Journal of Pre-Clinical and Clinical Research, 2011, Vol 5, No 1, 16-21
www.jpccr.eu

ORIGINAL ARTICLE

Whey administration modulates spatial memory in a water maze

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Abstract

Whey protein concentrate is an effective and safe cysteine donor for the cellular antioxidant glutathione (GSH). Depletion of this tripeptide in the brain may decrease with age and result in free radical-induced neuronal damage. The effect of supplementation with a whey-based cysteine donor (HMS90) was analysed in Morris water task performance in rats. Five-month- old Wistar rats received HMS90 orally at doses of 150 (HMS 150) and 300 mg/kg (HMS 300) from day 1 to day 21 of the experiment. A water maze paradigm was carried out for 4 days by training the rats using four training trials per day. In addition to acquisition latency, memory was assessed by a probe trial given 24 h after the last training trial. ANOVA for repeated measurements did not show any significant differences in acquisition in the water maze between the groups. However, in the probe trial on day 5, the HMS 150 group showed improved memory of the position of the platform, compared to the control and HMS 300 groups. Follow up data on brain content of monoamines and metabolites in the prefrontal cortex, hippocampus and striatum revealed significant differences between the groups. These findings suggest that improvement of cognitive performance in rats treated with HMS90 is associated with neuroregulation in the central nervous system. We have demonstrated that HMS 90 diet results in increased noradrenaline and dopamine concentration in the prefrontal cortex, and that some of the beneficial effects of bovine whey proteins intake are related with dopamine and serotonin metabolism.

Keywords

HMS90, whey, glutathione, spatial memory

INTRODUCTION

Whey, a major co-product of the cheese and casein industries, contains a multitude of biologically-active proteins and peptides, but for many years it has been viewed as a low-value product. A greater understanding of the characteristics of whey proteins and their interactions with other food components, together with the development of technology for whey processing, have expanded the new relevance base for whey protein ingredients. Whey is a rich source of essential amino acids, sulphur amino acids (methionine, cysteine), and in the branched chain – leucine, isoleucine and valine, with a great nutritional value. Today, whey proteins have been implicated in a variety of nutritional and physiological effects, including; antimicrobial actions, immune modulation, prevention and management cardiovascular disease, cancer, osteoporosis, muscular atrophy, body ageing, and infant nutrition [1].

HMS90 (humanised milk serum, 90 % protein) is a bovine whey concentrate rich in serum albumin, α-lactalbumin and lactoferin, which assists the body in maintaining optimal concentration of glutathione (GSH) by supplying the precursors required for its intracellular synthesis. The biological activities of GSH are partially associated with the thiol group of cysteine which takes part in redox state modulation. It has also been reported that in the central nervous system glutathione plays an important role in proteins and nucleic acids biosynthesis, stabilization of cell membranes and enzyme activity, modifies activity of ionotropic receptors, modulates levels of neurotransmitters and transport of amino acids [1-7]. There is emerging data demonstrating that free radicals scavengers such as glutathione can protect against excitatory amino acid neurotoxicity *in vitro* and *in vivo* [8-11].

Several studies have focused on the cognitive effects of bioactive milk proteins and their physiological roles in the peripheral and central nervous systems [12-14]. Supplementation with whey-derived protein – α-lactalbumin with relatively high content of tryptophan (serotonin precursor) – produces cognitive changes consistent with facilitation of sertoninergic neurotransmission [15,16]. Growing evidence suggests that whey proteins and their components ameliorate cognition and display an anxiolytic-like activity [17]. Some evidence indicates that this effect is a consequence of serotonin (5-HT) synthesis enhancement and release [18].

The present study was conducted to evaluate the effect of supplementation with a bovine whey-based cysteine donor (HMS90) on neurotransmission and spatial memory in rats. From the point of view of potential health-promoting properties, whey could be used as a valuable component in functional foods.

MATERIALS AND METHODS

Subjects. The effect of supplementation with a whey-based cysteine donor (HMS90) was analysed in a modified Morris water maze task in 5-month-old male Wistar Charles

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Received: 15 April 2011; accepted: 20 June 2011

River rats, weighing between 320-350 g, receiving orally for 3 weeks HMS90 at two doses of 150 and 300 mg/kg b.w./day. The animals were housed in a temperature-controlled room (maintained at 22–26°C), operating on a 12 h light/dark cycle, with food and tap water or solution HMS90 continuously available. Animals were given access to standard lab chow (Labofeed, Kcynia, Poland).

All experimental procedures involving the use of animals were carried out according to the Ethical Committee for Animal Experiments at the Medical University of Warsaw, which are in compliance with the ethical standards of the European Communities Council Directive of 24 November 1986 (86/609/EEC).

HMS90 treatment. Rats received bovine whey concentrate in drinking water for 3 weeks and during behavioural experiments. The solution of HMS90 (Immunotec Research Ltd., Montreal, Canada) in tap water were prepared fresh daily. The HMS90 was added directly to the drinking water and mixed carefully. Each rat drank about 30 mL of solution every day. This amount had been earlier estimated in pilot trials. Animals were divided into 3 groups and treated as follows:
1) drinking water (Control, n = 9);
2) HMS90 at dose 150 mg/kg b.w. (HMS 150, n = 10);
3) HMS90 at dose 300 mg/kg b.w. (HMS 300, n = 10).

Behavioural test. ***Water maze.*** The effect of supplementation with a whey-based cysteine donor (HMS90) on the Morris water task performance was analysed in this study by training rats using a 4 training trials per day procedure. In addition to acquisition latency, learning was assessed by a probe trial 24 h after last training trial. The experiment was performed during the light phase of the cycle (between 08.00-15.00).

Water escape task. Modified from the standard version of the Morris water maze [19]. The Morris water maze task was performed in a circular pool (140 cm diameter) divided into 4 quadrants (Northeast-NE, Northwest-NW, Southeast-SE and Southwest-SW), and filled with 30 cm of water at a temperature of 23°C.

The rats were trained to locate a transparent hidden plexiglas platform (10 cm × 10 cm, 29 cm high) submerged 1 cm beneath the surface of the water. The maze was located in a laboratory and surrounded by several prominent cues to spatial coordinates. All rats were given one session of 4 trials daily for 4 consecutive days. The location of the escape platform in the SE quadrant remained fixed throughout the 4 days of acquisition training. Escape latency (time to reach the platform) was automatically recorded.

On each experimental day, a trial was initiated by placing each rat in the water facing the side wall of the pool in one of the 4 equally-spaced starting positions, excluding the quadrant with the platform, and allowing the rat to swim freely to the escape platform. The order in which these starting points were used was determined randomly for each trial, and changed each day. A trial was complete when the rat reached and entered the platform, or after 60 sec elapsed. If the animal did not find the platform within this time, it was gently guided to the platform by the experimenter and allowed to remain on it for 15 sec before the next trial was initiated. Each animal was trained 4 times per day for 5 consecutive days.

The probe trial (memory test) was performed the day after the last training session (5th day) after removing the platform from the pool to measure the spatial bias of the rats. The time the rat spent in the 4 quadrants and annuli was measured for 60 sec. All rats started from the same start position, opposite the quadrant where the escape platform had been positioned during acquisition.

Data from the water maze was monitored *via* a camera mounted overhead, which relayed information, including escape latency to find the platform, distance travelled and time in quadrants to a video tracking system. The swimming activity of each rat was then recorded and analysed by a computerised video-tracking system (Chromotrack, San Diego Instruments).

Biochemistry. Regional brain concentrations of monoamines and metabolites were estimated in selected brain regions after completion of the behavioural tests.

Rats were decapitated 24 h after the last behavioural experiment, their brains removed and dissected into 3 regions (prefrontal cortex, hippocampus and striatum) on an iced plate [20].

To determine the level of neurotransmitters, the brains were freshly dissected, weighed, and quickly frozen (-80°C) for future examination. After deep-frozen storage, the tissues were homogenised in ice-cold 0.1 N $HClO_4$ and centrifuged at 13,000 g for 15 min. The supernatant was removed, filtered using a 0.2 μm pore size filter (Whatman, USA), and examined for neurotransmitter content. Tissue levels of Dopamine (DA; standard substance supplied by RBI), its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC; RBI, USA); 5-hydroxytryptamine (5-HT; Sigma, Germany); 5-hydroxyindolacetic acid (5-HIAA; Sigma, Germany); 3,4-dihydroxyphenylethanolamine (NA; Sigma, Germany) and homovanilic acid (HVA; Sigma, Germany) – were measured using high performance liquid chromatography with electrochemical detection (L-3500A detector, Merck, Germany) and a glassy carbon electrode. Neurotransmitters were detected at a potential of 0.8 V *vs.* a Ag/AgCl reference electrode. The mobile phase contained 58 mM sodium phosphate (Sigma, Germany), 31 mM citric acid (Sigma, Germany), 1 mM octane sulfonic acid (Sigma-Aldrich, USA), and 27 μM ethylenediaminetetraacetic acid (EDTA, Sigma, Germany) in deionised, 18.3 mΩ purified water containing 1 % acetonitrile (Merck, Germany) and 12 % methanol (Merck, Germany). Monoamines were separated on a C-18 column (250 mm × 4 mm reverse phase, Nucleosil, 5 μm particle size; (Macherey-Nagel, Germany), and the mobile phase flow rate was maintained at 0.8 mL/min.

Sample quantification was achieved by comparison with standard solutions of a known concentration using HPLC software, and the area under the peaks was quantified. The amount of each monoamine was determined with peak-area ratios using HPLC chromatogram analysis software Eurochrom 2000 for Windows (Knauer, Germany). Metabolic turnover was calculated as the ratio of metabolite to its specific monoamine to estimate the activity of metabolic pathway. Contents of neurotransmitters and metabolites were expressed as ng/g of fresh tissue. Comparison between neurotransmitters and metabolites of the groups was accomplished by one-way analysis of variance, followed by Student's t-test.

Statistical analysis. Significant differences between treatments during acquisition learning were determined by repeated measures analysis of variance ANOVA (treatment x day x trial). Values of all variables are presented as means ± SEM. All *post hoc* tests were performed using Student's t-test to identify any significant differences (Statistica version 9, StatSoft®).

Correlation coefficients between learning performance and the level of monoamines, their metabolites and neurotransmiters turnover in the selected brain regions, were determined using simple linear regression analysis according to Pearson's r correlation. All hypotheses tested used a minimum accepted level of statistical significance of 0.05.

RESULTS

Water maze results. *Aquisition trials (days 1-4).* The results of the acquisition in the water maze test (mean escape latency) are presented in Figure 1. In the acquisition days 1-4 the performance of all rats showed a reduction of latency with subsequent days of testing; however, no significant differences were noted between experimental groups (Con: 21.125 ± 2.3 sec; HMS 150: 25.20 ± 1.76 sec; HMS 300: 22.517 ± 2.2 sec) ($F_{(2,26)} = 1.113$, $p = 0.3405$).

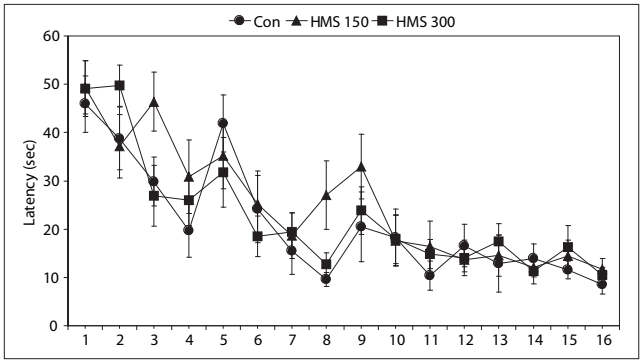

Figure 1. Mean escape latency (sec) during acquisition of the spatial navigation task for the control and rats that received bovine whey concentrate (HMS90). Data are shown as mean ± SEM. All rats showed reduction of latency with subsequent days of testing, however no significant differences were noted between experimental groups

The result did not show a significant main effect for swimming speed (Con: 0.235 ± 0.01 m/sec; HMS 150: 0.249 ± 0.01 m/sec; HMS 300: 0.248 ± 0.02 m/sec) ($F_{(2,26)} = 1.274$, $p = 0.2938$).

The probe trial (day 5). As shown in Figure 2, in the memory task on day 5, the group treated with HMS90 at a dose of 150 mg/kg b.w./day showed improvement in crossings for the position of the platform in the SE quadrant (4.6 ± 0.45 sec), compared to the control group (2.4 ± 0.53 sec) and animals pretreated with 300 mg HMS90 (3.3 ± 0.66 sec) ($F_{(2,26)} = 3.719$, $p = 0.0376$).

Rats treated with a low dose of HMS90 swam preferentially in the target SE quadrant where the platform had been placed previously (Figure 3). The *post-hoc* tests show that the HMS 150 group spent statistically more time in target quadrant SE, compared to the other experimental groups (Con: 18.81 ± 1.50 sec; HMS 150: 25.01 ± 1.89 sec; HMS 300: 22.30 ± 2.00 sec) ($F_{(2,26)} = 2.805$, $p = 0.0781$). Interestingly, rats from

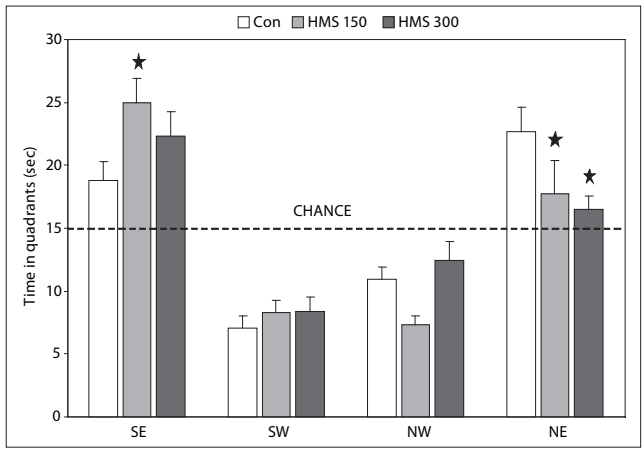

Figure 2. Spatial probe data from the quadrants area crossings of the control and HMS90 treated rats in the water maze task on day 5 (Trial 17). The test was run in the same manner as the acquisition trials, except that the target was absent and the trial was terminated after 60 sec. *p<0.05 HMS vs control

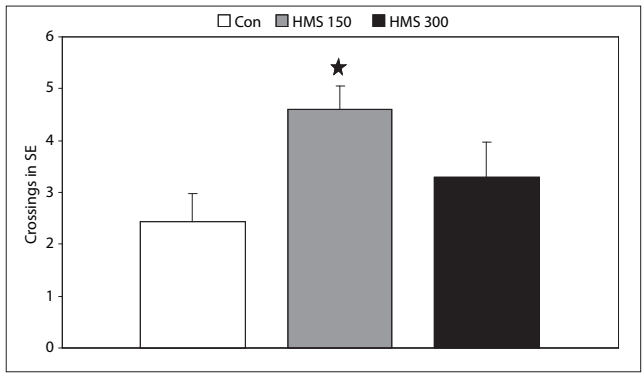

Figure 3. Spatial probe data from the platform area crossings of the control and HMS90 treated rats in the water maze task on day 5 (Trial 17). The test was run in the same manner as the acquisition trials, except that the target was absent and the trial was terminated after 60 sec. The measure given is platform crossings ± SEM.: the number of times the rat passed through a nominal area defining the oryginally correct platform position. *p<0.05 HMS vs control

the control group spent more time in the NE quadrant, compared to animals treated with bovine whey concentrate (Con: 22.653 ± 1.944 sec; HMS 150: 17.749 ± 1.262 sec; HMS 300: 16.503 ± 1.100 sec).

Regional brain monoamines levels. The levels of monoamines and their metabolites in the prefrontal cortex, hippocampus and striatum are summarised in Tables 1 and 2. ANOVA demonstrated significant differences in the content of monoamines and metabolites in the prefrontal cortex and striatum of rats between the HMS90 treatment groups, compared to control.

Noradrenaline (NA). ANOVA demonstrated statistically significant differences between the content of NA in the prefrontal cortex ($F_{(2,26)} = 17.436$, $p < 0.005$). *Post-hoc* comparisons showed a significant increase in the level of NA in the group pre-treated with the HMS90, compared to control ($p < 0.05$) (Table 1).

Dopamine (DA). HMS90 treatment significantly elevated DA levels in the prefrontal cortex ($F_{(2,26)} = 3.123$, $p < 0.05$) (Table 1).

3,4-dihydroxyphenylacetic acid (DOPAC). ANOVA showed that the content of DOPAC in the striatum was significantly higher in rats treated with 300 mg HMS90 *vs.* the control group ($F_{(2,26)} = 4.018$, $p = 0.0299$) (Table 2).

Table 1. Effects of HMS90 on brain tissue noradrenaline (NA), dopamine (DA) and serotonin (5-HT) levels in the prefrontal cortex, hippocampus and striatum

Brain region	Group	Monoamine levels in ng/g wet tissue		
		NA	DA	5-HT
Prefrontal cortex	Control	171.33 ± 29.19	976.44 ± 150.72	200.22 ± 29.91
	HMS 150	283.00 ± 18.74*	1248.60 ± 38.49*	264.10 ± 18.43
	HMS 300	346.60 ± 13.93••	1269.70 ± 58.32•	266.80 ± 25.89
Hippocampus	Control	491.56 ± 36.35	1191.67 ± 48.11	211.44 ± 41.12
	HMS 150	429.30 ± 41.72	1069.00 ± 73.16	142.80 ± 23.13
	HMS 300	405.80 ± 48.88	1175.70 ± 92.26	198.40 ± 23.24
Striatum	Control	153.33 ± 34.21	8173.44 ± 469.16	262.33 ± 26.24
	HMS 150	171.80 ± 41.73	9284.70 ± 485.16	276.70 ± 18.30
	HMS 300	122.80 ± 22.65	9014.70 ± 174.50	244.70 ± 16.60

Data are presented as mean ± SEM levels (ng/g wet tissue). *p<0.05 HMS 150 *vs* control, •p<0.05 HMS 150 *vs* HMS 300, •p<0.05 HMS 300 *vs* control.

Table 2. Effects of HMS90 on brain tissue metabolite levels: 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA) in the prefrontal cortex, hippocampus and striatum

Brain region	GroupMetabolite levels in ng/g wet tissue			
		DOPAC	HVA	5-HIAA
Prefrontal cortex	Control	-	-	570.11 ± 155.18
	HMS 150	-	-	674.60 ± 167.87
	HMS 300	-	-	1607.90 ± 228.11••
Hippocampus	Control	2513.00 ± 323.38	-	677.44 ± 47.84
	HMS 150	2313.40 ± 403.12	-	584.30 ± 117.14
	HMS 300	2722.90 ± 288.36	-	602.10 ± 65.00
Striatum	Control	1108.89 ± 111.26	502.00 ± 38.41	610.78 ± 29.74
	HMS 150	1470.40 ± 136.75	606.50 ± 48.80	666.50 ± 24.02
	HMS 300	1685.10 ± 169.69•	607.80 ± 38.86	667.10 ± 34.86

Data are presented as mean ± SEM levels (ng/g wet tissue). •p<0.05 HMS 150 *vs* HMS 300, •p<0.05 HMS 300 *vs* control.

DOPAC/DA ratio. There were no differences in the ANOVA analysis in dopamine turnover (DOPAC/DA) in any brain region ($F_{(2,26)} = 2.29$; $p = 0.124$).

Serotonin (5-HT). No statistical differences ware found in serotonin level in the selected brain regions, but in groups treated with HMS90 we observed higher concentrations of 5-HT in the prefrontal cortex (non-significant tendency) (Table 1).

5-hydroxyindolacetic acid (5-HIAA). Overall ANOVA showed significant differences between groups in 5-HIAA content in the prefrontal cortex ($F_{(2,26)} = 9.274$, $p = 0.0009$). High concentration of 5-HIAA was observed in the HMS 300 group ($p < 0.05$) (Table 2).

5-HIAA/5-HT ratio. There were no differences in the ANOVA analysis in 5-hydroxy-tryptamine turnover (5-HIAA/5-HT) in any brain region ($F_{(2,26)} = 3.028$, $p = 0.149$).

Monoamine levels and spatial memory correlation. The number of crossings over the previous position of the platform during the probe trial was compared with the levels of monoamines and their metabolites in the prefrontal cortex, hippocampus and striatum.

It has been shown that 5-HT concentration in the cortex of the HMS 150 group correlated with results of the probe trial ($r = 0.85$; $p < 0.01$). Hippocampal DA levels correlated positively with the mean annulus crossing of the HMS150 group ($r = 0.64$; $p < 0.05$) during the probe trial.

The accuracy of spatial memory was not reliably correlated with any monoamine, metabolites level, or neurotransmitters turnover in the hypothalamus and the striatum ($p > 0{\cdot}05$).

DISCUSSION

The aim of the present study was to investigate the influence of dietary whey proteins on memory and learning in rats. Many animal studies have examined the effect of whey and its components on neurotransmission, cognitive functions and behaviour.

Bovine milk is rich in protein with biological activity as caseins α-lactalbumin and β-lactoglobulin. Extensive research has presented the biological activity of each of these proteins [21-23]. The most abundant protein in milk – β-lactoglobulin, is a member of the lipocalin family. β-lactotensin derived from β-lactoglobulin is a natural ligand for neurotensin – 2 (NT2) receptors and has an anti-stress effect. This peptide also reduce sensitivity to pain, consolidate and promote the abolition of fear memory [24-26]. Major whey proteins components also show opioid and angiotensin I-converting inhibitory activity [27].

In this study, we have confirmed previous reports from animal model studies that the whey fraction of bovine milk can modulate neurotransmission and enhance spatial memory. However, in our experiment, no significant differences were observed in learning between groups, except in the memory task on day 5. The group treated with the bovine whey concentrate at a dose of 150 mg/kg b.w./day showed improvement in crossings for the position of the platform in the SE quadrant, compared to the control group and animals pre-treated with higher dose of HMS90 ($F_{(2,26)} = 3.719$, $p = 0.0376$). Rats treated with a low dose of HMS90 swam preferentially in the target SE quadrant, where the platform had previously been placed (Fig. 3.). The results of our study indicate that bovine whey concentrate exhibits non-linear biphasic activity, depending on the dose, and this effect could be described as an example of hormesis. An unexpected observation was the high frequency of rats from the control group in the NE quadrant (opposite the SE quadrant), compared to animals treated with bovine whey concentrate.

Similarly, dose-response biphasic effect on cognitive functioning was demonstrated for polypeptides isolated from early milk. The findings of Popik *et al* [12] indicate that colostrinin, a complex of polypeptides derived from colostrum of sheep, facilitates spatial learning and incidental memory in rats [12].

Whey protein contains high levels of cysteine, which is considered to be a rate limiting substrate for the glutathione synthesis. As a donor of cysteine, whey proteins provide optimal level of glutathione, which protect neurons from the toxicity of free radicals. There is some evidence that whey protein ingestion under acute and chronic conditions of oxidative stress improves GSH status [28-30]. GSH – an endogenous antioxidant molecule and redox modulator – plays multiple roles in the central nervous system. As a free radical scavenger and detoxifying agent, glutathione protect neurons against oxidative and free radical damage. In the brain, GSH plays a critical role as an enzyme co-factor, a cysteine storage form, a major redox buffer, and a neuromodulator.

In this study, we discovered that bovine whey concentrate significantly modified neurotransmitters concentration in the brain regions related to spatial memory. Major changes were observed in the prefrontal cortex – a structure which has been implicated in a variety of cognitive and executive processes, including working memory. The prefrontal cortex contributes to executive functioning, controlling cognitive processes necessary for optimal scheduling of complex sequences of behaviour (monitoring, planning and decision making), and plays a role in contingency perception [31].

Research in animals indicates that the prefrontal cortex is very sensitive to its neurochemical environment, especially to changes in catecholamine status [32,33]. Accumulating evidence from research has revealed that the catecholamines, such as DA and NA, play a critical role in the modulation of the spatial memory functions of the prefrontal cortex. A study by Rossetti and Carboni (2005) has demonstrated that elevations of catecholamines (NA and DA) in the prefrontal cortex is selectively increased during spatial working memory tasks [34]. This study supports the hypothesis that in the working memory task, DA is primarily associated with reward expectancy, whereas NA is necessary for actively maintaining the rules to achieve the goal [34, 35].

In our study, rats fed a whey protein diet exhibited a higher concentration of noradrenaline in the prefrontal cortex. In addition, a reduced content of DA in the prefrontal cortex of the control group *vs* groups treated with HMS90 was observed; however, there were no differences in dopamine turnover in any brain regions. Noradrenaline as a neurotransmitter and stress hormone is crucial for the regulation of various fundamental brain functions, especially attention, consolidation and retrieval of some types of memory. In the mammalian brain, NA is thought to act on neuronal/synaptic plasticity and plays an important role in the late phase of long-term memory consolidation [36]. Behavioural modulations by NA are closely related to anxiety, and cognitive impairment observed during chronic stress are a consequence of the down-regulation of NA transmission [37]. The literature highlights that the cognitive function of the prefrontal cortex is enhanced by moderate levels of NA engaging post synaptic α-2 adrenergic receptors, while high levels of NA released during stress impair cortical functions *via* α-1 and β-1 adrenergic receptors [38]. There is a growing body of evidence from behavioural studies suggesting that increased levels of this neuromodulator lead to better memory performance [39].

Fitzgerald (2010) suggests that serotonin plays a general role in activating the prefrontal cortex, whereas noradrenaline deactivate this brains region. These 2 neurotransmitter systems modulate the functional properties of the prefrontal cortex, and may have opposing effects on behaviour [40].

Our findings are in agreement with other studies where in rats α-lactalbumin ingestion enhanced serotonin release and induced anxiolytic effect [15]. investigations in humans have demonstrated that whey proteins improve cognitive function, especially in highly stressed individuals (13,14,41]. Decline in cognitive performance under chronic stress exposure may be mediated by reduced serotonin function in the brain. A rise in serotonin levels is thought to improve adaptation to stressful conditions. A diet rich in α-lactalbumin improves cognitive functions via increased serotonin precursor – tryptophan – an essential amino acid that comes from protein sources on diet [14].

The improved spatial memory and better navigation in rats treated with HMS90 may be the result of enhanced serotonin neurotransmission. In our experiment, we observed a non-significant tendency for an increase in the serotonin level in the prefrontal cortex. 5-HT levels in this tissue correlated positively with crossings over the target space during the probe trial. In addition, our data indicate that in rats fed a dietary whey protein concentrate exhibited also an elevated content of 5-HIAA – a serotonin metabolite in the prefrontal cortex. It has recently been shown that serotonin manipulations have reliable effects on cognition, emotional functioning and mood [18]. A study by Markus *et al* (2000) demonstrates that α-lactalbumin increased serotonin production in the brain, improved mood, and decreased cortisol levels [13]. Other authors found that ingestion of α-lactalbumin-enriched diets can enhance serotonin release, induce anxiolytic-like effects, and preserves lipid oxidation [15,42]. The intake of α-lactalbumin by human volunteers increased availability of plasma tryptophan, and improved morning alertness and attention [1]. In healthy elderly volunteers, whey protein supplementation improved cognition and reduced the rate of forgetting on the paragraph recall at 15 min., independently of elevations in blood glucose [43]. Unfortunately, whey – just as other milk products – has an allergenic potential. Despite of all the advantages and health benefits, many animal studies confirm immune and allergic responses to milk proteins [44].

In conclusion, the experimental results in this study indicate that administration of HMS90 in rats affects the neurotransmitter balance in the brain. The presented data show that ingestion of whey proteins has a beneficial effect on spatial memory, but the mechanisms of neuromodulation remain to be determined.

ACKNOWLEDGEMENT

The authors declare that they have no competing interests. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All authors approved the final manuscript.

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