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Study to gelatin peptide on anti-fatigue of central nervous system

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ABSTRACT

We feed gelatin peptide to fatigue mice for nutrition intervention, detect and evaluate the effect of gelatin peptide to delay and moderate of central fatigue. We want to develop the high value-added products of pigskin gelatin bioactive peptide, expense the scope of use of the product. Compare to the control group of feeding double distilled water, DA/5-HT of animal experimental group is 0.84, control group is 0. 14. Comparison of these two groups, the experimental group is 600% higher than the control group. Gelatin peptide has obviously increased the effect of central excitatory, alleviated the central fatigue.

KEYWORDS

Gelatin peptide; DA/5-HT; Central fatigue.

Mechanisms of exercise-induced fatigue are a complex and numerous sites of action of a physiological phenomenon. Because of the limitations of traditional measurement techniques, the study of exercise-induced fatigue is more concentrated in the peripheral fatigue; the function of the central nervous system in exercise-induced fatigue is less attention. The present study showed that monoamine neurotransmitters in the brain can change, affecting the central excitatory and inhibitory processes after prolonged exercise, which is one of the possible mechanisms leading to the central fatigue during exercise ; for different projects and intensity induced fatigue, researchers use physiologically active ingredient of some medicine or food to interventions implemented, can make the concentration of monoamine neurotransmitters in animal models and in different brain regions or whole brain metabolite changes, although the extent of these changes in the nature, aspects of metabolic pathways and molecular mechanism is unclear, but they illustrate the active ingredient in regulating brain monoamine neurotransmitters and their metabolites play an important role. This paper intends to implement the model animal nutrition intervention by gavage feeding gelatin peptides, and research anti- fatigue effect. We wish to provide a theoretical basis for the development of high value-added products gelatin peptides, expand the scope of application of pig products.

MATERIALA AND METHODS

Reagents and Instruments

KM outbred rates sterile type 50 (8 weeks old), 180 ~ 220g; purchased from Xi'an Jiaotong University animal testing center. Food grade gelatin (Shandong Zibo Bo Yan Biotechnology Co., Ltd.) ; trypsin (Wolsen); gelatin peptides (trypsin hydrolyzed gelatin homemade): standard DA,5-HT (Sigma Company) ; standard solution configuration: to 0.01mol/LHCl were dubbed the concentration of stock solution of 1g / *L*, and stored at 4 ℃ refrigerator, diluted before use. With DHBA as an internal standard, the concentration above. The rest are analytical grade reagents.

WatersTM600E HPLC, AS3120A to ultrasonic gas meter ; CHI832 electrochemical detector ; 5011 graphite carbon working electrode, a reference electrode was Ag / AgCl, parameters are set at + 0.72V (oxidation) and the electrode potential than +. 0 05V (reduction), the sensitivity of both 5nAFS; 4.6mm × 150mm column packing 5um Hypersil BDS (Dalian Elite scientific instrument Co., Ltd.) ; detection voltage is 700uv, sensitivity 5nA; Scientific software Ezchromelite analysis software ; 0.45um filter and pump filter (Millipore); Biofuge28RS speed refrigerated centrifuge (Heraens); Soniprep150 sonicator (Sanyo); 0.2mGHP Acid Sample Processor (Pall); SN-2 type stereotaxic instrument (Narishige).

Experimental Methods

Exhaustive loaded swimming test in rats

According to body weight rates were randomly divided into two groups, control group and test group. 3-5 per group are raise to prevent gavages or drowning accidents at sports training. Daily uncontrolled drinking water, gavages feeding 28d, administered before exercise schedule is 2h, the control group was given a double distilled water, the test group were given gelatin peptides, raising the amount of 3% of animal body weight. Swimming training once a day, every 20min, depth 100cm, temperature 30 ± 2 ℃, not weight. After the last administration 2h, arrange 3% weight in rats swimming sports, weight way wrap applications plastic of food package, then thread the tail in the mouse model system, the head submerged in the water for 10 seconds,they cannot leak out water oneself is exhaustive fatigue, sampling and testing.Sample extraction and purification

Sample extraction and purification

This test uses scissors decapitation, locator divided brain areas, direct access to the process of the brain. Rats were decapitated and open the cranial cavity at quiet state and after exercise fatigue immediately, take the striatum cerebellum and brain stem and other parts of the brain on the ice, wishing with the ice saline and dry by filter paper and weighed used salicylic acid treatment for 10 times(the pre-cooling 100g / L salicylic acid), set 1.5 mL centrifuge tube fully homogenized, adding tissue lysates (0.0l% cysteine, 0.2mmol/LHCIO, 0.5mmol / L Na_2EDTA), placed in a 1.5mL tube again fully homogenized and sonicated twice centrifuged 15min (14000r/min, 4 ℃); After, the supernatant set another centrifuge tube, add ice-cold 1:5 ratio of perchloric acid (0.1mol / L) to solution, the low temperature centrifuge 30min (10000r/min, 4 ℃) once, and take the supernatant, and precipitate protein,centrifuged, the supernatant was frozen in liquid nitrogen set -80 ℃ tested. When measuring sample, centrifuged 0.2mmol / L acid melted ice after the filter sample.supernatant to another tube set, the ratio 1:5 of perchloric acid was added the supernatant, solution of solution 0.1mol / L, the low temperature centrifuge 30min (10000r/min, 4 ℃) once, the after protein precipitation centrifuged, the supernatant was frozen in liquid nitrogen set -80 ℃ tested. When the sample was centrifuged and melted with 0.2mmol / L ice acid. The supernatant sample was joined from the automatic.detected dopamine, 5 - neurotransmitters serotonin levels using high performance liquid chromatography with electrochemical. Experimental data using SPSS11.5 software for statistical analysis, P 0.05.

Sample Test

1990s, the cyclic adenosine monophosphate (cAMP) was called second messengers by physiologist; now again monoamine neurotransmitters was called third messenger. Monoamine neurotransmitters including catecholamines and indoleamines, the dopamine (DA) has been studied more former and earlier; latter including 5 - hydroxytryptamine (5-HT).

They are carrier material with important information at the central nervous system of higher animals and humans'.accurately detecting the content in brain tissue, and studying its physiological function, the judgment of the function and status of the central nervous system has important significance. However, because the monoamine neurotransmitter release from peripheral neurons, rapid metabolism or reuptake, the fluid concentrations of these neurotransmitters in organizations is very low, coupled with the unstable nature of their chemical,their biological structure has similarities, as well as the possible presence of interference source was detected very easily. Common measurement techniques have high performance liquid chromatography, capillary electrophoresis etc., but these techniques for sample handling are more complex and more time-consuming. The approach of a variety of neurotransmitters simultaneously determinate is rare in the same way once treated samples. Meeusen, Zhao Huan Ying, Ye Wei Ling were improved the detection process from various aspects. This study build on existing research results, after repeated experiments, designed to detect the following scenarios:

(1)Sample pretreatment

The method selected the effective sample preparation is the key to measure all kinds of neurotransmitters successfully by HPLC. After preliminary experiments, we sure the 0.2 mol / L HClO is extract tissue samples of monoamine neurotransmitters protein denaturing agent ; while adding 0.0l% cysteine and 0.5 mmol / L Na2EDTA, to achieve the purpose of reducing the solute oxidative damage. Extracted during the operation in order to reduce the degree of inequality neurotransmitter levels caused by the difference in grinding, tissue samples were modest after sonication ; at 4 ℃, 15 min under (14000 r / min) and then centrifuged with acid the GHP 0.2M filter processing.

(2)Chromatographic conditions of selection and optimization

Column temperature: 30 ℃; injection volume: 20 mL; work potential selection 0 6V. After repeated experiments,we select pH is 5.0, the buffer concentration of citrate/ sodium citrate is 0.1mmol / L, the rate of flowing is 0.4g / l; the methanol is the mobile phase of an organic agent, Among the methanol contents of 8% (V / V); The rate of flowing was 1.0mL/min. Taking into account the monoamine neurotransmitters and metabolites difficult to effectively separate from the mixtures of simple by the method of non- gradient systems, the experiment adding 1 - heptane's suffocate to citric acid / sodium citrate buffer, as well as adding an appropriate amount of 二、三-ethylamine of 5mmol / L to stable pH and prevent peak tailing, the result showed two kinds of monoamine neurotransmitters to achieve a better separation. The levels of Dopamine and 5 - Determination (the standard use the internal standard) were measures the peak area and calculate the integral to determine content with the U.S. Walters's Millennium32 software automatically.

RESULTS AND DISCUSSION

The role and anti -fatigue effect of polygeline to adjust the DA in central

DA is an important brain monoamine neurotransmitters, the main role is to regulate muscle tension, so that the body prepare for movement, are an excitatory neurotransmitter. Modern medical research[1] shows that the reduction of substantial nigra - striatal DA will result in inhibition of movement. Bailey[2] studies have shown that the DA and its metabolites of the rat brain reduced at many parts areas after 3h movement. Song Ya-jun'[3] studies found that the levels of DA showed a specific changes in exhibit area. Chaouloff'[4] studies had found that the synthesis and metabolism of DA in rat brain maintained to delay the onset of exercise-induced fatigue. On the whole, the DA of brain have stimulate actuation, muscle coordination and endurance exercise performance, they are closely related; on the electrical activity of the nervous system stimulant, a high DA level of brain help delay the occurrence of fatigue. We establish the experimental animal models by gavages feeding gelatin peptides, and detect the reaction in different brain regions of this nutritional intervention, the results shown in TABLE 1, whether static or fatigue state, the DA levels of all brain were higher in the experimental group ; but the reaction is not consistent to nutritional intervention in various brain regions, there are some differences in the degree of DA increased ; the change of magnitude is nonlinear after exercise in reduction, which is most sensitive to the hippocampus reaction,the control group is 254% higher than the experimental group ; the ratio of the brainstem lack of statistically significant change ; The ratio of before/after in the telencephalon, diencephalon and the striatum higher than the experimental group. Polygeline improved the content of DA in different brain regions, and may be delay the onset of fatigue, but the content of DA have consistent response about the motion before/ the end in all regions of brain, Combined with our[5] preliminary research work, gelatin peptides not only has anti- fatigue effect in outer periphery, also regulate excitatory neurotransmitter levels by the way, delaying the occurrence of central fatigue.

The role and anti -fatigue effect of polygeline to adjust the 5-HT in central

5 - Serotonin is a inhibitory monoamine neurotransmitters in brain widespread, the main role is adjust accordingly the levels of blood hormone through the hypothalamic - pituitary - gland, so that the body was reduced the ability of movement and caused exercise-induced fatigue,it is the possibility of the medium for the occurrence of central fatigue [1]. Newsholme' [6] the studies had found that the level of 5-HT in brain to increasing can cause decreased exercise capacity, and accordingly the first time he had proposed a hypothesis of "central fatigue ". The experiments about drug-mediated show that [7], the use of 5-HT accelerator may reduce endurance exercise performance, causing appearance of fatigue early; while 5-HT antagonists can actually produce postpone fatigue. Davis'[8] researches reputed that the 5-HT in brain had synthesize; the body would reduce exercise capacity. Chaouloff' experimental study describe the treadmill running can increase the synthesis

and metabolism of 5-HT in specific brain areas of movement rat, and induced to produce exercise-induced fatigue. Song Ya-jun and other studies have shown that brain regions differ on the 5-HT response in acute endurance exercise. Blomstrand [9] and other studies have shown that the regular long exercise and the exhaustive exercise can increase the synthesis and updates several of 5-HT in the parts brain. In short change, 5-HT and central fatigue is closely related and consistent. Our nutritional intervention trials are shown in TABLE 2, in the static safety,the gelatin peptides may reduce the different levels of 5-HT in brain functional areas for the experimental, the animals maintain a high excitability; after fatigue, the content of 5-HT in the other functional areas also lower than the control group except the brain cortex and telencephalon the control group comparison of the experimental group, the levels had significantly different with statistical significance especially in the hippocampus, diencephalons, brainstem, hypothalamus and striatum,. The results showed that the central function of gelatin peptides had stable function; it can reduce or delay the neurotransmitter levels in motor stimulation caused, resulting in resistance to central fatigue effect.

TABLE 1 : Concentration of DA on the rat from the different tissue (n=10,ng/g, mean±SD)

Brain areas /DA	The control Group (former)	The experimental Group (former)	The control Group (after)	The experimental Group (after)	The control Group (former/after)	The experimental Group (former/after)
Cortex	1.36±0.19	1.84±1.16	0.98±0.016	1.61±1.23	1.39	1.02
Hippocampi	34.38± 3.21	63.45±5. 34	1.22±0.16	4.32±1.16	28.18	14.69*
Diencephalons	213.5±19.52	214.21±19.78	154±8.30	210.86± 11.67	1.39	1.02
Endbrain	0.542±0.083	0.997±0.129	0.39±0.022	0.65±0.049	1.39	1.53*
Diencephalons	1.20±0.09	1.33±0.12	0.75±0.11	0.82±0.07	1.28	1.62*
Brainstem	0.551±0.11	1.53±0.29	0.44±0.13	1.26±0.14	1.25	1.21
Hypothalamic	157.98± 12.47	201.11± 19.78	18.75±1.56	27.50±2.61	8.43	7.31
Striatal	1253.44± 131.86	2659.71± 223.56	23.42±3.91	32.09±3.14	53.52	82.88*

*** Indicates significant difference, P <0.05**

TABLE 2 : Concentration of 5 - HT on the rat from the different tissue

Brain areas /5-HT	The control Group (former)	The experimental Group (former)	The control Group (after)	The experimental Group (after)	The control Group (former/after)	The experimental Group (former/after)
cortex	10.11±1.36	7.36±0.69	13.47±1.13	14.25±1.31	0.75	0.52
hippocampi	25.78±2.96	17.56±1.66	234.11±25.21	124.3± 14.35*	0.11	0.14
diencephalons	1249.58± 100.79	1143± 125.26	1346.4±128.19	1185. ±109.75	0.93	0.96
endbrain	0.771±0.084	0.675±0.077	0.90±0.091	1.17±0.28	0.86	0.75
diencephalons	2.20±0.19	1.4277±0.15	3.52±0.31	1.76±0.19*	0.63	0.41
brainstem	2.26±0.36	1.44±0. 23	3.09±0.29	1.49±0.13*	0.73	0.47
hypothalamic	898.64±61.70	513.± 36.52	1152.5±127.97	871.0±104.08*	0.78	0.45
striatal	54.3±6.25	45.86±3.77	1716.43±257.38	911.56±186.4*	0.032	0.03

*** Indicates significant difference, P <0.05**

Analysis mechanism restrained central fatigue of polygeline

Central nervous system is nature's most complex feedback and control systems, the specific functional neurons in the brain overlap extensively, this multi -level arrangement between neurons can cause numerous physiological effects between neurotransmitters and so on. Current research shows that the release of hypothalamic dopamine, 5 - serotonin in the striatum etc. may restrict each other, and dominates the central part of the same or different. On the other hand, although the experiment found that central 5 - HT and DA concentrations is an important neurobiological factor to exercise-induced fatigue, but most reports only pay close attention the local brain or some neurological research group. Therefore, the changes

of monoamine neurotransmitters on movement would awareness at different levels, different levels should be integrate, in order to fully understand the mechanism of central fatigue.

Exercise-induced fatigue is a synergistic interaction, complex process between the outer periphery and the central, in which a class of neurotransmitters cannot reflect the changes in the central state of excitation and inhibition. The study found that long movement arising from fatigue and increase brain 5-HT and DA reduce associated[10]. Wang Bin'[11] studies showed that the high ratio on dopamine / 5 - serotonin in the brain may be wake motivation to improve neuromuscular excitability and, enhanced capacity coordination; while the low ratio of dopamine / 5 - serotonin may by weakening pro- action, reducing movement motive to induce mental fatigue and insomnia, resulting in reduced capacity, constitutes the central fatigue. Therefore, some scholars have suggested using the ratio between them to analyze the relationship between them. Normal body depends on the balance of movement regulation of 5-HT and DA. We use the ratios of DA / 5-HT to describe the relationship between the process of formation and development of fatigue, the changes shown in TABLE 3: although the metabolic status of DA and 5-HT, the rats in various brain regions, before and after exercise is not uniform. Intervention which gelatin peptides effect in the hippocampus, diencephalons, brainstem, hypothalamus, striatum and other parts of the performance are more remarkable; it may be the five sensitive area of central fatigue on longer exercise; namely the prolonged endurance exercise may will first cause changes of the monoamine neurotransmitters of the five parts, resulting in reduced exercise capacity, and cause the formation and development of sports fatigue. But overall, DA/5-HT ratios of each brain region are higher than the experimental group whether it is static or fatigue. The ratio is 600% higher especially in the brainstem in the experimental group than the control group, the effect is particularly significant. The result show polygeline possible reduce or delay the dramatic changes in neurotransmitters through the coordination of different neurotransmitter metabolism, and have anti- fatigue effect by the central function of the steady-state stability central.

TABLE 3 : The ratios in different brain regions DA/5-HT movement after three rats in each group

Brain areas	the control group (former) DA/5-HT	the experimental group(former) DA/5-HT	the control group(after) DA/5-HT	the experimental group(after) DA/5-HT
cortex	0.13	0.25	0.07	0.11
hippocampi	1.33	3.6	0.005	0.035*
diencephalons	0.17	0.19	0.11	0.178
endbrain	0.70	1.48	0.43	0.56
diencephalons	0.55	0.93	0.21	0.47*
brainstem	0.24	1.06	0.14	0.84*
hypothalamic	0.18	0.39	0.016	0.032*
striatal	23.08	57.99	0.014	0.035*

*** Indicates significant difference, P <0.05**

CONCLUSIONS

Many scholars believe that the occurrence of fatigue on short-term strenuous exercise is often associated with peripheral mechanisms of fatigue; while the fatigue was generated by prolonged moderate intensity exercise, it[s] foundation is protective suppression for the central nervous system. The experimental results show that the levels of 5-HT increased at brain in the process of exercise-induced fatigue, the function of brain have appears to suppress,the inhibit occurred in certain brain areas in the first place, rather than occurs simultaneously in the whole brain, this result is consistent with existing similar findings. From the change of monoamine neurotransmitters see, change to this suppress have closely related with the content of the DA and 5-HT in central nervous system, e.g. striatum, brainstem and hypothalamus, there are relatively independent on dopamine and 5 - serotonin in certain brain areas, but more work is the same, normal movement depends on monoamine neurotransmitters and a variety of other functional balance of neurotransmitters. Where the balance between dopamine and 5 - serotonin may be an important factor in maintaining endurance exercise capacity, the balance between them by stabilizing can effectively weaken the occurrence of fatigue.

According to the current research, many scholars wish to delay and defer the happen of exercise-induced fatigue by supplementing branched chain amino acids, taurine and other nutrients means and methods; there is little research for complementary peptides, through our experiments (complement the gelatin peptides on animal models), the DA catabolism is improve generally in each brain region, the hippocampus is most obvious, but the changes between 5-HT and DA are not consistent. When the sports fatigue occurs, the ratio of DA/5-HT decreases in whole brain, the process of inhibition is dominant, at the same time the occurrence of central fatigue accompanied by. Detailed analysis, the ratio of DA/5-HT was significantly decreased in the hippocampus, hypothalamus and striatum and other parts, this suggesting that these parts may be sensitive area of central fatigue in prolonged endurance exercise, If we further analyze the experimental results, it showed that adding foods gelatin peptides can effectively improve the ratio of DA / 5 - HT, enhanced excitability of the brain in

different brain regions, particularly the brainstem, the experimental group are 600% higher than the control group after exercise, it may be plays an effective role in mitigation on the motion of fatigue -induced central inhibition, we will consider the ratio of DA / 5 - HT as one objective indicators of the research and evaluation of central fatigue during exercise,the adding gelatin peptides would enhance central balance function in anti-fatigue food research and development, alleviate occurrence of fatigue by prolonged exercise.

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