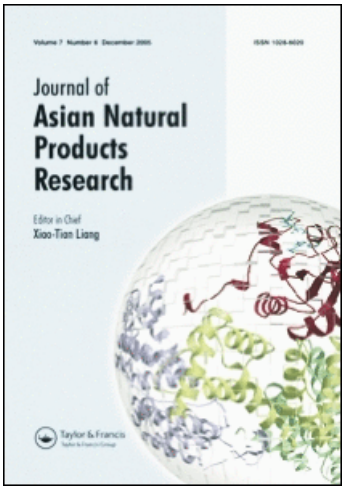

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STUDY ON THE NOOTROPIC MECHANISM OF (−)CLAUSENAMIDE – INFLUENCE ON THE FORMATION OF SYNAPSES IN MOUSE BRAIN

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The effects of (−)clausenamide (claus) on brain development were studied. Sixty kunming strain weaning mice were divided into 4 groups. One group was served as control administered with normal saline, the other 3 groups were treated with (−)claus 5, 10 and 20 mg kg^{-1} respectively by gavage once a day for 4 weeks. Results showed that (−)claus at dosage of 5 and 10 mg kg^{-1} facilitated learning and memory acquisition in step down and step through tests and increased thickness of cerebral cortex and synapse density significantly in the dentate cells over pyramidel cells in hippocampal region using quantitative technique of synapses analysis. These data provided the morphological basis of the nootropic effect of (−)claus.

Keywords: (−)Clausenamide; Nootropic; Synapses; Memory; Thickness of rat cerebral cortex

INTRODUCTION

Recently, it has become clear that in the mature nervous system the arrangement of synaptic contacts can undergo striking changes. The formation of the additional synaptic contacts plays an important role in acquisition of new behavioral experience and induction of synaptic structural plasticity [1]. In addition, plasticity of function is also accompanied by anatomical changes [2]. At present, one of the most promising ways to understand the synapse function is to establish correlation between the

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ultrastructure of synapse and its functional state. From our previous study [3,4], (−)claus improved amnesia induced by anisodine, facilitated learning and memory in normal mice, increased steady-state outward potassium current, enhanced LTP, indicating that (−)claus could promote synaptic plasticity.

In order to ascertain the basis of morphology of nootropic action, the effect of (−)claus on brain development was observed.

RESULTS AND DISCUSSION

Effects of (−)claus on body weight and amount of food consumption No significant difference in amount of food consumption between treated group and control group was found during 4 weeks of experiment. (−)Claus (5, 10 and 20 mg kg^{-1}) reduced gain of body weight in the first week, but no effect was found in the gain of body weight between control and treated groups in the next three weeks (see Table I).

Effects of (−)claus on brain weight and thickness of cerebral cortex (−)Claus (5, 10 and 20 mg kg^{-1}) increased thickness of cerebral cortex from 1.37 ± 0.14 mm to 1.53 ± 0.14 ($P < 0.05$), 1.54 ± 0.14 ($P < 0.05$), 1.49 ± 0.18 mm ($P > 0.05$) respectively, but no influence was observed on the brain weight (see Table II).

Effects of (−)claus on passive avoidance response – step down and step through tests Administration of (−)claus to normal mice for 4 weeks, (−)claus (10 mg kg^{-1}) increased latency of avoidance from 74.5 ± 15.9 to 106.6 ± 67.8 s ($P < 0.05$), reduced the number of errors from 2.8 ± 1.6 to 0.64 ± 0.7 s in step down test. Claus 5 and 10 mg kg^{-1} increased latency of avoidance from 55.0 ± 58.5 to 156.6 ± 124.2 s ($P < 0.05$), 111.5 ± 103.3 s ($P < 0.05$), 5 mg kg^{-1} reduced the number of errors from 3.0 ± 1.7 to 1.3 ± 1.1 s ($P < 0.05$) in step through test (see Table III).

TABLE I Effect of clausenamide on the body weight in mice

Group	*Dose* (mg/kg)	*Body weight* (g)			
		*w*1	*w*2	*w*3	*w*4
Saline		15.0 ± 2.1	19.0 ± 1.6	22.9 ± 1.6	24.9 ± 2.1
(−)Claus	5	13.6 ± 1.7*	18.3 ± 1.8	23.1 ± 2.3	23.5 ± 2.1
(−)Claus	10	12.8 ± 1.4*	18.4 ± 1.8	22.8 ± 2.0	25.7 ± 2.1
(−)Claus	20	11.9 ± 0.7*	18.2 ± 1.1	22.6 ± 1.3	23.5 ± 1.5

$x \pm$ SD ($n = 15$); *$P < 0.05$ vs saline.

TABLE II Effect of clausenamide on the brain weight and thickness of brain cortex and ratio of thickness of brain cortex (TC)/brain weight (BW) in mice

Group	*Dose* (mg/kg)	*Brain weight* (g)	*Thickness of cortex* (mm)	*Ratio of TC/BW*
Control		0.41 ± 0.02	1.37 ± 0.14	3.3
(−)Claus	5	0.40 ± 0.02	1.53 ± 0.14*	3.86**
(−)Claus	10	0.42 ± 0.02	1.54 ± 0.14*	3.64*
(−)Claus	20	0.42 ± 0.03	1.49 ± 0.18	3.55

$\bar{x} \pm$ SD ($n = 15$); *$P < 0.05$; **$P < 0.01$ vs control.

TABLE III Effects of clausenamide on the acquisition of memory in step down and step through tests after successive treatment for 4 weeks in weaning mice

Group	*Dose* (mg/kg)	*Latencies* (s)	*Number of errors*
Step down			
Control		74.5 ± 15.9	2.8 ± 1.6
(−)Claus	5	65.0 ± 22.1	2.2 ± 1.3
(−)Claus	10	106.6 ± 67.8*	0.64 ± 0.7*
(−)Claus	20	89.6 ± 57.8	1.4 ± 0.8
Step through			
Control		55.0 ± 58.5	3.0 ± 1.7
(−)Claus	5	156.6 ± 124.2*	1.3 ± 1.1*
(−)Claus	10	111.5 ± 103.3*	2.0 ± 1.2
(−)Claus	20	78.1 ± 62.5	1.8 ± 0.9

$\bar{x} \pm$ SD ($n = 15$); *$P < 0.05$ vs control.

Effects of claus on synapses density After the administration of (−)claus for 4 weeks to weaning mice, six mice were randomly sampled from 5 and 10 mg/kg and control group respectively for synapse quantitative analysis. The results showed that (−)claus (10 and 20 mg kg^{-1}) significantly increased synapse density in the hippocampal CA_3 region from 0.217 ± 0.095 to 0.282 ± 0.047 μm^2 ($P < 0.01$), 0.0298 ± 0.078 μm^2 ($P < 0.01$), respectively (see Fig. 1).

In our previous study, Claus was shown to improve learning and memory as well as potentiate LTP. It was interesting that the nootropic effect of Claus was much stronger than that of piracetam, a known nootropic agent. According to the existing theory, induction and facilitation of learning and memory may be attributing to the increase of synaptic function or changes of synaptic structure. Among them, generation of new synapses is especially important for neurotransmission, establishment of conditioned reflex and consolidation of memory. In addition, increase of cerebral cortex thickness is resulting mainly from increase of neurons and protein biosynthesis that were proved in our recent studies. There is a good reason to consider new protein as possible encoder of long term memory [5]. Why could Claus increase thickness of brain cortex and hippocampal synapses? Two factors

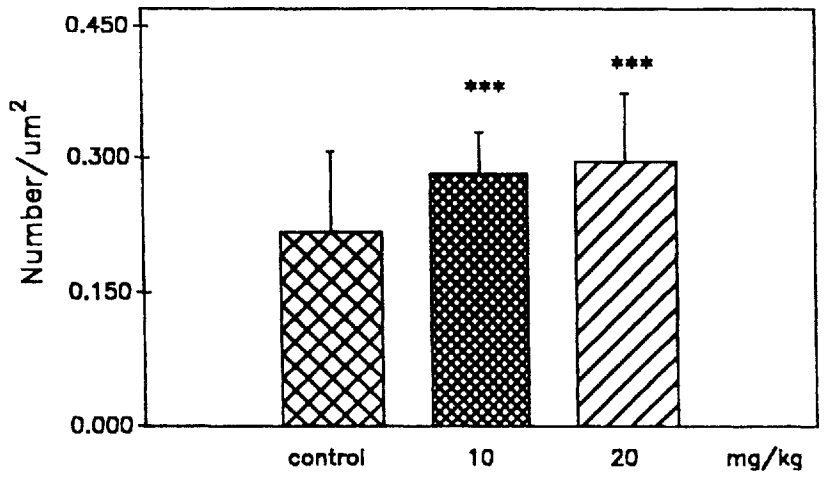

FIGURE 1 Effect of clauanamide on the number of synapses in the hippocampal CA_3 region after successive treatment for 4 weeks to weaning mice. ***$P < 0.01$

were taken into consideration, one is intracellular calcium concentration which is known to influence neuronal cytoarchitecture, and the other is excitatory amino acid which is believed to play a crucial role in a variety of physiological processes related to neuronal plasticity[6,7]. For example, activation of hippocampal N-methyl D-aspartate glutamate receptors triggers an increase in $[Ca^{2+}]_i$ and then induces growth of hippocampal neurons in tissue culture [8].

EXPERIMENTAL SECTION

General Experimental Procedures

Weaning mice (Kunming strain, $n = 60$) weighing 8 ± 0.5 g, supplied by Animal Center of Chinese Academy of Medical Sciences, were housed in a light, temperature and humidity controlled environment, and divided into 4 groups randomly. Group A (control): administered with normal saline, group B–D: treatment with (−)claus 5. 10 and 20 mg kg^{-1}, respectively. Drug was given by gavage once a day for 4 weeks. The animals were observed daily for any change in appearance or behavior. Food consumption and body weight were measured weekly. On day 27, the mice were trained to learn avoidance response to foot electric stimulation, then sacrificed for measurement of brain development. The mice were decapitated,

brains were weighed, thickness of cerebral cortex was measured by vernier caliper, dissected into two hemispheres, and rapidly frozen at −20°C.

Preparation of Electron Microscopic Specimen and Quantitative Analysis of Synapses [9]

(±), (−)Claus was synthesized by Department of Synthetic Chemistry in our institute. (±),(−)Claus was suspended in normal saline with a few drops of Tween80 and administered by oral gavage in a volume of 0.2 ml per mouse. Preparation was made fresh weekly.

Mice were anesthetized with ether and perfused through the heart with a solution of 2% formaldehyde, 3% glutaraldehyde in 0.1 mol L^{-1} PBS. Hippocampus was removed, CA_3 region was sectioned and placed in 3% glutaraldehyde for 2 h for prefixation. Then each slice was placed in 0.1 mol L^{-1} PBS for 24 h and on the next day, the samples were dehydrated in gradient ethanol solutions (35%, 50%, 70% and 100%). After a final dehydration in propylene oxide, the samples were embeded in Epon-Araldite in small dishes so that they could be oriented. Sections were cut perpendicular to the granule cell layer, stained with uranium citrate and photographed with a HITACHI-800 electron microscope. A series of consecutive photographs were taken at a primary magnification of 7000 × from the edge of the granular layer out to the exposed surface of the hippocampal fissure, then magnified additionally (1 × 4) using an enlarger during measurement.

Synaptic recognition was identified by the presence of pre- and postsynaptic membrane, synaptic cleft of 20–30 nm, synaptic vesicles in presynaptic membrane and terminal postsynaptic densities (PDS).

Memory Experiments

Step down and step through tests were carried out as in previous reports [10].

Statistical analysis of all parameters was performed by the Students' *t*-test.

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References

[1] Fifkova, E. and Harreveld, A.V. (1977) *J. Neurocytol.*, **6**, 216.
[2] Popov, V.I. and Bocharova, L.S. (1992) *Neuroscience*, **48**, 53.
[3] Huang, D.F. and Huang, L. (1991) *Chinese Chemical Letters*, **2**, 291.
[4] Zhang, J.T and Jiang, X.Y. In: *Annual Academic Papers Collection of CAMS & PUMC*. Beijing: Joint Press House of BMU & PUMC. 1994: 356–365.
[5] Keller, G. and Schnidt, J. (1986) *Advances in the Biosciences*, **59**, 129.
[6] Hua, Z.W. (1986) *Progress Physiological Science*, **17**, 33.
[7] Horn, G., Bradley, P. and McGabe, B.J. (1985) *J. Neurosci.*, **55**, 3161.
[8] Popov, V.I., Bocharova, L.S. and Bragin, A.G. (1992) *Neuroscience*, **48**, 45.
[9] Crain, B., Cotman, C., Taylor, D. and Lynch, G. (1973) *Brain Research*, **63**, 194.
[10] Petukhov, V.V. and Popov, V.I. (1986) *Neuroscience*, **18**, 823.