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Colostrinin, a Polypeptide Isolated From Early Milk, Facilitates Learning and Memory in Rats

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POPIK, P., B. BOBULA, M. JANUSZ, J. LISOWSKI AND J. VETULANI. *Colostrinin, a polypeptide isolated from early milk, facilitates learning and memory in rats.* PHARMACOL BIOCHEM BEHAV **64**(1) 183–189, 1999.—Initial observations in humans indicated that colostrinin, a complex of polypeptides derived from the colostrum of sheep, facilitates cognitive functioning in patients with Alzheimer's disease. Its effect on learning and memory in more controlled settings as well as the specificity of these effects were, however, unknown. The present experiments evaluated the effects of colostrinin on spatial learning (Morris water maze) and incidental memory (habituation test) in male Wistar rats of two age groups. Colostrinin, at a dose of 4 μg/rat IP, facilitated acquisition of spatial learning of 13- (aged) but not 3-month-old (young) rats. At the same dose, it improved incidental learning in aged rats, while the dose of 20 μg/rat attenuated it. Colostrinin did not change locomotor activity of rats. Taken together, the present findings indicate that colostrinin may have some beneficial effects on cognitive functioning, particularly in aged subjects. Given the fact that colostrum is the first nutritive agent of neonates, it might be speculated that its peptides may facilitate the early postnatal development of the cerebral neurons and their plasticity. © 1999 Elsevier Science Inc.

Memory Learning Locomotor activity Colostrum Colostrinin Proline-rich peptide Morris water maze Incidental learning

COLOSTRUM, the early milk produced during the puerperal period, plays an essential role in survival of the newborns, as it is loaded with antibodies and other immunoactive substances. Particularly in ungulates, in which mother antibodies do not cross the placenta, the colostrum is the main source of immunological defense in the first period of life. One of immunologically active agents from ovine colostrum was isolated by Janusz and colleagues (19–21), and was characterized as an 18 kD polypeptide, built of three 6 kD subunits, rich in proline (22%) and nonpolar amino acids. The peptide was named colostrinin, and was characterized as a new cytokine (18). The preparation was subsequently found to be rather a complex of proline-rich polypeptides (Georgiades and Kruzel, in preparation). Recently, the immunoactive properties of the human analog of ovine colostrinin were described (12,27).

Cognitive deficits including learning impairment and delayed amnesia are one of the most prominent debilitating consequences of the normal and pathological aging in humans. Among the most severe forms of the senile dementia is Alzheimer's disease, which is associated with numerous pathophysiological alterations of the CNS. Pathophysiological changes in Alzheimer's patients include, among others, accumulation of beta-amyloid plaques and neurofibrillary tangles (15). In addition to the profound impairment in cholinergic transmission, alterations in the function of cytokines and other factors associated with immune reactions have been reported (3,5,11).

Exploiting the possibility that immunological factors may play a role in the development of Alzheimer's disease, Inglot and coworkers (17) carried out a preliminary clinical experiment and demonstrated in a double-blind, placebo-controlled trial that three to six cycles of therapy consisting of oral administration of colostrinin (100 μg every second day for 3 weeks) resulted in the substantial improvement of cognitive functioning in 11 out of 16 patients as measured by the Mini Mental State score and the recent memory test.

Investigation of the cognitive effects of peptides has a long

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history. In the 1960s, De Wied found that removal of the posterior lobe of the pituitary in rats impaired the maintenance of avoidance behavior, and that this deficit could be restored by the treatment with vasopressin (6). These and other findings resulted in 1970s in the appearance of the "neuropeptide" concept and a hypothesis that the peptides are implicated in learning and memory processes. This hypothesis was latter supported by findings showing that numerous peptides affect learning and memory processes in various behavioral paradigms [for reviews see (7,37)]. Because colostrinin was demonstrated to produce improvement of cognitive functions in some Alzheimer's patients, the present experiments were aimed at evaluating its cognitive effects in a more controllable experimental setting.

METHOD

Animals

Male Wistar rats ~3 months of age [~200 g, (young subjects)] or ~13 months of age [~400 g (aged subjects)], purchased from a licensed dealer, were housed under standard laboratory conditions (lights on at 0600 h, lights off at 1800 h; room temperature 23 ± 1°) with pelleted food and tap water available ad lib. Rats were kept in 58 × 37 × 19 cm plastic cages (four to five rats per cage). All animals were used once.

Drugs

Colostrinin was obtained from sheep colostrum according to the procedure of Janusz and colleagues (21). Physiological saline (vehicle) served as placebo. All injections were done IP in the volume of 1 ml/kg. The selection of doses for current investigation was based on immunotropic effects of colostrinin in animals (16,39).

Experimental Procedures

Locomotor activity. The test was performed on young rats. The animals were placed individually in the Opto-Varimex activity chambers (Columbus Instruments, Columbus, OH), linked to a PC computer. The rectangular chambers were illuminated with dim light. Each chamber (43 × 44 cm) was equipped with 15 infrared emitters placed on adjacent walls and with an equivalent number of receivers on the opposite walls. The rats were kept in the chambers for a 30-min adaptation period and then injected with colostrinin (4 or 20 μg/rat) or placebo. Immediately after the injection, the recording of the horizontal activity begun and was continued for 90 min. The locomotion, defined as an interruption of three consecutive photobeams, was analyzed using Auto-track software (Columbus Instruments, Columbus, OH). Experimental groups consisted of 10 animals each.

Spatial learning (Morris water test). The test was carried out on 20 young and 20 aged rats. A gray metal circular pool (180 cm in diameter, 50 cm in height) was filled to a height of 25 cm with 22°C tap water, which was changed every day as described previously (29,30). Curtains, bright lamps, and other objects around and above the pool provided numerous stable extramaze cues. The total circular area of the pool was divided into four quadrants (NE, SE, SW, and NW) by means of the signs on the TV screen on which the training was observed.

Spatial learning consisted of the acquisition trials (days 1–12, one trial per day), the "transfer test" (day 13), and the visible platform (visual perception) test (day 14).

During acquisition trials, 30 min before each trial one-half of rats were injected with colostrinin (4 μg/rat), the other half received placebo injections. The trial consisted of manually placing a rat into the water, facing the wall of the pool, at one of the four starting positions (compass points N, W, S, or E) around the pool perimeter. Every day the starting position was changed. The rat was required to find a gray metal platform (10 × 10 cm) that was present in the SE quadrant in the middle between its center and the wall, with the upper surface submerged 1 cm below the water surface. If a rat escaped onto the platform it was permitted to remain there for 30 s. If a rat failed to find the platform within 120 s, it was placed onto the platform by hand and allowed to remain there for 30 s. After completion of the trial, the rats were put in the "drying" cage and heated by infrared lamp.

To measure the strength of the spatial memory, on day 13, a "transfer test" was performed, during which, rats were swimming for 1 min in the pool without platform. On days 13 and 14, no drugs were administered.

On day 14, the ability of placebo-treated, young and aged rats, to find a visible platform was tested. The platform was placed in the NW quadrant (opposite to that in which it was kept during the acquisition trials), and was raised 1 cm above the water surface so that the sides (marked white) were clearly discernible.

Swimming behavior during the acquisition trials and the "transfer test" was video-recorded using a commercial VCR and analyzed by a PC computer, using the EYE 1.3 (J. Długopolski, Kraków, Poland), TRACK-ANALYZER (40) and Wintrack software. In addition to the escape latencies, the analysis of swimming behavior recorded during the acquisition trials involved calculation of Whishaw's indexes, representing % of path swum within a straight "corridor," 16 cm wide, connecting the starting point and the escape platform (38). During the "transfer test" calculated were: 1) the time (in seconds) spent by a rat in the SE quadrant in which the platform was hidden during acquisition trials, and 2) the number of episodes of swimming exactly over the previous position of the platform (direct "hits").

Incidental learning. This experiment was carried out on the aged rats. The procedure used in this experiment was conceptually based on the habituation test, developed initially for mice (28). Briefly, each rat was exposed to an open field twice, and the decrease of exploratory activity on the second exposure served as the measure of incidental memory. The open field (50 × 50 × 50 cm) was made of the plywood and painted black. To make the open field more distinctive, in the two corners, a pieces of the blotting paper were attached to the wall. Each blotting paper was marked with few drops of the flavored oil NDC-0395-1913-91 or NDC-0395-2015-91 (Humco, TX). The level of illumination was kept as low as possible to allow videotaping. The exposures were videotaped using TV camera attached to the commercial VCR and a PC computer. After each exposure, the cages were cleaned and dried.

Two exposures, each 10 min long, were separated by various interexposure intervals (IEIs). The behavior of rats (horizontal activity) was analyzed using the EYE 1.3 (J. Długopolski, Kraków, Poland) and TRACK-ANALYZER (40) software. For each rat, the total length of the path traveled during both exposures was recorded. As the measure of incidental memory, the decrease of horizontal activity on the exposure #2, was used [the difference between length of the path during exposure #1 and exposure #2, (Δ exploration score)].

Two independent experiments were performed. The preliminary experiment served to establish the effect of the length of IEI on decline of incidental memory in drug-free rats. It was assumed that at a long IEI, the memory is weaker than at a short IEI. Subjects were placed in experimental cages and their exploratory activity was measured as described. Because incidental memory in mice lasts no longer than 7 days (28), rats ($n = 5$ or 7) were placed subsequently in their home cages for a IEI of 1 or 7 days. After that time, rats were investigated in the experimental cages again (exposure #2).

In the proper experiment, the effect of colostrinin (4 or 20 μg/rat) on the consolidation of incidental memory was investigated. Colostrinin was administered ~1 min after the exposure #1. The IEI lasted for 3 days. There were 12, 7, and 6 rats for colostrinin at doses of 0, 4, or 20 μg/rat, respectively.

Statistics

Repeated-measures two-way ANOVA followed by Newman–Keuls test was used for the comparison of escape latencies in the Morris water maze. To evaluate other effects, one-way ANOVA followed by Dunnet's test or Student's *t*-test were used, where appropriate.

All experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication No. 85-23, revised 1985), and were approved by the internal Bioethics Commission.

RESULTS

Locomotor activity

Table 1 shows the effects of colostrinin on the horizontal locomotor activity recorded for 90 min, right after its administration. Colostrinin did not influence horizontal locomotor activity in young rats.

Spatial Learning (Morris Water Maze) Test

Rats demonstrated rapid acquisition of the spatial navigation task, and by day 10, the latencies to escape onto the platform were less than 10 s, and did not decrease on the following days (Fig. 1). In the young rats, escape latencies and Whishaw's indexes were virtually identical in placebo- and colostrinin-treated subjects. However, the aged, placebo-treated controls demonstrated markedly longer swimming latencies on days 3 and 4 of the training than all other subjects. Statistically significant difference between aged placebo-treated and aged colostrinin-treated rats may suggest that colostrinin improves the ability of aged subjects to acquire the platform position. In addition, a detailed analysis of swimming behavior indicated that the aged placebo-treated rats showed impairment in the latter stages of spatial learning. Thus, they were finding the platform with less accuracy compared to all other groups, because they were not swimming toward it straight from the starting point (Fig. 2). Because no such impairment was noted in aged rats treated with colostrinin, this may suggest that colostrinin improved the ability of aged subjects to search for the platform more effectively. There were no differences between colostrinin-treated and control rats in the swimming speed (data not shown).

During the transfer test, rats were swimming in the vicinity of the place that contained escape platform during the acquisition trials. The treatment with colostrinin resulted in the higher precision of the search for the platform as revealed by the higher number of episodes of direct "hits" (Fig. 1, inset). The difference between the performance of aged placebo-treated and colostrinin-treated rats was significant. This spatial bias was not observed when the time spent in the "training" quadrant was calculated (data not shown). Mean swimming time in the quadrant previously containing the platform was 20–25 s for all groups.

The surface plots analysis of the time spent by rats in a 10 × 10 cm segments of the water maze during the "transfer test" (Fig. 3) has revealed that young rats, regardless of treatment, preferred both the place where the platform was positioned and the starting (north) point. On the other hand, placebo-treated, aged rats did not show any preference for a particular segment of the water maze. However, the aged rats receiving

TABLE 1

THE EFFECT OF COLOSTRININ ON THE HORIZONTAL LOCOMOTOR ACTIVITY IN 3-MONTH-OLD RATS

Colostrinin (μg/Rat)	Horizontal Activity
0	470 ± 130
4	530 ± 87
20	550 ± 130
ANOVA:	$F(2, 29) = 0.10, p > 0.05$

The activity was recorded for 90 min after colostrinin administration. The data are mean ± SEM of arbitrary units. The *n* for each group was 10.

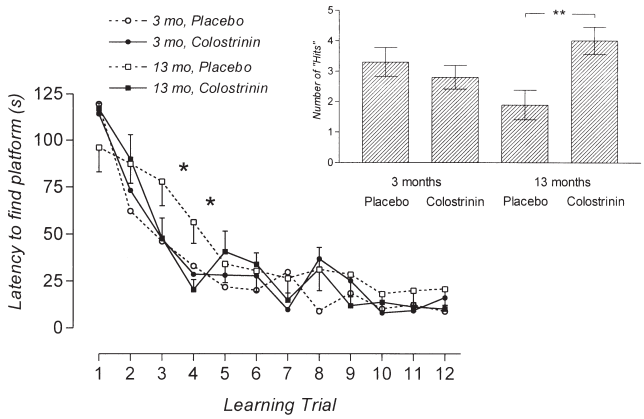

FIG. 1. Acquisition of spatial learning by young and aged rats treated with colostrinin. Rats were trained for 12 days with one trial per day, to navigate into the hidden platform. Presented are mean escape latencies to find the hidden platform. For aged subjects, the − and + SEM are shown for placebo and colostrinin-treated rats, respectively. For the sake of clarity, the SEM of young rats is not presented. Asterisks indicate statistically significant difference from all other groups [two-way repeated measures ANOVA: group, $F(3, 36) = 3.46$, $p < 0.05$, day, $F(1, 36) = 8.59$, $p < 0.01$]. This difference is attributed to the longer latencies of placebo-treated 13-month-old rats (post hoc Newman–Keuls test $p < 0.05$ vs. all other groups). The *n* for all groups was 10. Inset: Assessment of the strength of spatial memory during the "transfer test." Presented are mean ± SEM number of swims ("hits") over the place where the platform was located during training. Thirteen-month-old rats treated with colostrinin demonstrated higher number of "hits" compared to their agemates treated with placebo [$p < 0.05$ Dunnet's test followed by ANOVA, $F(3, 39) = 4.013$, $p < 0.025$].

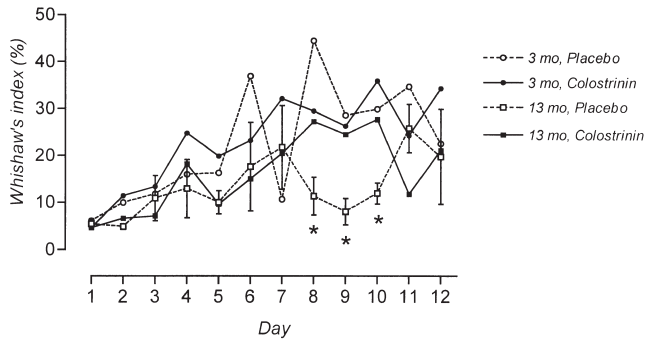

FIG. 2. Effects of colostrinin on the precision of swimming to the goal platform (Whishaw's indexes) of young and aged rats. The data are derived from the same experiment as that presented in Fig. 1. Rats were trained for 12 days with one trial per day to navigate into the hidden platform. Presented are mean Whishaw's indexes, representing % of path swum within a straight "corridor," 16 cm wide, connecting the start and the platform. Periods of inactivity were not considered. For all groups, except aged rats treated with placebo, the SEM are not presented for clarity. Asterisks indicate statistically significant difference from all other groups [two-way repeated measures ANOVA: group, $F(3, 36) = 3.68$, $p < 0.05$, day, $F(2, 72) = 0.69$, $p > 0.05$]. This difference is attributed to the lower values of Wishaw's indexes of placebo-treated 13-month-old rats (post hoc Newman–Keuls test $p < 0.05$ vs. all other groups).

colostrinin during training demonstrated the preference for the segments where the platform was located.

The placebo-treated rats. regardless of age, performed equally well in the test for perception of objects (swimming to the visible platform). The mean ± SEM latencies to reach the platform were 19.20 ± 2.25 s and 21.30 ± 5.47 s for young and aged rats, respectively, suggesting that the perception of objects was not compromised by the age of the subjects.

Incidental Memory

Drug-free aged rats exposed to the open field twice with a IEI of 1 day demonstrated marked decrease of horizontal activity on the second exposure [exposure #1 and #2: 1002 ± 163.5 and 676.7 ± 170.1 (mean ± SEM arbitrary units), respectively]. In contrast, rats that were exposed to the open field twice with a lEI of 7 days did not show such a decrease of horizontal activity on the second exposure (exposure #1 and #2: 1004 ± 96.68 and 950.5 ± 143.9). The Δ exploration scores for IEI of 1 and 7 days (325.0 ± 32.33 and 54.03 ± 72.15, respectively) appear to differ significantly, $t(10) = 2.99$, $p < 0.01$.

In this test, colostrinin administered after exposure #1 produced a significant reduction of the horizontal activity of aged rats recorded during exposure #2 only when given at the dose of 4 μg/rat (Table 2). The detailed analysis demonstrated that the Δ exploration score increased after a low (4 μg/rat), but decreased after a high (20 μg/rat) dose of colostrinin (Table 2). This may indicate that colostrinin has a biphasic effect on the consolidation of incidental memory, with low doses improving, and high doses deteriorating it.

DISCUSSION

The present study demonstrates that colostrinin, a complex of proline-rich polypeptides isolated from ovine milk and having immunomodulatory properties, may have potent behavioral effects in rodents. Of particular interest is that colostrinin apparently improved cognitive functions that were deteriorated in aged rats. Thus, at the dose of 4 μg/rat, it facilitates the acquisition of spatial learning in aged rats. bringing it to the level observed in young subjects. In addition, the dose of 4 μg/rat of colostrinin improves the consolidation of incidental memory in aged rats (increases the extent of between-trial habituation). Although the dose of 20 μg/rat of colostrinin produced an inhibitory effect on incidental memory, this agrees with the common finding that the procognitive drugs, as a rule, produce biphasic effect on learning and memory processes.

In the present study, the promnestic effects of colostrinin were demonstrated in two paradigms, including spatial learning in the Morris water maze (25) as well as in the incidental memory (habituation test) developed originally for mice (28). Habituation tests are based on the observation that an animal's reaction to a novel stimulus is reduced at the next exposure to the same stimulus. This paradigm is regarded as a rapid and effective screening test, but its use is compromised by the low predictive validity and difficulty in reproducing results due to the sensitivity to environmental factors. On the other hand, the Morris water maze is regarded as a test very sensitive to a number of treatments producing deficits in spatial learning, including brain lesions and a variety of amnestic agents [see (4) for review]. This test measures spatial memory, which is highly dependent on the presence of extramaze cues. These two tests differ also in other aspects. Thus, the habituation test appears to measure learning of an information acquired in the absence of an explicit reinforcement (other than curiosity) and involves implicit memory. The Morris water maze is dependent upon a potent motivation (escape from water) and involves explicit memory. Both tests appear to measure long-term memory, and both are independent on the level of food or water deprivation.

Investigators of "antidementia" drugs lack both a reliable model of human dementia and a standard, recognizable memory-facilitating reference compound. The lack of these two basic tools in the learning and memory research (as opposed to other areas of psychopharmacology) is a serious obstacle in drug development. This prompts for a development of a variety of animal models of dementia, of which none to date seems to be fully satisfactory [for a review see (32)]. Nonetheless, there is a common agreement that the best animal model of the normal and pathological aging in humans is the aging animal itself. Aged rodents show deficits in a number of learning and memory paradigms, including passive avoidance (2), habituation tests (1,26,36), and the Morris water maze (10,32). From this perspective, our attempt to investigate promnestic effects of colostrinin in aged rats seem to correspond well with the currently available and most broadly accepted animal models of human dementia.

As mentioned above, colostrinin produced a biphasic effect on the incidental memory in aged rats, because the dose of 4 μg/rat facilitated, and the dose of 20 μg/rat attenuated its consolidation. This finding is not surprising, because numerous observations indicate that the dose–response curve for the effects of promnestic agents on learning and memory is typically of an inverted-U shape in that low doses have opposite effects from high doses (24). Such effects were demonstrated for naloxone (8), neuropeptide Y (9), or oxytocin (31). It is also worth mentioning that similar dose–response relationship, characteristic for substances with regulatory properties, was observed in the case of studies on the effect of colostrinin on the immune responses (39).

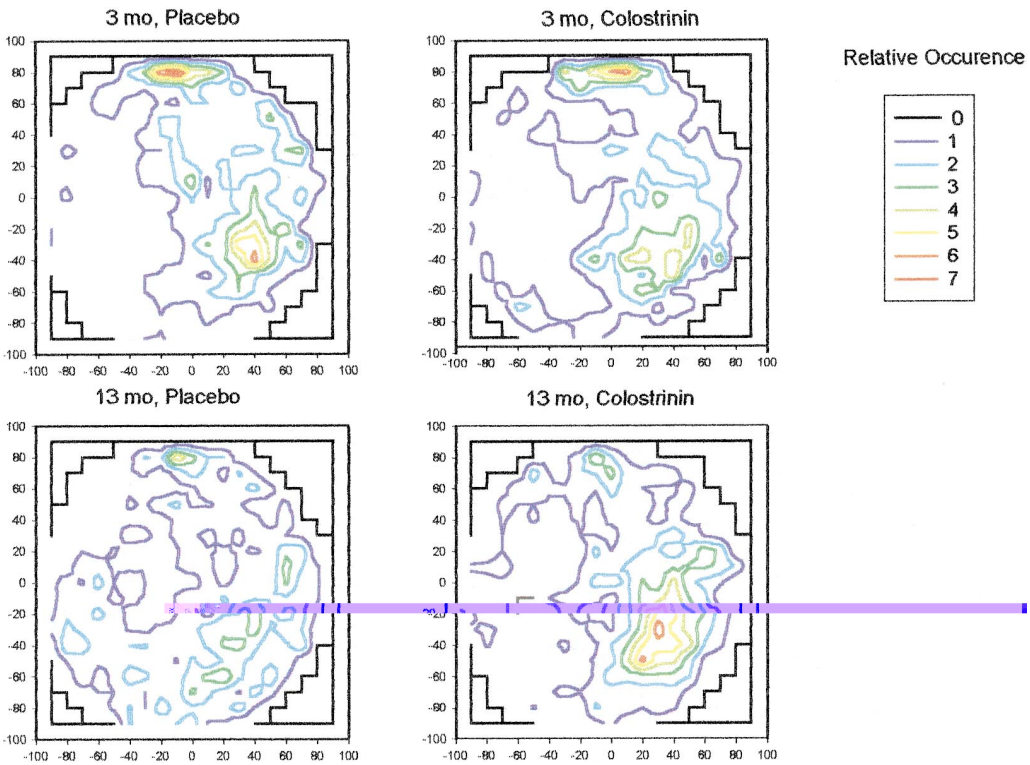

FIG. 3. Surface plot analysis of swimming behavior recorded during the "transfer" test of young and aged rats treated with colostrinin during acquisition trials. Presented are surface plots of spatial preferences of rats recorded on day 13 of training. The whole area of the tank was divided into 10 × 10-cm segments, and the mean number of times the rats swam in a particular segment are plotted. The platform was positioned in the SE quadrant in the middle way between the center of the pool and the wall. It appears that aged, placebo-treated rats did not swim in the vicinity of the platform to the same extent as all other rats did.

Regarding the specificity of the procognitive effects of colostrinin, two issues should be brought about. First, it is unlikely that the shorter latencies to find the platform of the colostrinin–treated aged rats were due to the possible "stimulatory" effect. This is because colostrinin did not influence locomotor activity in young rats (Table 1) nor the swimming speed of rats as measured in the Morris water maze (data not shown). Alternatively, aged rats might have impaired sense of sight and colostrinin might improve it. This explanation is also unlikely, because we found no difference between young and aged rats in the latency to find visible platform (visual perception test), performed on day 14.

TABLE 2

THE EFFECT OF COLOSTRININ ON THE CONSOLIDATION OF INCIDENTAL MEMORY AT INTEREXPOSURE INTERVAL OF 3 DAYS IN AGED RATS

Colostrinin (μg/Rat)	Δ Exploration Score
0	118.2 ± 93.08
4	357.5 ± 47.26*
20	−151.9 ± 113.5*
ANOVA	$F(2, 24) = 5.68, p < 0.05$

Administration of 4 μg/rat of colostrinin resulted in the higher (*$p < 0.05$) Δ exploration score; the dose of 2 μg/rat produced significantly (*$p < 0.05$) lower Δ exploration score compared to placebo-treated controls. The data are mean ± SEM. There were 12, 7, and 6 rats for colostrinin at doses of 0, 4, or 20 μg/rat, respectively. The mean horizontal activity of rats on the exposure #1 did not differ significantly among groups, and was 1050.6 ± 72.4 ($n = 25$) arbitrary units.

The present study supports clinical observations indicating that colostrinin may facilitate cognitive functioning in patients suffering from Alzheimer's disease (17). At present, we do not propose the mechanism of this action. It cannot be excluded, however, that the immunomodulatory effects of colostrinin, [reviewed by Janusz and Lisowski (19)] may be of importance. For example, it has been reported that spatial learning is impaired in mice suffering profound deficits in immunological responses (35). The interferon gamma-inducing activity of colostrinin (16) might thus play a role in the mechanism of promnestic effects of this preparation. The findings of Schmitt et al. (34) and Ringheim et al. (33) demonstrating that interferon gamma inhibits production of the Alzheimer's amyloid beta precursor protein are in favor of this idea. However, whether or not the immunomodulatory effects of colostrinin are of importance for the improvement of cognitive functions in rats and humans, remains to be established.

One of the central issues in the cognitive effects of peptides is the question of whether these effects are centrally mediated (6,7,37). As far as colostrinin is concerned, at present

no data on its permeability through the blood–brain barrier are available. However, the high content of hydrophobic amino acids, resulting in lipophilicity of colostrinin may suggest that it might penetrate into the central nervous system.

Colostrum, because of its immunoactive peptides, offers the immunological protection to the newborns, before they can start producing their own antibodies. Converging lines of evidence demonstrate the favorable effect of breast feeding on mental development of babies. A better development of brain and retina resulting in the improved cognition and visual function, intelligence, and neurological development has been frequently associated with breast feeding, especially when it is done within the first months, or even first 2 weeks of life (13,14,22,23). According to some hypotheses, human milk is more suitable than artificial formulations, owing to the proper content of essential fatty acids, indispensable for neuronal membrane formation (13,14,23). However, the particular importance of breast feeding within short period after parturition emphasizes the effects of compounds present in colostrum. It is interesting that in ovine colostrum the highest content of the colostrinin complex was found in the early colostrum (6–12 h after the delivery), and was significantly lower in samples of colostrum or milk collected within several days after the delivery (21). It cannot be excluded that the peptides contained in colostrum play a unique function. In support, as pointed by Golding et al. (13), there is evidence that breast milk that has been pasteurized before feeding does not produce beneficial effects on cognitive functioning, whereas the fresh breast milk is effective whether it is delivered by the tube or by the breast. The present findings, demonstrating the procognitive effects of colostrinin, together with the literature data, may thus open an intriguing possibility that colostrum peptides may facilitate the early postnatal development of the cerebral neurons and their plasticity.

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REFERENCES

1. Barnes, C. A.; Markowska, A. L.; Ingram, D. K.; Kametani, H.; Spangler, E. L.; Lemken, V. J.; Olton, D. S.: Acetyl-l-carnitine. 2: Effects on learning and memory performance of aged rats in simple and complex mazes. Neurobiol. Aging 11:499–506; 1990.
2. Bartus, R. T.; Dean, R. L.: Animal models for age-related memory disturbances. In: Animal models of dementia. New York: Alan R. Liss, Inc.; 1987:69–79.
3. Bauer, J.; Ganter, U.; Strauss, S.; Stadmuller, G.; Frommberger, U.; Bauer, H.; Volk, B.; Berger, M.: The participation of interleukin-6 in the pathogenesis of Alzheimer's disease. Res. lmmunol. 143:650–656; 1992.
4. Brandeis, R.; Brandys, Y.; Yehuda, S.: The use of the Morris water maze in the study of memory and learning. Int. J. Neurosci. 48:29–69; 1989.
5. Cacabelos, R.; Alvarez, X. A.; Franco-Maside, A.; Fernandes-Novoa, L.; Caamano, J.: Serum tumor necrosis factor (TNF) in Alzheimer's disease and multi-infarct dementia. Methods Find. Exp. Clin. Pharmacol. 16:29–35; 1994.
6. De Wied, D.: The influence of the posterior and intermediate lobe of the pituitary and pituitary peptides on the maintenance of a conditioned avoidance response in rats. Int. J. Neuropharmacol. 4:157–167; 1965.
7. De Wied, D.; Diamant, M., Aodor, M.: Central nervous system effects of the neurohypophyseal hormones and related peptides. Front. Neuroendocrinol. 14:251–302; 1993.
8. Flood, J. F.; Cherkin, A.; Morley, J. E.: Antagonism of endogenous opioids modulates memory processing. Brain Res. 422:218–234; 1987.
9. Flood, J. F.; Hernandez, E. N.; Morley, J. E.: Modulation of memory processing by neuropeptide Y. Brain Res. 421:280–290; 1987.
10. Frick, K. M.; Baxter, M. G.; Markowska, A. L.; Olton, D. S.; Price, D. L.: Age-related spatial reference and working memory deficits assessed in the water maze. Neurobiol. Aging 16:149–160; 1995.
11. Fundenberg, H. H.; Singh, V. K.: Alzheimer's "syndrome" prognosis of subsets with different etiology and preliminary effects of immunotherapy. Drug Dev. Res. 15:165–169; 1988.
12. Georgiades, J.; Gelder, F.; Inglot, A. D.: Isolation and preliminary characterization of a new cytokine present in human colostrum. Its similarity to ovine Colostrinin. Eur. Cytokine Netw. 7:511; 1996.
13. Golding, N.; Rogers, I. S.; Emmett, P. M.: Association between breast feeding, child development and behaviour. Early Hum. Dev. Suppl. 49:S175–S184; 1997.
14. Gordon, N.: Nutrition and cognitive function. Brain Dev. 19:165–170; 1997.
15. Hyman, B. T.; Van Hoesen, G. W.; Damasio, A. R.: Memory-related neural systems in Alzheimer's disease: An anatomic study. Neurology 40:1721–1730; 1990.
16. Inglot, A. D.; Janusz, M., Lisowski, J.: Colostrinine: A proline-rich polypeptide from ovine colostrum is a modest cytokine inducer in human leukocytes. Arch. Immunol. Ther. Exp. 44:215–224; 1996.
17. Inglot, A. D.; Leszek, J.; Janusz, M., Lisowski, J.: Colostrinin for treatment of Alzheimer's disease. Eur. Cytokine Netw. 7:458; 1996.
18. Janusz, M., Inglot, A. D.; Lisowski, J.; Piasecki, E.; Krukowska, K.; Georgiades, J.: Colostrinin identified as new cytokine. Eur. Cytokine Netw. 7:512; 1996.
19. Janusz, M., Lisowski, J.: Proline-rich polypeptide (PRP)—an immunomodulatory peptide from ovine colostrum. Arch. Immunol. Ther. Exp. 41:275–279; 1993.
20. Janusz, M., Lisowski J.; Franek, F.: Isolation and characterization of proline-rich polypeptide from ovine colostrum. FEBS Lett. 49:276–279; 1974.
21. Janusz, M., Staroscik, K.; Zimecki, M., Wieczorek, Z., Lisowski, J.: Chemical and physical characterization of prolin-rich polypeptide from sheep colostrum. Biochem. J. 199:9–15; 1981.
22. Johnson, D. L.; Swank, P. R.; Howie, V. M.; Baldwin, C. D.; Owen, M.: Breast feeding and children's intelligence. Psychol. Rep. 79:1179–1185; 1996.
23. Lanting, C. I.; Fidler, V.; Huisman, M., Touwen, B. C.; Boersma, E. R.: Neurological differences between 9-year-old children fed breast-milk or formula-milk as babies. Lancet 344:1319–1322; 1994.
24. McGaugh, J. L.: Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. Annu. Rev. Neurosci. 12:255–287; 1989.
25. Morris, R. G.: Spatial localisation does not depend on the presence of local cues. Learn. Motivat. 12:239–260; 1981.
26. Pelleymounter, M. A.; Cullen, M. J.: Effects of idebenone on information processing in aged Long–Evans rats. Pharmacol. Biochem. Behav. 46:415–421; 1993.
27. Piasecki, E.; Inglot, A. D.; Winiarska, M., Krukowska, K.; Janusz, M., Lisowski, J.: Coincidence between spontaneous release of interferon and tumor necrosis factor by colostral leukocytes and the production of colostrinin by human mammary gland after normal delivery. Arch. Immunol. Ther. Exp. 45:109–117; 1997.
28. Platel, A.; Porsolt, R. D.: Habituation of exploratory activity in mice: A screening test for memory enhancing drugs. 1982. Psychopharmacology (Berlin) 78:346–352; 1982.

29. Popik, P.; Mamczarz, J.; Vetulani, J.: The effect of electroconvulsive shock and nifedipine on spatial learning and memory in rats. Biol. Psychiatry 35:864–869; 1994.
30. Popik, P.; Nalepa, I.; Mamczarz, J.; Vetulani, J.: Retrieval associated cholinergic activity and its inhibition by memory updating. Life Sci. 54:1251–1257; 1994.
31. Popik, P.; Vetulani, J.; VanRee, J. M.: Low doses of oxytocin facilitate social recognition in rats. Psychopharmacology (Berlin) 106:71–74; 1992.
32. Porsolt, R. D.; Roux, S.; Wettstein, J. G.: Animal models of dementia. Drug Dev. Res. 35:214–229; 1995.
33. Ringheim, G. E.; Szczepanik, A. M.; Burgher, K. L.; Petko, W.; Heroux, J. A.; Cavalieri, F.: Transcriptional inhibition of the beta amyloid precursor protein by interferon gamma. Biochim. Biophys. Res. Commun. 224:246–251; 1996.
34. Schmitt, T. L.; Steiner, E.; Klinger, P.; Sztankay, H.; Grubeck-Loebenstein, B.: The production of an amyloidogenic metabolite of the Alzheimer amyloid beta precursor protein (APP) in thyroid cells is stimulated by interleukin 1 beta, but inhibited by interferon gamma. J. Clin. Endocrinol. Metab. 81:1666–1669; 1996.
35. Sei, Y.; Arora, P. K.; Skolnick, P.; Paul, I. A.: Spatial learning impairment in a murine model of AIDS. FASEB J. 6:3008–3113; 1992.
36. Stoll, S.; Haitmann, H.; Cohen, S. A.; Muller, W. E.: The potent free radical scavenger alpha-lipoic acid improves memory in aged mice: Putative relationship to NMDA receptor deficits. Pharmacol. Biochem. Behav. 46:799–805; 1993.
37. VanRee, J. M.; Bohus, B.; Versteeg, D. H. G.; De Wied, D.: Neurohypophyseal principles and memory processes. Biochem. Pharmacol. 27:1793–1800; 1978.
38. Whishaw, I. Q.: Formation of a place learning-set by the rat: A new paradigm for neurobehavioral studies. 1985. Physiol. Behav. 35:139–143; 1985.
39. Wieczorek, Z.; Zimecki, M.; Janusz, M.; Staroscik, K.; Lisowski, J.: Proline-rich polypeptide from ovine colostrum: Its effect on skin permeability and on the immune response. Immunology 36:875–881; 1979.
40. Wolfer, D. P.; Lipp, H.-P.: A new computer program for detailed off-line analysis of swimming navigation in the Morris water maze. J. Neurosci. Methods 41:65–74; 1992.