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Effect of succinyl cholecystokinin heptapeptide on synaptosomal ^{45}Ca uptake of mouse hippocampus

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AIM: To study the effect of a new peptide, succinyl cholecystokinin heptapeptide (SCH), on the uptake of ^{45}Ca by synaptosomes from mouse hippocampus, and compare it with that of sincalide. **METHODS**: Protein concentration was determined by the dye method. ^{45}Ca uptake was determined by adding $^{45}CaCl_2$ into synaptosome suspending solution. Samples were incubated and terminated by filtration through glass microfiber filters and finally counted in a Beckman LS 9800 liquid scintillation spectrometer. RESULTS: SCH 0.125 — 2 $\mu mol \cdot L^{-1}$ dose-dependently depressed synaptosomal ^{45}Ca uptake, and the depressive extent was similar to that of sincalide. The suppressive effect induced by either SCH or sincalide on synaptosomal ^{45}Ca uptake was antagonized by proglumide, which itself did not affect ^{45}Ca uptake of synaptosomes. The inhibition of synaptosomal ^{45}Ca uptake induced by SCH or sincalide was blocked by β-endorphin. **CONCLUSION**: The replacement of the *N*-terminal aspartic acid residue of sincalide does not influence its effect on ^{45}Ca uptake of hippocampal synaptosomes.

KEY WORDS hippocampus; synaptosomes; calcium radioisotopes; cholecystokinin; proglumide; β-endorphin; sincalide

Sincalide (cholecystokinin octapeptide, Cho-8) exists in brain as a main form of cholecystokinin (Cho) and regulates many functions of brain[1,2]. We synthesized a new peptide, succinyl cholecystokinin heptapeptide (SCH, Suc-Tyr (SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2). SCH increased the contraction of gall bladder[3]. Hippocampus is not only the part of high-containing Cho, but also the region concerning brain high functions, such as learning and memory. In this experiment, the effect of SCH on ^{45}Ca uptake by synaptosomes prepared from mouse hippocampi was examined, and the antagonism of proglumide, B-type antagonist of Cho, against SCH was also studied.

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MATERIALS AND METHODS

Materials Kunming strain mice ($n = 40$), weighing $23 \pm s$ 1 g, were provided by the Animal Breeding Center, Nanjing Institute of Dermatology, Chinese Academy of Medical Sciences. SCH was synthesized by Department of Biochemistry, Nanjing University. Proglumide was purchased from Nanjing Pharmaceutical Factory. β-Endorphin was the product of Organon Co, Holland. $^{45}CaCl_2$ (specific activity 1406 $MBq \cdot g^{-1}$) was purchased from Institute of Atomic Energy, Chinese Academy of Sciences.

Preparation of hippocampus synaptosomes[4,5] The resulting pellets were resuspended to 0.5 mL in physiological buffer solution (NaCl 143, KCl 4.7, $CaCl_2$ 1, $MgCl_2$ 1.2, $NaHCO_3$ 24.9, Tris-base 20 and glucose 10 $mmol \cdot L^{-1}$, pH 7.4)[6]. Protein concentration was determined by the dye method[7].

Determination of ^{45}Ca uptake ^{45}Ca uptake was determined according to the method[8] with some modifications. Synaptosomes from 40 mice were resuspended in physiological buffer solution at a protein concentration of about 0.4 $g \cdot L^{-1}$ and drugs were added. Parallel controls contained drug vehicles. The volume of each sample was adjusted to 0.5 mL by physiological

buffer solution. These synaptosomes were incubated at 30℃ for 10 min. $^{45}CaCl_2$ was diluted in the buffer solution and added (0.5 mL, 18.5 kBq). Samples were incubated for a further 10 min at 30 ℃, which was terminated by filtration through glass microfiber filters (Yuguang Pure Materials Co, Shanghai) prewashed with 10 mL of ice-cold physiological buffer solution. The synaptosomes retained on the filters were washed with 10 mL of the prewished solution. The filters were dried, placed in 5 mL of scintillation fluid (0.5 % PPO, 0.03 % POPOP), and counted in a Beckman LS 9800 liquid scintillation spectrometer.

Results were expressed as $\bar{x}\pm s$ and compared by t test.

RESULTS

SCH (0.125 to 2 μmol·L^{-1}) inhibited ^{45}Ca uptake of hippocampal synaptosomes in mice (Fig 1).

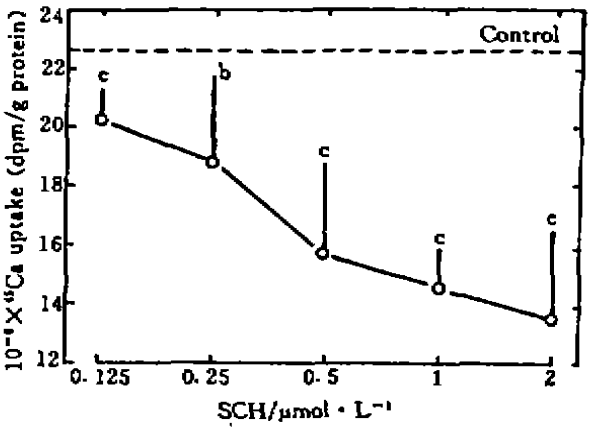

Fig 1. Effect of SCH (0.125 − 2 μmol·L^{-1}) on ^{45}Ca uptake by synaptosomes of hippocampus. n=8 experiments, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ vs control.

Proglumide itself did not alter ^{45}Ca uptake of hippocampal synaptosomes, but proglumide 1 μmol·L^{-1} antagonized the suppressive effect induced by SCH (1 μmol·L^{-1}) on ^{45}Ca uptake of synaptosomes (Tab 1). The depressive effect induced by sincalide on synaptosomal ^{45}Ca uptake was similar to that by SCH and was also antagonized by proglumide.

Tab 1. Interaction of Cho with proglumide or β-endorphin on synaptosomal ^{45}Ca uptake of mouse hippocampus. All doses were 1.0 μmol·L^{-1}. n=6−10 experiments, $\bar{x}\pm s$.
$^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs control.

	n	$10^{-6}\times$dpm/g protein
Control	8	22.5±1.4
SCH	10	14.6±1.6^c
Sincalide	10	14.9±3.2^c
Proglumide	6	24.1±4.7^a
SCH+Proglumide	6	22.7±1.0^a
Sincalide+Proglumide	6	23.7±2.3^a
β-Endorphin	8	26.2±3.7^b
SCH+β-endorphin	8	28.1±4.1^c
Sincalide+β-endorphin	8	25.7±3.8^a

When β-endorphin (1 μmol·L^{-1}) and SCH (1 μmol·L^{-1}) or sincalide (1 μmol·L^{-1}) were added together, the depressive effect induced by SCH or sincalide on synaptosomal ^{45}Ca uptake was blocked by β-endorphin (Tab 1).

DISCUSSION

Cho exists in brain with various fragments and has complex functions. Tyrosine residue and methionine residue of sincalide could greatly affect biological activity[9]. When N-terminal aspartic acid residue of sincalide was replaced by succinyl radical, a new peptide was obtained. The peripheral effect of SCH was significantly superior to that of sincalide[3]. However, Our experimental results showed that the suppressive effect induced by SCH on ^{45}Ca uptake of hippocampal synaptosomes was similar to that by sincalide. The extents of antagonism by proglumide, which is an antagonist of Cho receptor, against the effect of SCH and sincalide on synaptosomal ^{45}Ca uptake were also identical. These results suggested that the replacement of the N-terminal aspartic acid residue of sincalide do

not influence its effect on ^{45}Ca uptake of hippocampal synaptosomes.

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琥珀酰缩胆囊素七肽对小鼠海马突触体摄取 ^{45}Ca 的影响

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目的：探讨琥珀酰缩胆囊素七肽(SCH)对小鼠海马突触体摄取^{45}Ca的影响，并与辛卡利特作了比较. **方法**：蛋白质浓度的测定用染料法. ^{45}Ca的摄取测定采用$^{45}CaCl_2$加入突触体悬液中孵育后用玻璃微纤维过滤器过滤，用液闪光谱仪计数. **结果**：SCH是辛卡利特异构物. 实验表明在0.125 － 2 μmol·L^{-1}的剂量范围内SCH呈剂量依赖性地抑制小鼠海马突触体摄取^{45}Ca. 其抑制的程度与辛卡利特近似. SCH与辛卡利特的这种抑制效应能被丙谷胺、β-内啡肽所拮抗. **结论**：辛卡利特*N*端天冬氨酸残基的改变对海马突触体摄取^{45}Ca无明显影响.

关键词 海马；突触体；钙放射性同位素；缩胆囊素；丙谷胺；β-内啡肽；辛卡利特