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ORIGINAL CONTRIBUTION

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Effects of a tryptic hydrolysate from bovine milk α_{S1}-casein on hemodynamic responses in healthy human volunteers facing successive mental and physical stress situations

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Summary *Background* Preclinical results in rats have demonstrated anxiolytic-like effects of a tryptic bovine α_{S1}-casein hydrolysate. *Aim of the study* We investigated the putative effects of this tryptic hydrolysate on systolic (SBP), diastolic (DBP) blood pressures, heart rate (HR) values and plasma cortisol concentrations (CC) in human healthy volunteers facing successive stress situations. *Methods* The subjects were (double blind) randomly allocated to ingest three times, 12 hours apart, two capsules containing either 200 mg of α_{S1}-casein hydrolysate (TS) or bovine skimmed milk powder as a placebo (CS). On the morning of the test day, a first blood sample for baseline measurement of CC was taken before the subjects were submitted to the Stroop test (ST) and, after a 30-min rest, to a Cold Pressor test (CPT). SBP, DBP, and HR were continuously recorded for 5 min before the ST and during each stress situation. A second blood sample was taken 15 min after the end of the CPT condition. *Results* ST and ST + CPT combined test situations increased SBP, DBP and HR. The significant "Treatment x SBP" and "Treatment x DBP" interactions indicated the lower percentage changes in SBP and DBP of the TS. In addition, the results showed a significant decrease of the CC in the TS but not in the CS throughout the ST + CPT combined stress tests. HR remained stable in TS between the initial rest period and the CPT unlike what happened in CS. *Conclusion* On the basis of blood pressure and cortisol changes, these results suggest an antistress profile of this α_{S1}-casein hydrolysate in human subjects.

Key words milk α_{S1}-casein hydrolysate – blood pressure – heart rate – cortisol – stress

Introduction

Numerous bioactive peptides have been identified in milk proteins and may be released after enzymatic digestion [1, 2]. Some of them exhibit for example antithrombotic [3] or opioid [4] activity. Many peptides stemming specifically from bovine α_{S1}-casein have already been identified as having biological activity. For example, α_{S1}-CN [(43–58), (57–70), (66–74)] sequences facilitate mineral absorption [5] while α_{S1}-CN [(23–27), (23–34) and (194–199)] peptides (α_{S1}-5 casokinin, α_{S1}-12 casokinin and α_{S1}-6 casokinin, respectively) display an anti-hypertensive efficacy [6].

Few observations have been made on the neuropsychopharmacological activity of milk peptides. Although newborns are often quiet after drinking milk, the link between this effect and any bioactive milk peptide remains partly unknown. However, it has been shown that this quieting effect was related not only to suckling but also to milk itself [7].

The milk α_{S1}-casein hydrolysate and the bioactive de-

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capeptide [α_{S1}-CN(f91–100)], a fragment of this hydrolysate that has been spatially modeled [8, 9], have already shown an anxiolytic-like profile in the conditioned defensive burying test and in the elevated plus-maze in rats [10], two models used to study anxiolytic agents in rodents.

The main objective of the present study was to investigate whether oral intake of this α_{S1}-casein hydrolysate by healthy human subjects would modify some physiological variables during imposed experimental stress tasks: Stroop test (ST), a mental conflict situation [11] and cold pressor test (CPT), a physical stress situation [12]. Hemodynamic parameters, including systolic (SBP), diastolic (DBP) blood pressures, and heart rate (HR), were followed as physiological indicators of the stress state, as well as plasma cortisol concentration (CC), cortisol being a steroid hormone released from the adrenal cortex in response to a stress [13].

Subjects and methods

Subjects

A total of 42 healthy male subjects participated after informed consent in the present study, approved by the Ethics Committee of the Necker-Enfants Malades Hospital of Paris, France and in accordance with the Helsinki Declaration of 1975 as revised in 1983. Enrolled subjects were free of any problem or medication known to affect the sympathetic nervous system or the activity of the hypothalamic-pituitary adrenocortical axis; they had no history of cardiovascular disease or circulatory disorders, no excessive consumption of dairy fermented food, or no allergy to milk products.

Tested products

The milk α_{S1}-casein hydrolysate (lactium™) and the placebo, consisting of bovine skimmed milk powder, were supplied by INGREDIA (Arras, France). Both were administered in exactly the same capsule form containing either 200 mg of α_{S1}-casein hydrolysate (CH) or 200 mg of placebo (P).

Experimental design and hemodynamic parameter analysis

Study design

The study was conducted in the Clinical Investigation Center (CIC) at the Necker-Enfants Malades Hospital, Paris. Each subject was randomly assigned to ingest capsules containing either CH (treated subjects or TS) or P (control subject or CS). On Day 0, each subject ingested at home two capsules of either CH or P at 8:00 am and two others at 9:00 pm. On Day 1, each subject reported to the CIC at 8:00 am and was placed for 30 min in a sound-attenuated, temperature-controlled room. An intravenous catheter was inserted into an ante-cubital vein and the subject was left recumbent for at least 30 min, before the blood sample (T0) for baseline measurement of CC was drawn. Then, the subject ingested a third dose of two 200 mg-capsules corresponding to the assigned treatment code (CH or P). The subject was then left lying quietly on his bed for two hours and only allowed to read. After this rest period, the blood pressure monitor was set and instructions regarding the Stroop test and the CPT were recalled.

SBP, DBP, and HR recordings were obtained with a noninvasive automated method, by using a finger cuff, attached to the third finger of the non-dominant hand and linked to a computerized physiological response monitoring system (Finapres 2300, Ohmeda, USA) [14] recording continuously each of the cardiovascular parameters for 5 min at each of the 3 phases of the experiment (baseline, during the ST and, after a 30 min rest, during the CPT). The continuous digitalization of the finger blood pressure signal was re-analyzed off-line using the Model flow software Beatscope 1.0 (TNO-TPD-Biomedical Instrumentation, Amsterdam, The Netherlands). The second blood sample was obtained 15 min after the end of the CPT.

Biochemical analyses

Blood was collected into prechilled tubes containing EDTA for cortisol determination. Blood samples were placed on ice and centrifuged at 2500 rpm for 15 min at 4 °C. Plasma was stored at –80 °C until analyzed. Plasma cortisol concentration was assayed in duplicate using a radio-immunometric assay method.

Experimental stress

The ST and the CPT have been used extensively as mental and physical experimental stress situations. During the ST, words appeared on a computer screen at a progressively faster pace. These words were written in a color that did not always match the word. The subjects were asked to press with the index of the dominant hand one or another key on the computer keyboard, depending on whether the word matched or not its color. The task had to be performed for 5 min and each error was signaled by an electric bell. During the CPT situation, the subjects immersed their right hand for 5 min up to the wrist, in slushy ice water (2 °C).

Statistics

Only the data from subjects who completed the entire course of each test were used for the present analysis: the number of subjects was 20 for CS and 18 for TS during the ST, and was 18 and 16, respectively, during the CPT. For the CC comparison, the number of subjects was 20 in each group.

ANOVA, for repeated measures, was used to analyze the hemodynamic changes of the two groups between the baseline and the stress situations. A paired t-test (2-tailed) was used to compare the CC and the hemodynamic variables in each group. SBP, DBP, HR and cortisol mean percentage changes of the two groups were compared using an unpaired t-test (2-tailed). Data were reported as mean ± standard error of the mean (SEM). Differences were considered to be significant when $P < 0.05$. Statistical analyses were carried out using the Statview 5 package (SAS Institute Inc., USA).

Results

Baseline characteristics did not differ between CS and TS for the hemodynamic variables: SBP: 127.1 ± 1.4 mmHg vs. 124.1 ± 2.4 mmHg, respectively: t = 1.41, NS; DBP: 69.1 ± 1.5 mmHg vs. 68.7 ± 1.5 mmHg, respectively: t = 0.18, NS and HR: 67.4 ± 1.6 bpm vs. 67.6 ± 1.7 bpm, respectively: t = 0.08, NS).

Hemodynamic variables

Stroop test

SBP and DBP significantly increased in both TS and CS groups between the baseline and the ST ($F_{(1,36)} = 259.96$; $P < 0.0001$ and ($F_{(1,36)} = 194.58$; $P < 0.0001$, respectively). However, the ANOVA of the hemodynamic variables yielded significant "Treatment x SBP" ($F_{(1,36)} = 8.12$; $P = 0.007$) and "Treatment x DBP" ($F_{(1,36)} = 4.36$; $P = 0.04$) interactions. The comparisons revealed that SBP and DBP mean percentage changes were higher in the CS than in the TS group (Table 1).

HR of both CS and TS groups was increased between the baseline and the ST ($F_{(1,36)} = 58.99$; $P < 0.0001$). However, the "Treatment x HR" interaction was not significant ($F_{(1,36)} = 0.02$; $P = 0.90$) and no significant difference was observed in the mean percentage change in HR between the two groups (Table 1).

Stroop and cold pressor combined tests

Both SBP and DBP significantly increased in the CS and TS groups between the baseline and the CPT condition ($F_{(1,36)} = 100.29$; $P < 0.0001$). The ANOVA of hemodynamic variables yielded a significant "Treatment x SBP" interaction ($F_{(1,36)} = 5.14$; $P = 0.03$) and a trend in the "Treatment x DBP" interaction ($F_{(1,36)} = 3.14$; $P = 0.08$). The comparisons revealed that SBP and DBP mean percentage changes were higher in the CS than in the TS group (Table 2).

The HR significantly increased in the CS and TS groups between the baseline and the CPT task ($F_{(1,36)} = 6.60$; $P < 0.015$). Although the "Treatment x HR" interaction was not significant ($F_{(1,36)} = 0.56$; p = 0.46), it remained stable in the TS group between the rest period before testing and the CPT task period (t = 1.14; $P = 0.27$), whereas that of the CS group significantly increased (t = 2.67; $P = 0.016$) (Table 2).

Plasma cortisol

CC showed a significant decrease in the TS group by 20.69 % (t = 3.73; $P = 0.001$), whereas it only decreased by 3.39 % in the CS group (t = 1.05; $P = 0.30$) between the first and the second blood sampling (Table 3).

Table 1 Effects of the α_{s1}-casein hydrolysate on SBP, DBP and HR of CS (n = 20) and TS (n = 18) in the Stroop test[a]

Parameter	Treatment group[b]	Baseline (before Stroop test)	Stroop test	Change (%)[c]
SBP (mmHg)	CS	135.03 ± 4.12	163.29 ± 4.80***	21.25 ± 1.78
	TS	139.27 ± 3.13	159.05 ± 2.53***	14.65 ± 1.67[2]
DBP (mmHg)	CS	69.18 ± 1.88	83.79 ± 2.44***	21.24 ± 1.84
	TS	71.89 ± 3.09	82.69 ± 2.54***	15.26 ± 1.87[1]
HR (bpm)	CS	65.9 ± 1.6	75.1 ± 2.7***	13.89 ± 2.72
	TS	66.5 ± 1.9	76.0 ± 2.4***	14.41 ± 1.96

*** p < 0.001: paired t-test: baseline mean values vs. Stroop stress mean values

[a] Values are mean ± SEM

[b] *CS* control subjects; *TS* treated subjects

[c] Change is expressed as the percentage of variation of SBP, DBP and HR values between the Stroop test and baseline; [1] p < 0.05; [2] p < 0.01: unpaired t-test (2-tailed): TS vs. CS

Table 2 Effects of the α_{S1}-casein hydrolysate on SBP, DBP and HR of CS (n = 18) and TS (n = 16) in the cold pressor test[a]

Parameter	Treatment group[b]	Baseline (before Stroop test)	CPT test	Change (%)[c]
SBP (mmHg)	CS	134.72 ± 4.45	168.57 ± 5.37***	25.93 ± 3.14
	TS	140.66 ± 3.36	162.01 ± 3.86***	15.66 ± 2.79[1]
DBP (mmHg)	CS	69.33 ± 2.09	90.17 ± 2.80***	21.24 ± 1.84
	TS	72.52 ± 2.37	86.57 ± 3.48***	15.26 ± 1.87[2]
HR (bpm)	CS	65.4 ± 1.7	69.4 ± 2.6*	6.01 ± 2.20
	TS	68.1 ± 1.6	70.3 ± 1.9	3.80 ± 3.14

* $p < 0.05$; *** $p < 0.001$: paired t-test: baseline mean values vs. CPT stress mean values
[a] Values are mean ± SEM
[b] *CS* control subjects; *TS* treated subjects
[c] Change is expressed as the percentage of variation of SBP, DBP and HR values between the CPT test and baseline; [1] $p < 0.05$; [2] $p < 0.10$: unpaired t-test (2-tailed): TS vs. CS

Table 3 Effects of the α_{S1}-casein hydrolysate on cortisol plasma concentration of CS (n = 20) and TS subjects (n = 20)[a]

Parameter	Treatment group[b]	Baseline (before Stroop test)	After CPT test	Change (%)[c]
Cortisol (ng/dl)	CS	17.23 ± 0.94	16.05 ± 1.04	−3.39 ± 7.32
	TS	20.01 ± 0.91	15.34 ± 1.01***	−20.69 ± 6.35[1]

*** $p < 0.001$: paired t-test: baseline mean values vs. after CPT stress mean values
[a] Values are mean ± SEM
[b] *CS* control subjects; *TS* treated subjects
[c] Change is expressed as the percentage of variation of cortisol concentration values between the end of the CPT test and baseline; [1] $p < 0.09$: unpaired t-test (2-tailed): TS vs. CS

Discussion

The present study aimed to assess the effects of an orally taken α_{S1}-casein hydrolysate containing the α_{s1}-CN-(f91–100) on reliable biological indicators of stress in healthy human volunteers. One major finding of this study was that the percent increase in both SBP and DBP were significantly higher in CS than in TS during the ST, in spite of similar baseline values in the two groups. In the same condition, no significant difference between groups was observed for the percent increase in HR. On the basis of previous findings [15, 16], the presently observed pressure response may be caused by increased cardiac output.

Moreover, even if the CPT elicited significant increases of SBP and DBP in both groups, the SBP increased less and the DBP tended to increase less in TS than in CS. These results are in agreement with other reports of hemodynamic effects of this physical stress paradigm [17] and could indicate that the CPT was more powerful as a diastolic pressure stimulus [18].

In contrast to what happened during the ST, the HR of TS remained stable between baseline and testing, whereas that of CS was increased, possibly due to the lasting effects of the Stroop conflict condition. Indeed, the cold pressor test essentially induces a vascular response with an increased total peripheral resistance, but without changing HR, stroke volume, and cardiac output [15–17].

In agreement with this hypothesis is the increased CC throughout the experiment, a well-established method of estimating the influence of different stressors [19]. The cortisol response after the cold pressor test may have been obscured by the decline in CC that occurs naturally in the morning as part of the normal cortisol diurnal rhythm [20]. However, under our conditions, the mean CC value obtained at the end of the CPT was not diminished in CS, indicating no efficient coping with the successive stressful situations. At the same time, the plasma cortisol concentration of the TS significantly decreased, despite the anxiogenic and painful situation, following the normal diurnal rhythm.

It is generally considered that, facing experimental stress situations, the subjects who exhibit lower cardiovascular responses are less anxious than those displaying strong responses. α-casozepine, the α_{s1}-CN(f91–100) fragment present in the hydrolysate, as well as its original hydrolysate, have been demonstrated to exhibit anxiolytic-like effects in the elevated plus-maze and in the conditioned defensive burying paradigm in rats [10]. It could therefore be considered that the anxiolytic-like effects of the α-casein hydrolysate were due to this active decapeptide.

This study provides preliminary evidence that the bovine milk α_{S1}-casein hydrolysate displays an anti-stress activity in human subjects. It will be now of great interest to investigate the effects of this product in other experimental conditions, for example after long-term ingestion at low doses. Therefore, further preclinical and

clinical behavioral studies are needed to understand the still unknown mechanism of action of this α_{S1}-casein hydrolysate and whether this product may be used as a functional ingredient and as a way to cope better with the stress of every day events with a possibility of minimal side effects.

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