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Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men

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Summary. The effect of chronic administration of phosphatidylserine derived from brain cortex on the neuroendocrine responses to physical stress has been examined in a placebo-controlled study in 9 healthy men.

Phosphatidylserine 800 mg/d for 10 days significantly blunted the ACTH and cortisol responses to physical exercise ($P = 0.003$ and $P = 0.03$, respectively), without affecting the rise in plasma GH and PRL.

Physical exercise significantly increased the plasma lactate concentration both after placebo and phosphatidylserine.

The results suggest that chronic oral administration of phosphatidylserine may counteract stress-induced activation of the hypothalamo-pituitary-adrenal axis in man.

Key words: Phosphatidylserine, Stress; ACTH, cortisol, lactate, growth hormone, prolactin

Recent awareness of the active participation of cell membrane structural components in the modulation of such highly complex biological phenomena as neuronal excitability, message transduction and neurotransmitter activity [Wheeler and Whittam 1970, Raese et al. 1976, Nishizuka 1984] has concentrated interest on the physiological role of phospholipids.

Phosphatidylserine is a typical representative of a large class of natural lipids, which are essential components of biological membranes, and which occur in the internal layer of the cell membrane. Exogenous phosphatidylserine affects the number and affinity of membrane receptor sites, such as central benzodiazepine receptors and muscarinic receptors [Stockert et al. 1989, De Robertis et al. 1989], and influences cell membrane transduction mechanisms, such as phosphatidylinositol turnover and specific protein kinase activation [Canonico and Scapagnini 1989].

Since information transduction systems play a fundamental role in cell-to-cell communication, they are crucial in homoeostatic adaptation to perturbing stimuli, such as stress. In consequence, phosphatidylserine, by modulating functional events in the cell membrane, may influence the response of the body to stress.

It has recently been shown in healthy male volunteers that a single i.v. injection of phosphatidylserine derived from bovine cerebral cortex partly counteracted the activation of the hypothalamo-pituitary-adrenal (HPA) axis caused by physical exercise [Monteleone et al. 1990]. This suggested that phosphatidylserine might have an acute effect on the stress-induced release of adrenocorticotrophic hormone (ACTH) and cortisol.

In the present study the effect of repeated administration of phosphatidylserine on the neuroendocrine responses to physical stress has been assessed by measuring hormonal secretion after physical exercise in healthy volunteers, who had taken phosphatidylserine or placebo orally for 10 days.

Subjects and methods

Informed written consent was obtained from nine healthy subjects. They were men aged 18–40 y (mean and SD 29.2 ± 2.2 y), who smoked fewer than 10 cigarettes per day, and who took no part in any kind of sport or regular physical activity. They had normal endocrine and metabolic functions, no family history of diabetes mellitus, a body weight within 15% of ideal, and were drug-free. Followup a double-blind, randomized, cross-over design, at two-week intervals each subject underwent three 10-day courses of treatment with placebo, phosphatidylserine 200 mg b.d., or phosphatidylserine 400 mg b.d. The drug and placebo were prepared in water-soluble, granular formulations and were free from viral contamination (FIDIA S.p.A., Abano terme, Italy). At the end of each treatment the subjects exercised on a bicycle ergometer as described below.

Experimental sessions started between 08.30 and 09.30 h, after an overnight fast. One hour after the last morning dose of drug or placebo a butterfly needle was inserted into an antecubital vein and was kept patent by a slow infusion of saline. After 10 min and 40 min blood samples were collected, and immediately after the last sample physical exercise was started on a bicycle ergometer: 6 min at 1.5 $W \cdot kg^{-1}$, 1 min rest, 6 min at 2 $W \cdot kg^{-1}$, 1 min rest, and 6 min at 2.5 $W \cdot kg^{-1}$. Further blood samples were collected at the end of each work period and 40 and 80 min after the exercise. Blood pressure and heart rate were recorded at the same times. Plasma was separated by centrifugation and was stored at −20 °C until assayed.

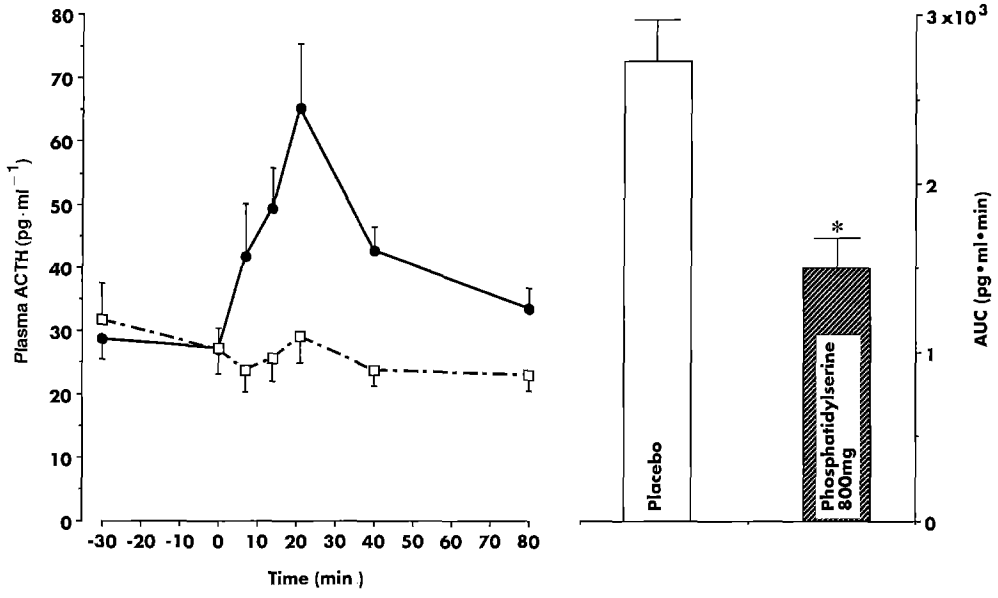

Fig. 1. Plasma ACTH response to exercise after 10 days of oral treatment with placebo (–●–) and 800 mg/d phosphatidylserine (–□–) of healthy volunteers.
* $P < 0.0007$ versus placebo

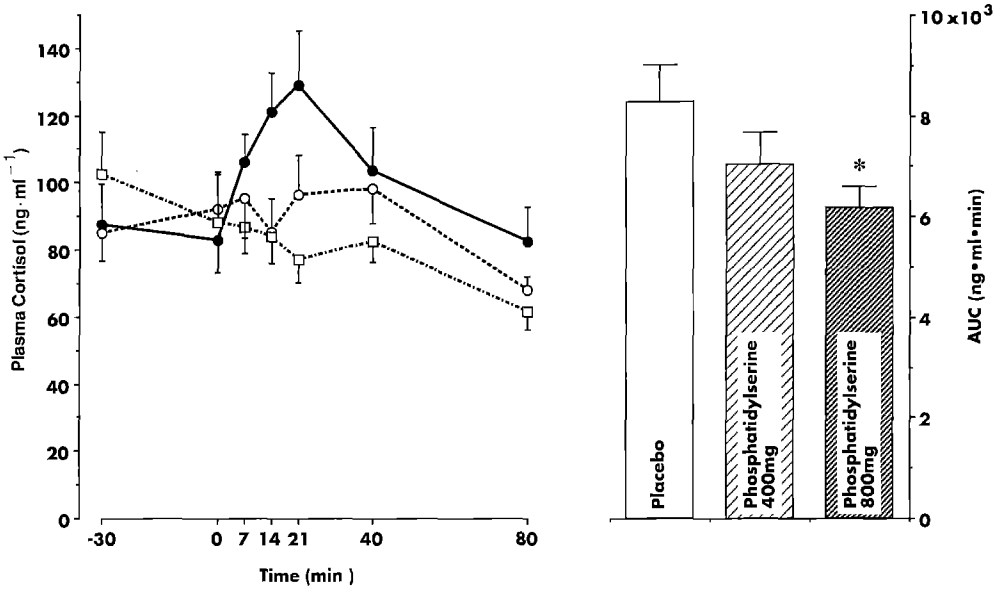

Fig. 2. Plasma cortisol response to exercise after 10 days of oral treatment with placebo (–●–) and 400 (–○–) and 800 (–□–) mg/d phosphatidylserine of healthy volunteers.
* $P < 0.009$ versus placebo

Plasma growth hormone (GH), prolactin (PRL) and cortisol were determined by radioimmunoassay, using commercial kits (Serono Diagnostics, Milan, Italy). Inter- and intra-assay coefficients of variation (CV) were 7.2% and 4.0% for GH, 5.8% and 4.2% for PRL, and 9.4% and 3.4% for cortisol. Plasma ACTH was determined by RIA, using a commercial kit (INCSTAR, Stillwater, Minn, USA). The inter- and intra-assay CVs were 6.3% and 6.0%. Plasma glucose was determined by the hexokinase method of Bondar and Mead (1974). Plasma lactate was measured by a commercial enzymatic UV method (Sigma Diagnostics, St. Louis, MO, USA).

The results are expressed as mean (SEM) and were analyzed by two-way repeated measures analysis of variance (ANOVA). When the ANOVA showed a significant difference between treatments, the areas under the curve (AUC) were calculated and were compared by Student's t-test for paired data.

Results

ACTH and cortisol

For ACTH, because of technical difficulties, data were available from only eight subjects and only after placebo and 800 mg/d phosphatidylserine. Therefore, since ACTH and cortisol secretions are strictly interrelated, in the analysis of cortisol results data were excluded from subjects for whom there were no ACTH data.

Physical exercise led to a significant increase in the plasma ACTH concentration after placebo, but not after phosphatidylserine 800 mg/d (Fig. 1). Two-way ANOVA with repeated measures showed a significant effect for both treatment ($F = 14.0$, $P = 0.002$) and time ($F = 5.7$, $P = 0.0002$) and a significant treatment X time interaction ($F = 3.9$, $P = 0.003$). The secretion of ACTH (evaluated as AUC) was significantly lower after phosphatidylserine 800 mg/d ($P < 0.0007$).

The cortisol concentration in placebo-treated subjects rose significantly after physical exercise. The increase was significantly blunted by phosphatidylserine 800 mg/d but not by 400 mg/d (Fig. 2). There was a significant effect of both treatment ($F = 6.3$, $P = 0.02$) and time ($F = 3.9$, $P = 0.003$), and a significant treatment X time interaction ($F = 2.6$, $P = 0.03$). The AUC of plasma cortisol was signi-

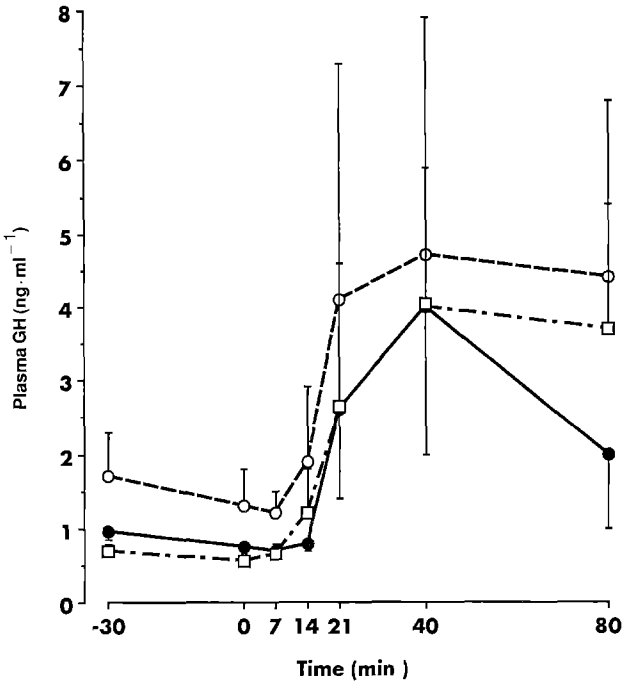

Fig. 3. Plasma GH response to exercise after 10 days of oral treatment with placebo (–●–) and 400 (–○–) and 800 (–□–) mg/d phosphatidylserine of healthy volunteers

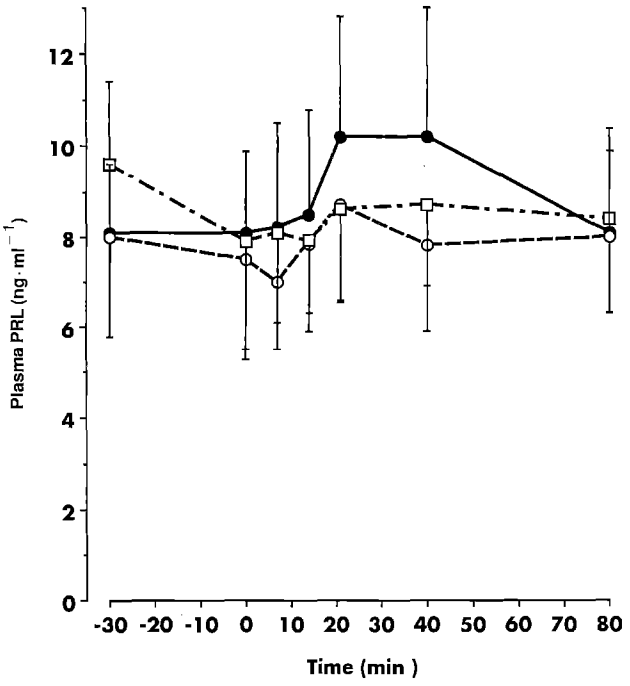

Fig. 4. Plasma PRL response to exercise after 10 days of oral treatment with placebo (–●–) and 400 (–○–) and 800 (–□–) mg/d phosphatidylserine of healthy volunteers

ficantly lower after the higher ($P < 0.009$) but not the lower dose of phosphatidylserine.

GH and PRL

Plasma GH and PRL rose significantly after exercise, and these increases were not affected by phosphatidylserine (Figs. 3 and 4). There was no significant effect of treatment nor a significant treatment X time interaction, but there was a significant effect of time ($F = 4.1$, $P = 0.001$ for GH; $F = 4.6$, $P = 0.0007$ for PRL), suggesting that the timing of the GH and PRL responses to physical exercise was not different in the three treatment groups.

Lactate and glucose

After exercise there was a significant increase in the plasma lactate concentration, with a peak at the end of exercise. This occurred after chronic administration both of 400 mg/d and 800 mg/d phosphatidylserine and after placebo, suggesting that the intensity of stress in the three groups was similar (Fig. 5).

Blood glucose concentrations did not differ significantly in the three groups.

Physiological measurements

Blood pressure and heart rate in the three groups did not differ at any time.

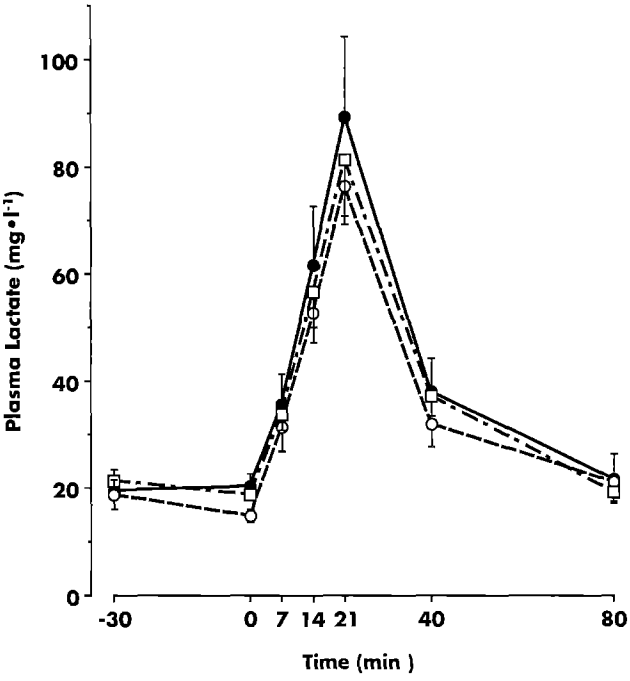

Fig. 5. Plasma lactate concentrations after 10 days of oral treatment with placebo (–●–) and 400 (–○–) and 800 (–□–) mg/d phosphatidylserine of healthy volunteers during and after physical exercise

Discussion

The physical stress used here significantly increased plasma ACTH, cortisol, GH, PRL, and lactate, but not blood glucose. Chronic treatment with phosphatidylserine

800 mg/d blunted the responses of ACTH and cortisol to physical exercise. The dose of 400 mg/d had no effect on the cortisol response.

The activation of the HPA axis after physical stress is a well-known homoeostatic response (Axelrod and Reisine 1984), which is presumably mediated by the hypothalamic corticotropin-releasing factor (CRF). Depending on the type of stress experienced, hypothalamic CRF may have either a stimulatory action or a permissive role on the ACTH and cortisol responses (Gaillard and Al-Damluji 1987).

There may be several ways by which phosphatidylserine can blunt the ACTH and cortisol response to stress.

After oral administration of radiolabelled phosphatidylserine to rats, increasing blood concentrations of the drug have been observed between 1 and 4 h with the peak at 24 h (Toffano et al., 1987). Phosphatidylserine is widely distributed in the body. It penetrates the blood-brain barrier, reaching the brain, where an affinity for the hypothalamus has been noted, and where its concentration is within the range required for pharmacological activity (Toffano et al. 1987).

Phosphatidylserine is known to influence receptor-ligand interactions by interfering with lipid microviscosity in the cell membrane. The relative viscosities of phospholipids affects the position of membrane proteins with enzymatic activity, perhaps modifying their interactions with ligands [Hirata and Haxelrod, 1980, Shinitzky 1984]. Phosphatidylserine may affect the number of receptors in the cell membrane [Stockert et al., 1989; De Robertis et al., 1989] or interfere with the activity of protein kinase C, which controls hormone-receptor transduction mechanisms [Canonico and Scapagnini 1989]. It is possible, therefore, that chronic treatment with phosphatidylserine may alter CRF receptor interactions, resulting in reduced activation of the HPA axis after stress.

Alternatively, phosphatidylserine may alter the activity of the various neurotransmitter systems involved in regulation of the HPA axis [Tuomisto and Mannisto, 1985]. In experimental animals phosphatidylserine caused a marked increase in the release of acetylcholine from the cerebral cortex [Vannucchi and Pepeu 1987] and in the stimulation of noradrenaline turnover in the hypothalamus [Toffano and Bruni 1980].

In conclusion, the present results and previous observations [Monteleone et al. 1990] suggest that both single i.v. and chronic oral doses of phosphatidylserine, may blunt the response of the HPA axis to stress in humans.

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