[Biol. Pharm. Bull., 23, 231-234 (2000)]

[Lab. of Pharmacognosy]

## Molecular Cloning and Characterization of a cDNA for *Glycyrrhiza glabra* Cycloartenol Synthase.

Hiroaki HAYASHI, \* Noboru HIRAOKA, Yasumasa IKESHIRO, Tetsuo KUSHIRO, Masayo MORITA, Masaaki SHIBUYA and Yutaka EBIZUKA

A cDNA clone (GgCAS1) encoding cycloartenol synthase (CAS) has been isolated from *Glycyrrhiza glabra* (licorice) by cross-hybridization with that of *Pisum sativum* CAS as a probe. The deduced amino acid sequence of GgCAS1 exhibits 89%, 83%, and 81% identities to those of *Pisum sativum*, *Panax ginseng*, and *Arabidopsis thaliana* CASs, respectively. CAS activity has been detected in the homogenate of the yeast transformed with the expression vector containing the open reading frame of GgCAS1. Southern blot analysis suggested that at least two CAS genes exist in the licorice genome. In Northern blot analysis, the strong signal for CAS mRNA is detected in the cultured licorice cells of all growth phases, but no significant increase of CAS mRNA expression was observed in the cells treated with the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor, pravastatin.

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[Lab. of Molecular Biology]

## Dietary n-3 Fatty Acid Deficiency Decreases Nerve Growth Factor Content in Rat Hippocampus.

Atsushi IKEMOTO, Atsumi NITTA, Shoei FURUKAWA \*, Masayo OHISHI, Akira NAKAMURA,

Yoichi FUJII and Harumi OKUYAMA

Dietary deprivation of  $\alpha$ -linolenic acid (n-3) through two generations has been shown to lower performance in an operant-type brightness-discrimination learning test in rats. Here, we examined a possible correlation between NGF content and n-3 fatty acid status in the brain. Female rats were fed a semipurified diet supplemented with safflower oil (n-3 fatty acid-deficient) and their offsprings were fed a diet supplemented with either 3% safflower oil (Saf group) or a mixture of 2.4% safflower oil plus 0.6% ethyl eicosapentaenoate (Saf+EPA group) after weaning. The brain docosahexaenoic acid (22:6n-3, DHA) content in the Saf group was less than half of that in the Per group fed a diet supplemented with 3% perilla oil (n-3 fatty acid-sufficient) throughout the duration of the experiment. The DHA level of the Saf+EPA group was restored to the level of the Per group. However, the NGF contents in the hippocampus of the Saf and Saf+EPA groups were half that of the Per group. In the piriform cortex, the NGF content tended to be higher in the Saf and Saf+EPA groups than in the Per group. These results indicate that dietary n-3 fatty acid deficiency and restoration affect NGF levels differently among different brain regions.

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[Lab. of Molecular Biology]

## Neurotrophic Function of Conditioned Medium from Human Amniotic Epithelial cells.

Saiko UCHIDA, Yukiko INANAGA, Makiko KOBAYASHI and Shoei FURUKAWA\*,

Makoto ARAIE and Norio SAKURAGAWA

Human amniotic epithelial cells (HAEC) may have pluripotent function because they are formed from the epiblast cells at the 8th day of fertilization. Previously, we reported that HAEC have the capacity to synthesize and release acetylcholine and catecholamine associated with the binding sites of catecholamine receptors. We show the neurotrophic function of a conditioned medium from HAEC using cultured cortical neurons of E18 rats. Extensive analyses with various techniques demonstrated that HAEC and immortalized HAEC synthesize and release BDNF, NT-3 and NGF. Other neurotrophic factors were not detected in a cultured medium of HAEC by enzyme immunoassay. Various neurotrophic factors or growth factors did not show neurotrophic effects on E18 rat neuron except for EGF. Because EGF was not detected in the conditioned medium of HAEC, these data indicate an unidentified neurotrophic factor presently that is synthesized and released from HAEC. The amniotic membrane may have a significant role in supplying neurotrophic factors to the amniotic fluid as well as neurotransmitters, suggesting an important function to the early stages of neural development in the embryo.

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[Lab. of Molecular Biology]

## Two Novel Diterpenoids, Erinacines H and I from the Mycelia of Hericium erinaceum.

Eun Woo LEE, Kazue SHIZUKI, Satoshi HOSOKAWA, Masami SUZUKI, Hiroyuki SUGANUMA, Takahiro INAKUMA, Jingxuan LI, Mayumi OHNISHI-KAMEYAMA, Tadahiro NAGATA,

Shoei FURUKAWA\* and Hirokazu KAWAGISHI

Novel diterpenoids, erinacines H and I, were isolated from the cultured mycelia of *Hericium erinaceum*. The structure of the compounds were determined by interpretation of the spectral data. Erinacine H showed stimulating activity of nerve growth factor (NGF)-synthesis.