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MDR 细胞株 K562r 细胞内药物积聚的恢复并不代表其抗药性的逆转

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摘要 本文探讨细胞内药物积聚与细胞抗药性的相关性. MTT 法揭示 K562r 细胞对 Dox、Epi、Dau 及此三药混合物的 IC_{50}分别比 K562 细胞提高22、16、10及20倍. K562r 细胞分别与 Dox、Epi 或 Dau (2—32 $\mu g \cdot ml^{-1}$)温育1 h，其细胞内药物含量显著低于 K562细胞. 但细胞与三药混合物温育1 h，二者细胞内药物积聚除3个例外均无显著差异，提示 K562r 细胞内药物积聚与其药敏无相关性.

关键词 阿霉素；表柔比星；柔红霉素；合并用药；肿瘤细胞培养；抗药性

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Protein c-*fos* induction in rat brain and spinal dorsal horn by sincalide and opioids *in vitro*

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ABSTRACT Sincalide (CCK-8) is an antiopioid substance. In this study, we investigated the effects of sincalide and opioids on c-*fos* expression and their interaction in brain and spinal dorsal horn *in vitro*. Immunoprecipitation was used for detection of c-*fos* protein. The results indicated that 0.1 $\mu mol \cdot L^{-1}$ sincalide induced c-*fos* expression markedly in both brain (a 3.8-fold increase in c-*fos* protein level) and in spinal dorsal horn (a 3.6-fold increase). NDAP (a κ receptor agonist) 0.1 $\mu mol \cdot L^{-1}$ showed some activating effects on c-*fos* expression, the c-*fos* protein level increased 2.7 and 2.6 times respectively in brain and spinal dorsal horn. Ohmefentanyl (Ohm, a μ receptor agonist) 0.1 $\mu mol \cdot L^{-1}$ also exhibited an inducing effect on c-*fos* protein production. Sincalide and NDAP exerted some additive effects on c-*fos* protein production in spinal cord. In contrast, the effect of sincalide on c-*fos* protein production is antagonistic to that of Ohm. The results suggested that there were changing patterns of interaction on c-*fos* expression between sincalide and opioids.

KEY WORDS proto-oncogene proteins c-*fos*; sincalide; narcotics; brain; spinal cord; precipitin tests; autoradiography.

Sincalide (CCK-8) showed antiopioid activities in pain modulation[1], food intake[2], and brain dopamine turnover rate[3]. Sincalide could modify κ and μ opioid receptors[4], and reverse the inhibitory effect of opioids on synaptosomal Ca^{2+} intake[5]. Nuclear proto-oncogene c-*fos* protein function as a part of

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the third messenger system, involved in the process of stimulus-transcription coupling[6]. As a "third messenger" molecule in the signal transduction system, it would couple short-term intracellular signals elicited by a variety of extracellular stimuli to long-term responses by altering gene expression.

The aim of this work was to examine the patterns of c-*fos* expression in rat brain and spinal cord tissues *in vitro* treated with sincalide and opioids, and to study the interaction between them attempting to obtain evidences on proto-oncogene expression of sincalide and opioids.

MATERIALS AND METHODS

Materials *L*-[^{35}S]methionine (41.75 GBq·mol^{-1}) was made by New England Nuclear Inc, USA; c-*fos* antibody (purified rabbit antibody raised aganist the peptide corresponding to residures 4 to 17 of human c-*fos*) was obtained from Oncogene Science Inc, USA; Dulbecco's modified Eagle medium (DMEM), methione-free DMEM, immunoprecipitatin (formalin-fixed *Staphylococcus aureus*) and molecular weight protein standards were made by Gibco. Sincalide was donated by Squibb, USA. The κ opioid receptor agonist (*N*-Methyl-Tyr^1, *N*-methyl-Arg^7, *D*-leu^8)dynorphin1-8 ethyl amide (NDAP) was donated by the Tsukuba Research Lab of Eisai Co, Japan. The μ receptor agonist *N*-[1-(beta-hydroxy-beta-phenethyl)-3-methyl-4-piperidyl]-*N*-phenylpropionamide (ohmfentanyl HCl, Ohm) was obtained from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Tissue incubation Adult Wistar rats ($n = 3$) were killed by cerical dislocation. The brain (without cortex and cerebellum) and spinal dorsal horn were cut into 1-mm^3 pieces aseptically, then transferred to sterile Petri dishes, and incubated in DMEM medium (100 mg tissue·ml^{-1}) under 95 % air + 5 % CO_2 at 37 ℃. After 2 h of preincubation, the culture was transferred to an 1 ml methionine-free DMEM medium containing the testing agents, and *L*-[^{35}S]-methione (3.7 kBq·ml^{-1}). The control was incubated in methionine-free DMEM with solvents. Cultures were incubated for another 2 h.

Cell lysate and immunoprecipitation[7] The cell nuclei were isolated for preparing the nuclear lysates in RIPA buffer [Tris-HCl, 10 mmol·L^{-1}, pH 7.5, NaCl, 150 mmol·L^{-1}, 1 % Nonidet-P40, 1 % sodium deoxycholate, 0.1 % SDS, 1 % aprotinin, and phenylmethylsulfonyl fluoride (PMSF), 0.25 mmol·L^{-1}]. The lysate 1 ml was incubated with 10 μl (1 : 100) c-*fos* polyclonal antibody for 24 h at 4 ℃, followed by a further 1-h incubation with 10 μl of goat anti-rabbit IgG. Immune complexes were collected with 250 μl of 10 % suspension of fixed *Staphylococcus aureus* cells (Pansorbin). Immunoprecipitated proteins were separated on 10 % SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were dried and processed by autoradiography. Quantitative analysis of autoradiographic bands was performed by IBAS (Image Analysis System), Opton Co, Germany. The density of bands (Absorbance) was obtained for calculating the relative changes of c-*fos* protein.

RESULTS

Induction of c-*fos* expression by sincalide and/or NDAP in brain In the control group, very little c-*fos* protein was detected (Tab 1).

Tab 1. Effects of sincalide, NDAP, and Ohm on c-*fos* protein production in rat brain and spinal cord tissues *in vitro*. $n=3$ **rats,** $\bar{x}\pm s$. $^cP<0.01$ *vs* **control.** $^eP<0.05$, $^fP<0.01$ *vs* **corresponding group.**

0.1 μmol·L^{-1}	c-*fos* protein/Absorbanc	
	Brain	Spinal cord
Control	0.103±0.005	0.105±0.011
Sincalide	0.364±0.019^c	0.356±0.012^c
NDAP	0.268±0.031^c	0.273±0.012^c
Sincalide+NDAP	0.355±0.032^c	0.564±0.016^c
Ohm	0.212±0.003^c	0.166±0.010^c
Sincalide+Ohm	0.325±0.008^c	0.268±0.015^c

Sincalide (0.1 μmol·L^{-1}) triggered a dramatic induction of c-*fos* protein in brain, c-*fos* protein level being 3.8 times *vs* control group. In the presence of NDAP (0.1 μmol·L^{-1}), the c-*fos* protein production was 2.7 times *vs*

control group. When sincalide (0.1 μmol·L^{-1}) and NDAP (0.1 μmol·L^{-1}) were added simultaneously, the c-*fos* protein level was similar to that induced by sincalide alone.

Induction of c-*fos* expression by sincalide and/or NDAP in spinal cord The c-*fos* protein production increased 3.6 times and 2.6 times under the treatment of sincalide (0.1 μmol·L^{-1}) and NDAP (0.1 μmol·L^{-1}), respectively. Sincalide plus NDAP evoked a marked induction of c-*fos* expression. Its level was about 5.6 times *vs* control (Tab 1).

Induction of c-*fos* exprtession by sincalide and/or Ohm in brain Ohm 0.1 μmol·L^{-1} increased c-*fos* protein production in brain about 2.1 times. The combined action of sincalide (0.1 μmol·L^{-1}) and Ohm (0.1 μmol·L^{-1} induced a c-*fos* protein prodcution at a level intermediate between those induced by sincalide or Ohm alone.

Induction of c-*fos* expression by sincalide and/or Ohm in spinal cord Results were similar to those observed in the brain. Ohm induced a moderate increase in c-*fos* expression, whereas a combined action of sincalide and Ohm resulted in a c-*fos* protein level less than that induced by sincalide alone.

DISCUSSION

Induction of c-*fos* has been described in different areas of the CNS after a wide variety of physiological or pharmacological stimuli and it has been proposed as a marker for neuronal activities[8]. c-*fos* protein forms heterodimers with Jun protein and participates in the formation of transcriptional complex AP-1, which modifies the expression of target genes[9].

Recent immunocytochemical studies have shown the presence of c-*fos*-immunoreactive neuronal nuclei in brain tissue of experimentally manipulated rat and, to a lesser degree, in control rats. Following the application of diverse stimulations, an increase in c-*fos* was observed in different regions of the brain[10,11]. In the spinal cord, expression of c-*fos* was not detectable in control animals, but could be elicited through experimental stimulation[12,13]. In the present study, there was very little c-*fos* protein detected in the rat brain and spinal tissues, implying that the animals were not vigorously excited during the experimental procedure.

Results of the present study clearly indicated that CCK-8 at 0.1 μmol·L^{-1} concentration caused a 3—4 fold increase in c-*fos* protein expression in rat brain and spinal cord tissues. To our knowledge, this is the first report showing such an effect using *in vitro* immunoprecipitation detection of c-*fos* protein. Very few data were available about the expression of c-*fos* in brain and spinal cord under the treatment of sincalide. Concerning the effects of opioid on c-*fos* expression, our data showed that both κ opioid agonist NDAP and μ opioid agonist Ohm are effective in inducing c-*fos* protein. At a same molar concentration (0.1 μmol·L^{-1}), the κ agonist NDAP induced a 2—3 fold increase, whereas the μ agonist Ohm a 2 fold increase. In other words, the opioids and the antiopioid peptide sincalide are working here in the same direction, which is in contrast with their effects on intracellular free calcium [Ca^{2+}], opioids decrease while sincalide increases the cellular [Ca^{2+}] level.

In the case of sincalide plus NDAP, their effects on c-*fos* expression in spinal tissue were additive, but not in the brain, a combined effect of sincalide and NDAP being not more potent than the effect of sincalide alone. In the case of sincalide plus Ohm, the combined effects were less than those of sincalide alone, but higher than those of opioids alone. So we are facing a complex profile with three

different patterns. More studies are needed in order to find out a general rule governing the interaction between sincalide and opioids in affecting c-*fos* expression in CNS.

Several studies indicated that an increase of dynorphin and enkephalin and their corresponding mRNAs in dorsal horn neurons was preceded by the expression of c-*fos*[14]. The current results demonstrated the κ and μ opiate receptor activation could directly induce c-*fos* expression at different levels in rat brain and dorsal horn tissues.

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辛卡利特和阿片肽诱导大鼠脑和脊髓背角 c-*fos* 蛋白表达

R965.2

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摘要 通过免疫沉淀法检测 c-*fos* 蛋白，研究辛卡利特和和阿片肽对大鼠脑和脊髓 c-*fos* 表达的影响。结果表明，辛卡利特和 NDAP 可刺激脑和脊髓 c-*fos* 的表达（P 均<0.01），羟甲芬太尼也有诱导作用（$P<0.01$）。而辛卡利特和 NDAP 对脊髓 c-*fos* 的表达有协同作用（$P<0.01$），相反，羟甲芬太尼则拮抗辛卡利特对脑和脊髓 c-*fos* 的表达（$P<0.05$）。结果提示在 c-*fos* 水平辛卡利特与不同的阿片肽起不同作用。

关键词 原癌基因蛋白 c-*fos*；辛卡利特；麻醉剂；脑；脊髓；沉淀试验；放射自显影