1.3 29.3 ± 2.2

FIGURE 1   *One male subject withdrew during the study (available TSST data in this group only for 19 subjects).

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, norepinephrine and epinephrine 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 400 mg/day PAS, the third group 600 mg/day PAS and the fourth group 800 mg/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 "100 mg" PAS capsule consists of 100 mg phosphadityl-serine (PS) and 125 mg phosphatic-acid (PA), plus 270 mg of other inert phospholipids (PC, PI, PE, Lyso Phospholipids) and 5 mg 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 with the Patient Health Questionnaire (PHQ; Spitzer *et al.*, 1999; Loewe *et al.*, 2002) ($F(33, 174) = 0.80$; $p = 0.77$) 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 (haemoglobin, haematocrit, mean corpuscular volume, 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 study finally included healthy male and female non-smoking subjects between the age 20 and 45.

Subjects were assigned to one of the four treatment groups. Ten males and 10 females were randomly assigned

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).

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.

Trier Social Stress Test (TSST)

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" (Steyer *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) by Laux *et al.* (1981). The MDBF consists of 24 items (each with a five-level response scale) measuring three bipolar dimensions
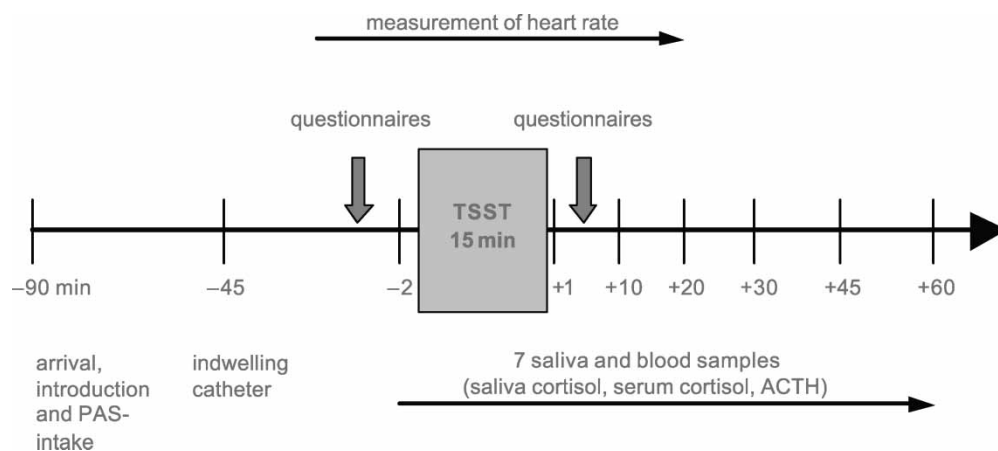

FIGURE 2 Experimental procedure.

of acute psychological well-being: "good–bad disposition" (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 post-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 immediately after the stress test. At the end, the subject was debriefed, by being informed about the nature of the experiment and the behaviour of the experimenters.

ACTH, Cortisol and Heart Rate Measurements

As shown in Fig. 2, blood and saliva samples were collected 2 min before, and 1, 10, 20, 30, 45 and 60 min after the TSST. Salivary and serum cortisol levels were assessed in all samples, while ACTH was only determined at -2 and $+1$ min, respectively. For ACTH, two blood samples were collected in EDTA-Monovettes (Sarstedt, Nümbrecht). After centrifugation for 10 min at 6°C and 1000 g, plasma aliquots were stored at -20°C until analysis. ACTH levels where determined via chemiluminiscent immunoassay (Nichols Institute Diagnostics, Bad Nauheim, Germany). Monoclonal mouse-ACTH antibodies for immobilisation, and biotinylated polyclonal goat-ACTH with a chemiluminescent avidin-complex were added to the sample. Luminescent detection of the hormone-antibody sandwich was measured using a commercial readout system (Auto-CliniLumat LB 952, Berthold, Bad Wildbad, Germany). This assay has a lower and upper detection threshold of 0.5–1550 pg/ml respectively, inter-assay variation ranged from 4.6 to 7.0% and intra-assay variation was between 3.4 and 3.8%. Serum cortisol was assessed by a commercial ELISA kit (IBL, Hamburg). The intra- and interassay variabilities were below 5 and 10%, respectively.

Subjects obtained saliva samples using Salivette sampling devices (Sarstedt, Nümbrecht, Germany). Saliva samples were stored at -20°C until assay. After thawing, saliva samples were centrifuged at 3000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. Cortisol concentrations were determined employing a commercial kit immunoassay with luminescence detection (IBL, Hamburg). This assay has a detection limit of 0.4134 nmol/l, an intra-assay variability of 4.5–7.7% and an inter-assay variability of 6.2–11.5%; to reduce error, all samples of one participant were analysed in one assay.

Heart rate was recorded by Polar Vantage NV heart rate measurement devices. Data were transmitted by a Polar-Electro interface to a personal computer and imported to the Polar Precision Performance SW program (Version 4.00.020, Polar Electro Oy 2003).

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 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 salivary cortisol concentrations in response to the psychosocial stressor (TSST), we compared differences between treatment groups and the placebo group by one-tailed tests of significance.

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 seen from a significantly blunted ACTH response (Effect of group: $F(1, 32) = 6.58$; $p = 0.008$) 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 $F(1, 30) = 2.84$; $p = 0.05$; Fig. 4), which was even more pronounced for the biologically active, free steroid fraction, as assessed in saliva (Main Effect Time $F(2, 65.1) = 4.21$; $p = 0.001$ and Main Effect Group $F(1, 33) = 5.24$; $p = 0.015$). Here, the 400 mg treatment group showed only about 20% of the salivary cortisol response when compared to placebo (Fig. 5). In both measures there was no significant interaction for group by time (serum cortisol $F(2.8, 84.3) = 0.62$, $p = 0.38$; saliva cortisol: $F(2, 65.1) = 0.574$, $p = 0.28$), indicating that treatment with 400 mg PAS dampens the serum and salivary cortisol response to the TSST to all time points.

Comparing 600 mg PAS daily with placebo, no treatment effects were found for the increase in ACTH ($F(1, 32) = 1.17$, $p = 0.29$), or the increase in serum cortisol ($F(1, 32) = 0.20$, $p = 0.66$), or the increase in salivary cortisol concentration ($F(1, 35) = 1.73$, $p = 0.20$). The same was found for subjects treated with 800 mg PAS daily. Comparisons with placebo treated subjects showed no significant treatment effects for the increase in ACTH ($F(1, 32) = 1.2$, $p = 0.28$),
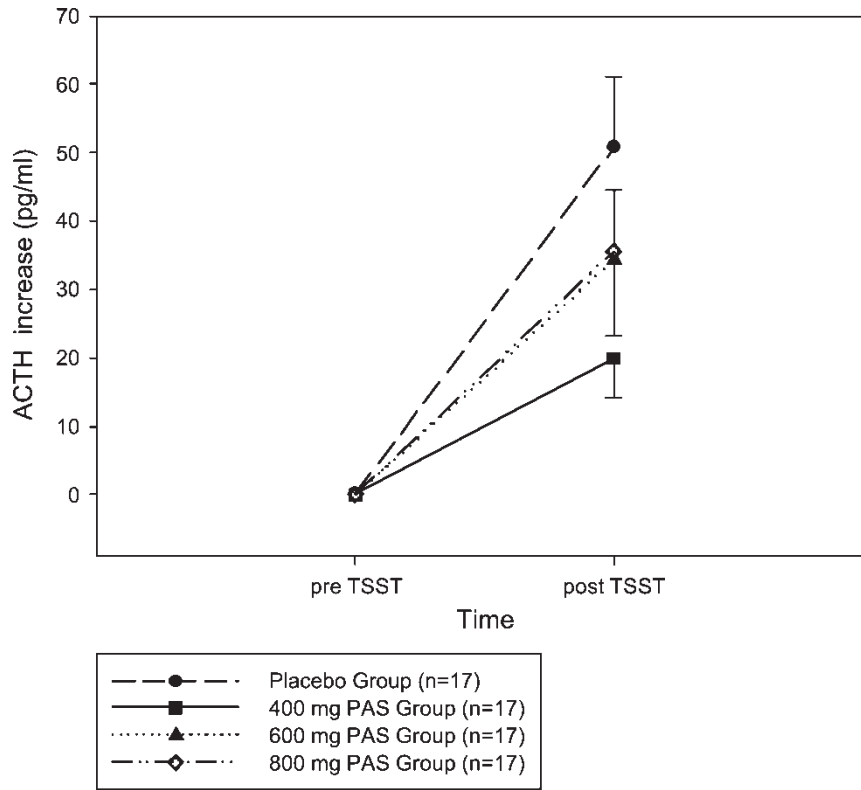
FIGURE 3   Effects of PAS on the ACTH response to the TSST. Baseline levels were 26.57 pg/ml (placebo), 21.3 pg/ml (400 mg), 21.29 pg/ml (600 mg) and 25.28 pg/ml (800 mg), respectively.
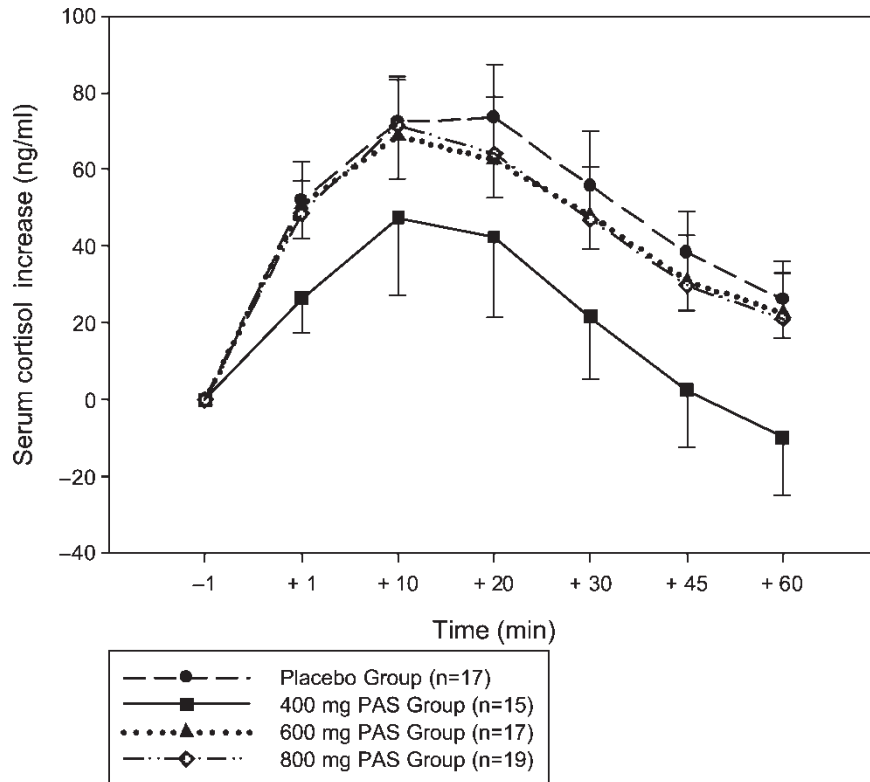

FIGURE 4   Effects of PAS on the serum cortisol response to the TSST. Baseline levels were 122.45 ng/ml (placebo), 129.54 ng/ml (400 mg), 107.13 ng/ml (600 mg) and 121.54 ng/ml (800 mg), respectively.
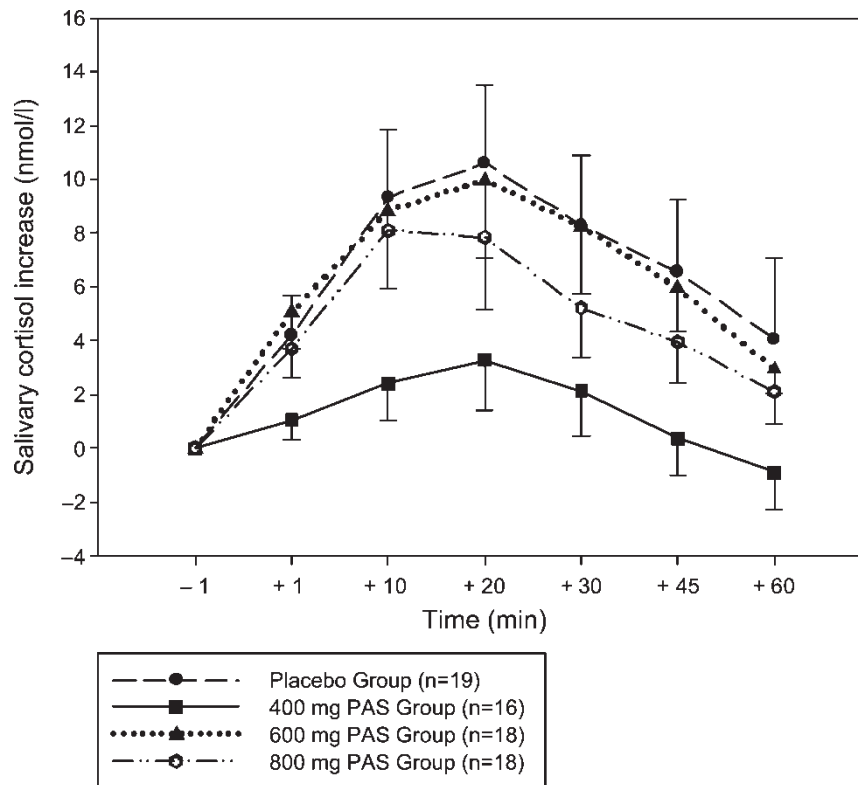
FIGURE 5   Effects of PAS on the salivary cortisol response to the TSST. Baseline levels were 11.58 nmol/l (placebo), 10.54 nmol/l (400 mg), 8.49 nmol/l (600 mg) and 12.38 nmol/l (800 mg), respectively.

or 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 STAI. 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 = 1.51/7.6% 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: $t(71) = 1.941$; $p = 0.056$).

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
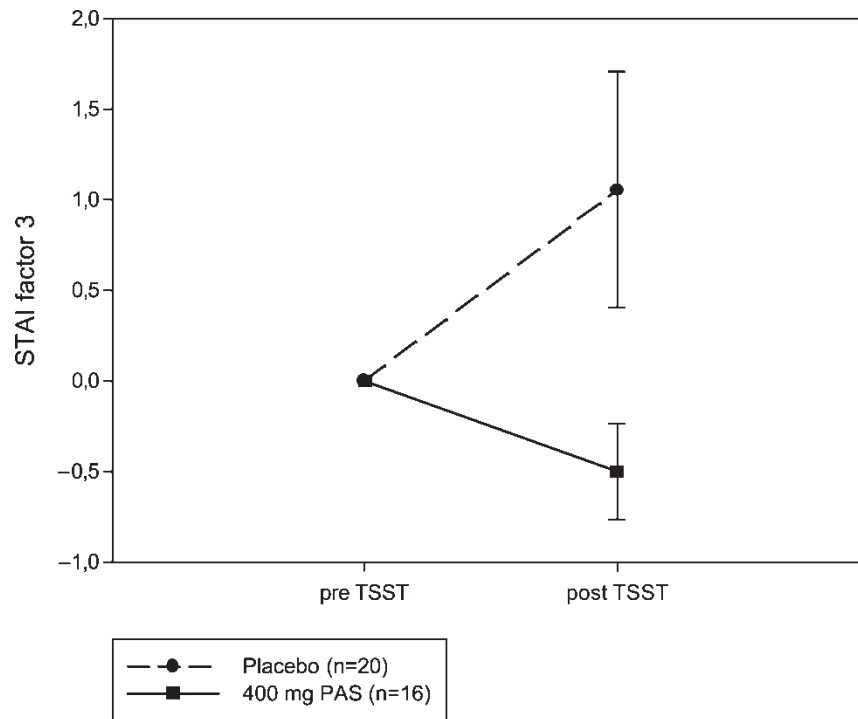
FIGURE 6 Change in STAI subscale "distress" before and after TSST. Baseline levels were 8.2 (placebo) and 8.81 (400 mg), respectively. Values are means ± s.e.m.

have been undertaken to understand the gender differences of the stress response. However, sex differences have not been explained by effects of sex steroids—they even persist in postmenopausal women (see, for review, Kudielka *et al.* (2004)). Consequently, the magnitude of the PAS effect was expected to be smaller in women, but still in proportion to the changes in men. As mentioned above, we did not expect gender effects on PAS action. Thus, group size and statistical error did not allow the necessary analyses. However, with the evidence available to date, we cannot exclude that PAS exerts stronger actions in men.

The mechanisms by which PAS affects HPA reactivity are still unknown. Interestingly, the heart rate response to stress was not dampened, questioning whether PAS has an effect at the hypothalamic level. It is important to note that PAS dampens but does not eliminate stress reactivity. Furthermore, basal cortisol levels remained unaffected. Thus, PAS does not seem to interfere with the integrity of the pituitary–adrenal axis.

Uncontrollability, unpredictability and uncertainty, resulting in strain and worry, combined with ego-involvement, are considered the key psychological elements of both distress and HPA axis activation. From this viewpoint, 400 mg PAS daily seems to exert a rather specific effect on this biobehavioral response to the TSST, as shown for a distress subscale, derived from the Spielberger State Anxiety Inventory. While the placebo group showed the expected increase in distress, the 400 mg 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 400 mg 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

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