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The expression of c-fos and HSP70 by the Capsaicin injection in the spinal cord(dorsal horn)

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Capsaicin 적용 후 손상된 흰쥐 척수내 c-fos와 HSP70의 발현

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〈 국문초록 〉

C-fos는 원종양유전자(proto-oncogene)인 v-fos의 세포 동족체로써, 성장인자나 신경전달 물질에 의해 수분 내에 다양한 형태의 세포에서 활성화된다. Fos 단백질은 스트레스와 통증 과정의 신호전달기전에서 세포활동을 조절하는 3차 전령으로 활동한다.

열충격 단백질(Heat shock protein : 이하 HSP)은 계통발생학적으로 초기 척추 동물에서부터 발현되며 생체방어체계의 중요한 인자로 세포가 고열, 외상, 허혈 등의 스트레스에 직면했을 때 발현이 증가하는 단백질로 알려져 있다.

본 연구에서는 캡사이신(capsaicin)으로 말초 신경병변을 유발시킨 후 통각신경활성의 지표로 이용되는 원종양 유전자인 c-fos의 발현과 열 또는 스트레스로 야기되는 손상에 대한 조직의 방어작용으로 발현되는 HSP 70의 발현을 동시에 관찰함으로서, 급성으로 유발된 말초 신경병변의 확인과 동시에 실험동물 체내에서 방어적인 역할을 밝히는 일환으로 이 실험을 실시하였다.

본 실험의 결과는 다음과 같다;

1. 척수 등쪽뿔 천층(Laminae Ⅰ and Ⅱ)에서 각각 c-fos와 HSP70을 항원으로 하는 면역조직화학적 방법으로 염색

한 표본에서 0.9% NaCl 투여 2시간 후 c-fos와 HSP70의 양성을 나타내는 세포는 전혀 없음을 알 수 있었다.

2. 척수 등쪽뿔 천층에서 c-fos 단백질을 항원으로 하는 면역조직화학적 방법으로 염색한 표본에서 Capsaicin 투여 2시간 후 c-fos 단백질에 양성을 나타내는 세포가 많이 발현됨을 육안적 관찰로서 알 수 있었다.

3. 척수 등쪽뿔 천층에서 HSP70을 항원으로 하는 면역조직화학적 방법으로 염색한 표본에서 Capsaicin 투여 2시간 후 HSP70의 양성을 나타내는 세포가 보통수준으로 발현됨을 육안적 관찰로서 알 수 있었다.

이 실험의 결과로 볼 때, 화학적인 신경병변 유발물질에 의한 손상을 방어하기 위해서 체내에는 내인성 물질이 형성될 것이라는 추측과 c-fos 가 다른 유전자의 발현을 유도한다는 점을 함께 고려 하였을때, Capsaicin에 의한 말초 신경병변에서 c-fos 발현이 많이 나타나는 것은 손상을 방어하는 물질의 생성에 관여하기 때문이며, 방어물질 중 이 실험에서 본 HSP70도 증가한 내인성 방어물질의 하나라고 할 수 있을 것이다.

I. Introduction

Pain is one symptoms which is usually complained by patients. The process of pain transmission is well known in the anatomy of nerves, but there are many things not yet known about the mechanism or relieving symptoms or the analgetic effects(Besson & Chaouch, 1987). However, recent animal models of pain syndrome resulting from various insults were developed and widely used for pain research(Lee et al, 2000; Lee et al, 1998; Palecek et al, 1992; Kim & Chung, 1992; Seltzer et al, 1990).

Capsaicin is a neurotoxic substance accompany by chemical pain in humans(Caterina et al., 1997; Blackshaw et al., 2000), therefore neurogenic inflammation caused by capsaicin(Miao et al., 2000). Capsaicin's action on afferents is traditionally regarded as involving two phases: initial excitation which leads to transmitter release, followed by desensitization and damage after prolonged or repeated exposure(Caterina et al., 1997). Capsaicin is ,305.42(molecular weight), manifested activation of the recently cloned VR1 vanilloid receptor(David, 1997).

Capsaicin induces chemical acute pain as well as causes peripheral nerve damage, selectively. Capsaicin also reduces pain(Hayes & Tyers, 1980). When capsaicin is injected for the first time, we feel severe pain. But as time passes it becomes unsensitivity in response to stimuli of the same degree. So we can't feel pain any more. These analgesic effects are in proportion to the quantity of capsaicin.

C-fos which is homology of v-fos, proto-oncogene, is activated in cells of various form within minutes by a growth factor or neurotransmitter(Greenberg et al, 1985). Fos protein acts as a "third messenger" molecule in signal transduction systems of pain and stress(Curran, 1988; Naranfo et al, 1991). Once expressed, c-fos protein is translocated to the nucleus and interacts with DNA to regulate the transcription of other genes(Sambycetti & Curran, 1986). These lead to longterm adaptive responses and plastic responses(Goelet, 1986).

Heat shock protein is expressed ontogenetically from early vertebrae(Schlesinger et al, 1986). It is known that its expression is increased when the cell is faced with stress such as heat, trauma, and ischemia, as important factors in our protective system(Bienz & Pelham, 1987; Lindquist, 1986; Brown, 1990).

In this situation, we know that everything is expressed through the test at in vivo and in vitro(Herrera & Robertson, 1996; Brown, 1990).

This response is particularly characterized by an increase in the expression of a group of gene families, the heat shock stress, which is essential for a rapid recovery from the stress with minimal damaging(Shpund & Gershon, 1997)

Therefore, HSP is usee molecular marker of

metabolic stress, biochemical marker of damages(Massa, 1996).

HSP is named according to molecular weight. HSP of eucaryotic cell includes HSP 110, HSP 95, HSP 84, HSP 70, HSP 60, small HSP and so on. In general, HSP is present in the cytoplasm in unstress, but the expressions are increased when these are stimulated from outside and reduced primarily in recovery stage HSP 70 family included HSP 72, HSP 73, glucose-regulated protein(GRP) 75, GRP 78. Especially, HSP 72 rapidly shows the change of expression by stimulation quantitatively.

Cells survive in high temperature exposures by inducing the expression of heat shock proteins(Nwaka et al., 1996; Schlesinger, 1990)

The important action of HSP is folding of polypeptide and acting of molecular chaperone supporting assembly(Beckmann et al., 1990)

Research on relationship between HSP and c-fos has been done in other regions, not in the spinal region. Scammell et al(1993) identified that fos immunoreactive neurons are significantly increased in median preoptic nucleus and preoptic areas by exposure from the heat rather than from general situations or the cold.

In this study, we observed both c-fos expression which is marker of pain after peripheral nerve damage induced by capsaicin and HSP 70 expression which is expressed to defend tissue about damage resulting from heat or stress. We try to identify the role of defense in the animals' body after inducing nerve damage through expression of c-fos and HSP 70, respectively.

Ⅱ. Materials and methods

1. Animals

A total of 8 male Sprague-Dawley rats(weight of 200-250g) at 6 weeks of age were used. The test group was divided into control and experimental group. Control group is shamed group(c-fos and HSP70 expression after 0.9% saline treatment). The experimental group was divided into two subgroups: one is experimental group I (c-fos expression after capsaicin treatment), and the other is experimental group II (HSP70 expression after capsaicin treatment).

2. Methods(treatment and immunohistochemistry)

To induce pain and heat stress, four male rats(experimental group) were administered 50㎎/㎏ of capsaicin(8-methyl-N-vanillyl-6-nonenamide :sigma) with dissolved in 10% ethanol, 10% Tween-80 and 80% saline in subcutaneousness of hamstring muscles. Four other non-capsaicin treated rats(control group) were administered 50 ㎎/㎏ of 0.9% saline at the same region.

After two hours, rats perfused transcardially with a fixative solution containing 8% paraformaldehyde in 0.1M phosphate-buffer(pH 7.4) after anesthesia and post-fixed for 2h in the same fixative solution before transferring to 25% sucrose overnight for cryoprotection.

Transverse sections were cut through the spinal cord at one level, spinal segments L2-L6. Sections were cryosectioned at 25㎛ at the very low temperature(-35℃), and reacted for 24 hours at room temperature with anti-c-fos (1:200, sigma), anti-HSP70(1:100, Sigma) in 0.03% Triton X-100 and 0.2% normal goat serum. Sections were then washed thrice with 0.01M phosphate-buffer, incubated in biotinylated rabbit anti-sheep IgG for 90min at room temperature. After washing 3 times, the sections were treated with an avidin-biotinylated peroxidase complex for 60 min at room temperature (Vectastain ABC kit, Vector Labs, Burlingame, CA94010) and followed by 0.04% DAB(3' 5' -diaminobenzidine) in PBS with 0.03% H2O2(Wasserstoffperoxid 30%) for 10 min. Counterstaining was performed with Mayer's

haematoxylin and sections were dehydrated in an ethanol series and dried to the air, examined using an Olympus BX50 microscope at 10×40 magnifications

To manage results, we based on reaction per unit area under light microscope. Visual views represent marked change, prominent, present, absent at morphorlogic analysis as +++, ++, +, 0, respectively.

Ⅲ. Results

1. Control group(expression group of c-fos and HSP70 after 0.9% NaCl injection)

The expression of c-fos and heat shock protein 70(HSP 70) in superficial dorsal horn was not observed for 2 hours after 0.9% NaCl injection(Table. 1).

2. Experimental group I(expression group of c-fos after Capsaicin injection)

The number of c-fos immunoreactive neurons in superficial dorsal horn was increased markedly 2 hours after capsaicin injection(Table. 1, Fig. 1).

3. Experimental group II(expression of HSP70 after Capsaicin injection)

The expression of HSP 70 in superficial dorsal horn was observed ordinarily 2 hours after capsaicin injection(Table. 1, Fig. 2).

Table 1. Changes of control and experimental group after administer a dose

	control group		experimental group	
	c-fos	HSP70	c-fos	HSP70
2 hours	0	0	++	+

control group = expression of c-fos and HSP70 after 0.9% NaCl injection
experimental group = expression of c-fos and HSP70 after Capsaicin injection
+++ = marked change, ++ = prominent, + = present, 0 = absent

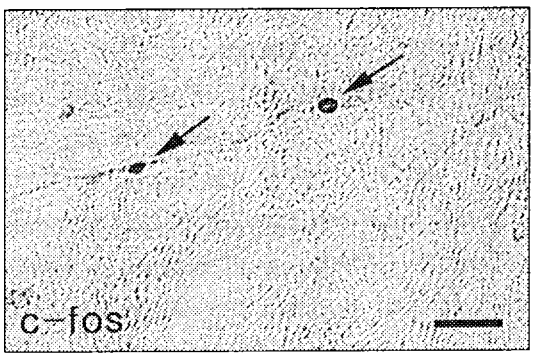

Fig.1 Photograph of the expression of c-fos immunoreactive neurons(arrow) in superficial dorsal horn at 2 hours after Capsaicin injection.
※ bar=20㎛ (×400)

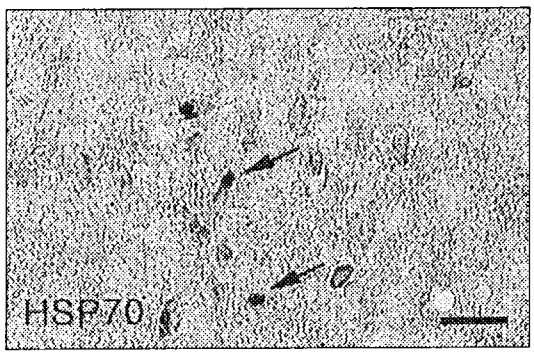

Fig.2 Photograph of the expression of HSP 70 immunoreactive neurons(arrow) in superficial dorsal horn at 2 hours after Capsaicin injection.
※ bar=20㎛ (×400)

Ⅳ. Discussion

The stress-provoked induction of HSP genes is specifically mediated by the heat-shock factor 1(HSF 1). In unstressed cells, HSF 1 is present in the cytoplasm as a monomer or forming

heteromeric complexes. Upon stress, this transcription factor homotrimerizes, translocates to the nucleus, and binds to its DNA recognizing sequence(Galan et al, 2001).

Much of the molecular damage exerted by these forms of stress involves conformational changes and patrial denaturation in protein molecules in the affected cells leading to aggregation and inactivation of the molecules(Shpund & Gershon, 1997). In cellular level, stress prevents transcription of large group of gene, interrupts mRNA process, and causes collapse of cytoskeleton(Welch & Georgopoulos, 1993).

HSP is important in the recovery of cAMP inner cells after heat shock. Zensho et al.(1998) reported that stress suppressed cAMP levels and HSP72 expression restored the cAMP formation. Calderwood et al.(1985) observed although heat shock leads to transient increases in cAMP, heat shock did not produce a pulse in cAMP level on heating. Also, heat shock increased the rate of arachidonic acid release and inositol phosphate, activated stimulation of a phospholipase A2 activity and phospholipase C(Calderwood, 1989).

HSP may aid repair of damaged tissue after injury through molecular chaperoning, protein folding, protein translocation or inhibition of apoptosis.(Allen & Chase, 2001).

Many researches had been done to observe the expression of c-foss and HSP 70 in the brain of central nervous system(Aquino et al, 1993), dorsal root ganglia of spinal cords, (Costigan et al, 1998), spinal motor neurons(Sakurai et al, 1997), and skeletal muscle.

But if the expression is appeared in central nerve system, it is certainly also expressed in spinal cord, especially the superficial layer which transmits pain or chemical neuropathy induced by capsaicin into the brain. As known from other researches, the process of pain transmission is the same with that of heat transmission(Clapham, 1997). Capsaicin can evoke acute pain simultaneously resulting from chemical neuropathy and heat from selective fattic oxydation.

Therefore, we observed both c-fos and HSP in spinal dorsal horn, which act as transcriptional factor of defense mechanism against the body.

Consequently, we know that the expression of HSP 70 is increased in peripheral nerve damage caused by capsaicin. This acute peripheral nerve damage was confirmed by c-fos which is marker of nerve activation. In a previous experiment, we observed c-fos expression each time after injecting capsaicin. The result is that c-fos expression is the highest at 2 hours and as time passes c-fos expression is reduced gradually.

Therefor, considering both presumption which intrincic substance might form in our body to defend damage by chemical substance inducing neuropathy and point that c-fos may lead to expression of other genes, we can assume that increasing c-fos expression in peripheral nerve neuropathy means that because it' s related to formation of substance to defend damage. HSP 70 expression increased in this study is considered one of the intrincic defense substances.

Ⅴ. Conclusion

We used c-fos known to be expressed under certain stimuli to promote the expression of a specific gene, and heat shock protein 70(HSP70) whose expression is increased under heat, trauma and ischemia to protect the cell from damage.

1. The expression of c-fos and heat shock protein 70(HSP70) in superficial dorsal horn was not observed for 2 hours after 0.9% NaCl injection.

2. The number of c-fos immunoreactive neurons in superficial dorsal horn was increased markedly 2 hours after capsaicin injection.

3. The expression of HSP70 in superficail

dorsal horn was observed ordinarily 2 hours after capsaicin injection.

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