关键词 黄皮酰胺；培养的细胞；胆碱乙酰转移酶；阿糖胞苷；前脑叶；大脑皮质

目的：研究左旋，右旋黄皮酰胺对培养脑皮层神经元发育有无促进作用. 方法：用比色法测定胆碱乙酰转移酶(ChAT)活性，用 Folin 酚法测定蛋白含量，细胞生长发育状态在倒置相差显微镜下动态观察. 结果：左旋黄皮酰胺(0.001 – 10 μmol·L^{-1})能促进皮层神经元细胞发育，光镜下看到细胞密度增加，突触生长旺盛；培养细胞中 ChAT 活性及蛋白含量较对照组明显增高. 右旋黄皮酰胺却无神经营养作用，且在高浓度时对培养神经元有损伤作用. 结论：左旋黄皮酰胺促进中枢胆碱能神经元发育，易化突触结构的可塑性.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Jul; 19 (4): 336 – 338

Effect of *Coriaria* lactone on cytosolic free calcium of cultured neurons from rat cerebral cortex[1]

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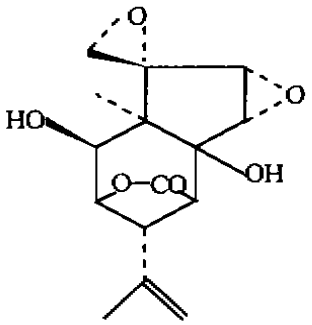
KEY WORDS *Coriaria*; lactones; neurons; cultured cells; calcium; cerebral cortex

AIM: To study the effect of *Coriaria* lactone (CL) on cytosolic free calcium ($[Ca^{2+}]_i$) of cultured neurons from cerebral cortex. **METHODS**: Primary neuron culture (14 d) and AR-CM-MIC cation measurement system were used, the $[Ca^{2+}]_i$ were measured. CL effect was observed by loading egtazic acid. **RESULTS**: The $[Ca^{2+}]_i$ of cultured neurons (99.4 – 103.4) nmol · L^{-1} was elevated concentration-dependently by CL (25 – 500) μmol·L^{-1} ($P < 0.01$). This effect disappeared after loading egtazic acid 5 mmol · L^{-1}, but reappeared after adding $CaCl_2$ to 1 mmol · L^{-1}. **CONCLUSION**: The $[Ca^{2+}]_i$ of cultured neurons was elevated by CL, depending on extracellular Ca^{2+}.

Epilepsy is a common disorder. The animal models evoked by *Coriaria* lactone (CL) was suitable to study the epileptogenesis[1]. The elevation of cytosolic free calcium ($[Ca^{2+}]_i$) is the key step in the process of neuronal injure and death[2]. Calcium regulation is a core problem in the study of epileptogenesis and the results were analysed easily using the cultured neurons. The present study was designed to investigate the effect of CL on $[Ca^{2+}]_i$.



Coriaria lactone

MATERIALS AND METHODS

Agents and equipments CL (West China Medical University Pharmaceutical Factory, Lot № 8338, purity 98 %, pH 3.5 – 5.5, melting point 211 – 2 ℃, $[\mu]_D$ + 10, $C_{15}H_{18}O_6$). AP_5, verapamil, and Fura 2-AM (Sigma Co). AR-CM-MIC cation measurement system (Spex Co). Diapho-TMD fluorescence microscope

[1] Project supported by the National Natural Science Foundation of China, № 39330210.

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Received 1996-08-19 Accepted 1997-10-07

(Nikon Co).

Neuron culture Cerebral cortical neurons were prepared from 18-d-old rat fetuses, the meninges were removed. The brain was mechanically disrupted in Ca^{2+}-Mg^{2+}-free *Hanks' balance salt solution*. The cell suspension was spun at $100 \times g$ for 5 min. The pellet was suspended with DMEM/F_{12} containing 15 % fetal calf serum and filtered through a nylon mesh (size 60 um), suspension contained 5×10^6 cells in 1 mL was cultured for 50 min, the cells from culture suspension were seeded again onto 24 mm × 24 mm slides coated for 24 h with poly-L-lysine 25 mg·L^{-1}, and cultured at 37 ℃ in a humidified 5% CO_2 + 95 % air. The culture medium was renewed every 4 d. The neurons were cultured for 14 d. The typical pyramid-like neurons were used.

Measurement of $[Ca^{2+}]_i$ The neurons were loaded with Fura 2-AM 3 μmol·L^{-1}, at 37 ℃ for 45 min. The loaded neurons were washed twice in Mg^{2+}-free Hanks' balance salt solution, and then were laid on Mg^{2+}-free Hanks' balance salt solution 1 mL to measure the calcium concentration of single neuron. The $[Ca^{2+}]_i$ was determined from the ratio of the fluorescence emission using λ_{ex} 340 nm and 380 nm and λ_{ex} 550 nm with AR-CM-MIC cation measurement system. The $[Ca^{2+}]_i$ was calculated according to the formula[3]:

$$[Ca^{2+}]_i = K_d \cdot \beta \cdot (R - R_{min})/(R_{max} - R)$$

RESULTS

The $[Ca^{2+}]_i$ of cultured neurons (99.4 – 103.4 nmol·L^{-1}) was elevated by CL (25 – 500) μmol·L^{-1} in a concentration-dependent manner (Fig 1).

$[Ca^{2+}]_i$ level of each experimental group had an obvious difference compared with the control (Tab 1).

The effect of CL disappeared after the addition egtazic acid 5 mmol·L^{-1}, but reappeared after the addition of $CaCl_2$ to 1 mmol·L^{-1} in the medium (Fig 2).

DISCUSSION

Calcium ions (Ca^{2+}) has an important role in many cellular events including signal transduction, regulation of cellular excitability and neurotransmitter release. Ca^{2+} is an universal second messenger. Mean while, Ca^{2+} also involved in the pathogenesis of many kinds of disease[4], neuron death had association with sustained increase of $[Ca^{2+}]_i$. Epilepsy may be defined as an intermittent derangement of the nervous system due presumably to a sudden, excessive, disorderly discharge of cerebral neurons. The relationship between the Ca^{2+}

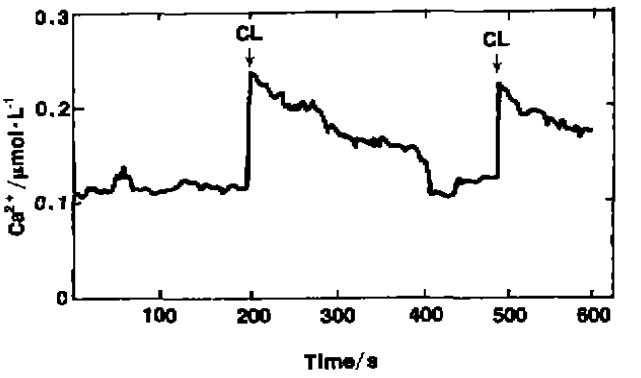

Fig 1. Effect of *Coriaria* lactone (CL) on cytosolic free Ca^{2+} of single cultured neurons.

Tab 1. Effect of *Coriaria* lactone on cytosolic free calcium. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Coriaria lactone/ μmol·L^{-1}	Cells	$[Ca^{2+}]_i$/μmol·L^{-1}
0	20	0.101 ± 0.002
2.5	15	0.12 ± 0.08
25	11	0.24 ± 0.10^c
100	15	0.31 ± 0.14^c
500	5	0.36 ± 0.09^c

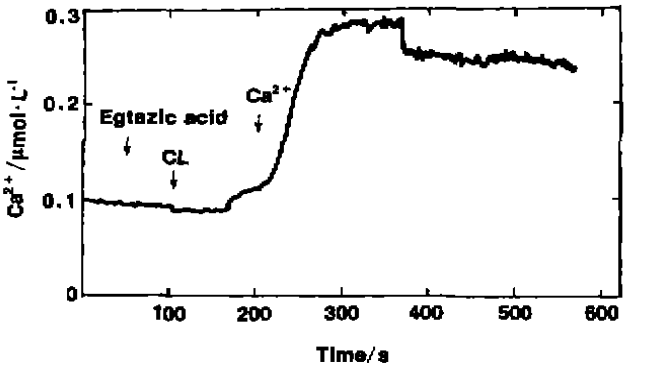

Fig 2. Influence of extracellular calcium on *Coriaria* lactone (CL) effect.

regulation and the epileptogenesis has become the focus of study in recent years. The decrease of extracellular calcium concentration was prior to the convulsive discharge, that indicated Ca^{2+} entered to neuron excessively had pacemaker effect in convulsive activities of some neurons[5]. The present study suggested that the $[Ca^{2+}]_i$ of cultured neurons from cerebral cortex was elevated by convulsant CL and had an obvious dose-effect relationship in the concentration of 25 – 500 $\mu mol \cdot L^{-1}$. The effect disappeared when adding excessive egtazic acid to the medium, that indicated CL effect was mainly dependent on extracellular calcium. The curve of CL effect was divided into up-phase and down-phase, the calculation results of many neurons identified the obvious dose-effect relationship, but the top of curve located in the same level, that indicated CL effect was associated with the Ca^{2+}, Mg^{2+}-ATPase activity inhibition[6]. Elevating $[Ca^{2+}]_i$, resulted in the increase of neurotoxicity following the Ca^{2+} dependent release of the presynaptic excitatory neurotransmitter[7]. CL induced LTP through elevated $[Ca^{2+}]_i$, that may be contributed to kindling mechanism of animal models.

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马桑内酯对培养大鼠大脑皮质神经元胞内游离钙的作用[1]

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关键词 马桑; 内酯类; 神经元; 培养的细胞; 钙; 大脑皮质

目的: 研究致痫剂马桑内酯对大脑皮质神经元胞内游离钙($[Ca^{2+}]_i$)的作用及影响因素. **方法**: 利用培养 14 天的大鼠大脑皮层神经元和 AR-CM-MIC 阳离子检测系统, 观察了不同浓度马桑内酯对 $[Ca^{2+}]_i$ 的作用及细胞外钙对其作用的影响. **结果**: 培养神经元的基础钙水平为 (99.4 – 103.4) $nmol \cdot L^{-1}$, 马桑内酯使 $[Ca^{2+}]_i$ 水平升高, 在 (25 – 500) $\mu mol \cdot L^{-1}$ 范围内, 量效关系明显, 加入 egtazic acid 5 $mmol \cdot L^{-1}$ 至介质中马桑内酯作用消失, 补充 $CaCl_2$ 1 $mmol \cdot L^{-1}$ 作用又出现. **结论**: 马桑内酯可升高神经元 $[Ca^{2+}]_i$, 其升高 $[Ca^{2+}]_i$ 的作用依赖于细胞外钙.