行政院國家科學委員會專題研究計畫成果報告

Cytokines 與癲癇機制之研究

The involvement of cytokines in the epileptogenesis or in neuroprotection?

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一、中文摘要

Cytokines，例如: interleukin-1 (IL-1)、interleukin-6、tumor necrosis factor (TNF)，已經廣泛被認為除了免疫系統能分泌外，亦存在於中樞神經系統以調節中樞功能。先前的研究發現抑制 corticotropin-releasing hormone (CRH) 作用後所增加的睡眠 (slow wave sleep; SWS) 主要是經由增加中樞內生性 cytokine --- IL-1 所產生的作用。而 IL-1 在此的作用為神經調節物質，已知在中樞神經系統可能借由 NF-κB, nitric oxide (NO) 等訊息傳遞改變睡眠。以往的文獻顯示癲癇發作後，無論是在人類或是動物，中樞內生性 cytokine 的 mRNAs 表現在中樞或周邊有增加的現象，而此增加的現象可能與癲癇相關機制有關。但至今對 cytokine 所扮演的角色究竟是 neuroprotection 或是 epileptogenesis 還不明確。因此本計畫藉由觀察睡眠行為的改變來進一步釐清癲癇所誘發 cytokines 的作用。我們以 amygdaloid kindling 誘發 temporal lobe seizures 的動物模式來決定 kindling 所誘發的癲癇是否改變腦內 cytokine mRNAs 的表現，以及 sleep-wake activity 的改變。我們的結果發現 amygdaloid kindling 誘發 Racine's stage 5 seizure 的老鼠在腦內 hippocampus 及 cortex 的 IL-1α, -1β, 及 TNF-α mRNAs 的表現在 12h:12h light:dark cycle 中的 dark period 的第一及第二小時均有增加的現象。非快速動眼期睡眠(non-REM sleep)的量在 dark period 有明顯的增加，而此增加的睡眠能被腦室投與 IL-1 receptor antagonist (IL-1ra)所阻斷。睡眠的增加可防止外在傷害的產生及增進能量的恢復，可視為中樞保護機制的一指標。因此我們的結果顯示癲癇所誘發中樞 cytokines 的增加可能是 protective 的作用。

關鍵詞：癲癇、睡眠、cytokine、amygdaloid kindling

Abstract

It is widely accepted that cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), etc, produced not only by cells of the immune system but also by cells of the central nervous system to modulate central function. Our previous observation indicates that the enhancement of endogenous cytokine, IL-1 in four discrete brain structures, acting physiologically as a neuromodulator, mediates the increase of slow wave sleep in response to the blockade of central corticotropin-releasing hormone (CRH) actions (2). IL-1 alters sleep-wake activity via signal transduction cascades of NF-κB and/or nitric oxide (NO) (9). This proposal focuses on the actions of cytokines in the regulation/modulation of epileptic activity, since we are aware of literature indicating that seizure enhances cytokines expression in the central nervous system (7,8,13,14,15,16) or in the peripheral (12), and that the enhancement of endogenous cytokines may be involved in seizure-associated process. However, it still remains controversial on how and whether cytokines exhibit neuroprotective or epileptogenetic actions after inducing electroencephalographic (EEG) seizures.

Sleep was used as an indication to

evaluate cytokine's neuroprotective or epileptogenetic actions, and amygdaloid kindling was applied to mimic temporal lobe seizure. Kindling stimulation was given at the beginning of the dark onset everyday for 4 weeks until reach Racine's stage 5 seizure scale. Our result indicates that the mRNA expression of IL-1α, -1β, and TNF-α in hippocampus and cortex are increased at hour 1 and hour 2 after the dark onset. Time spent in slow wave sleep (SWS) during the 12-h dark period increases and it could be blocked by ICV administration of IL-1 receptor antagonist (IL-1 ra) in a dose-dependent manner. These results suggest that the increased central cytokines induced after kindling contribute to the alteration of sleep activity, and the incremental SWS implies the neuroprotection of cytokines.

Keywords: epilepsy, sleep, cytokine, amygdaloid kindling

二、緣由與目的

The term cytokine refers to a group of regulatory proteins that are produced by a large number of cell types in response to a variety of stimuli, and include substances such as the interleukins (IL), tumor necrosis factor (TNF), and interferons (IFN). Cytokines are key mediators of many of the physiological responses to infection or trauma, and these responses are collectively referred to as the acute phase response. The role of cytokines in the complex physiological changes of the acute phase response has been extensively reviewed elsewhere [5,6]. The behavioral changes associated with the acute phase response include altered vigilance. Cytokines however have been reconsidered as a neurotransmitter and/or neuromodulator, since evidence indicates that the gene coding for theses cytokines and their accessory proteins are expressed by neurons, in addition to glial cells, in normal brain [see review [17]]. In addition, the cytokine receptors are present on all neural cell types in response to its signal. A great deal of evidence suggests that cytokines involves in the regulation of sleep behavior via signal transduction cascade of NF-kB and nitric oxide (NO) [9]. Blockade of IL-1 action by IL-1 receptor antagonist (IL-1 ra) [10] or neutralized IL-1 by antibody [2,11] reduces spontaneous sleep. This indicates that cytokines likely modulate in the brain behavior of a normal organism. Evidence also suggests that cytokines may involve in synaptic plasticity, neural transmission, and calcium signal [see review [17]]. Thus, these observations strongly suggest that cytokines perform neural functions in normal brain, in addition to their predominant role as inflammatory mediators.

Some observations indicate that EEG-seizure induces an increase in cytokine expression. For example, focal application of kainic acid or bicuculline induces EEG-seizure and enhances IL-1β immunoreactivity [16]. In addition, an audiogenic seizure induced by noninvasive sensory stimuli on the DBA/2J audiogenic seizure-susceptible mouse strain increases IL-1α expression in hypothalamus [8]. Furthermore, evidence indicates that cytokine production increases in peripheral [12] or in brain [15] in human subject. These observations suggest a role of cytokines in an adaptive mechanisms associated with generalized seizure activity, with implications for neuroprotection, neural dysfunction, or vulnerability associated with epileptic activity. However, it remains controversial on whether the enhancement of central cytokines induced by EEG-seizure involves in neuroprotection or epileptogenesis. Evidence supports cytokines implicated in neuroprotection such as transforming growth factor (TGF)-beta, a cytokine implicated in metabotropic glutamate receptor 3-mediated neuroprotection, was upregulated during the first three weeks after status epilepticus throughout the hippocampus [1]. However, observation that intrahippocampally injection of human recombinant IL-1β 10 min before kinate enhances the time spent in seizure [16] indicates cytokines may contribute to the epileptogenesis. Furthermore, IL-1β has been reported to inhibit post-synaptic NMDA receptors [4], but other suggests that the neurotoxicity induced by TNF-α or IL-1β is mediated by NMDA receptors to increase

nitric oxide (NO) production [3]. Therefore, this proposal is trying to further identify the action of central cytokines induced by amygdaloid kindling.

三、結果與討論

Racine's stage 5 seizure appears after 3- to 4-week kindling stimulation given at 20-min before the dark onset of 12:12h light dark cycle everyday. Rats were sacrificed at 1 hour and 2 hours after the beginning of dark period, and series of cytokine mRNAs were detected by ribonuclease protection assay (RPA). Our result indicates increases of IL-1α, -β and TNF-α mRNAs in hippocampus and cortex at both hour 1 and hour 2 after dark onset (Figure 1). Expressions of IL-1α mRNAs (value represents optic density [O.D.] ± SEM) were increased from 0.78 ± 0.22 to 2.52 ± 0.4 (independent-samples T-test, $p<0.05$), and from 1.08 ± 0.17 to 2.95 ± 0.23 (independent-samples T-test, $p<0.05$) in hippocampus at hour 1 and hour 2, respectively. In cortex, IL-1α mRNA was increased from 1.02 ± 0.09 to 2.89 ± 0.32 (independent-samples T-test, $p<0.05$), and from 1.05 ± 0.12 to 2.95 ± 0.43 (independent-samples T-test, $p<0.05$) at hour 1 and 2, respectively. IL-1β mRNA was significantly altered in cortex at hour 1 and hour 2, OD increases from 2.05 ± 0.43 to 6.95 ± 2.52 (independent-samples T-test, $p<0.05$), and from 2.01 ± 0.21 to 7.97 ± 1.24 (independent-samples T-test, $p<0.05$), respectively. There was a strong tendency for an increase in IL-1β mRNA expression in hippocampus at hour 1 and 2. In contrast, IL-6 mRNA was variably detected in both brain areas. TNF-α mRNA expression was not consistently altered by kindling, although there was an increase tendency for TNF-α in hippocampus at hour 1 after kindling protocol (Table 1). These results suggest certain cytokines, such as IL-1α and -1β in the brain, especially in the areas of hippocampus and cortex, are altered during the dark period after kindling seizure developed.

In addition, we found that SWS was increased during the 12-h dark period after the kindling protocol; the time spent in SWS increases from 23.77 ± 2.18 % to 32.08 ± 1.60 % (ANOVA, $p<0.05$, Figure 2A) and the time spent in wakefulness decreases from 72.29 ± 2.48 % to 65.90 ± 1.87 % (ANOVA, $p<0.05$, Figure 2A). The total time spent in REM sleep was not altered by kindling protocol. ICV administration of IL-1 receptor antagonist (IL-1ra) dose-dependently blocks the sleep alteration induced by kindling (Figure 3). High dose (100 ng) of IL-1ra significantly reduces the total time spent in SWS from 32.08 ± 1.60 % to 21.59 ± 1.45 % (ANOVA, $p<0.05$, Figure 2B) and increases wakefulness from 65.90 ± 1.87 % to 77.86 ± 1.52 % (ANOVA, $p<0.05$, Figure 2B). REM sleep was also reduced by ICV IL-1ra. These results provide pharmacologically the involvement of increased cytokines, especially IL-1 in brain contributing to the kindling-induced sleep-wake alteration.

Sleep could be served as indication to evaluate the neuroprotective or epileptogenetic actions of cytokines induced after kindling protocol. It's well known that SWS increases after infected by pathogen and the increase of SWS is essential for animal to recover from infectious state. The infection-induced increase of SWS could be mimicked by ICV administration of IL-1 and could be blockade by IL-1ra and/or suppressed by anti-IL-1β antibody. Increase of SWS avoids animals from the environmental harm and restores energy; in addition, the increase of IL-1 also elevates thermoregulatory set point and consequently increases body temperature and inhibits growth of pathogens. Although IL-1 action in peripheral primarily mediates inflammation, in the central nervous system IL-1 possesses a protective action during the recovery period after infection. Therefore, our results might suggest the protective action for IL-1 induced by kindling because of the contribution of IL-1 in SWS increase.

四、計畫成果自評

Our results suggest that amygdaloid kindling given at 20 min prior to the beginning of dark period induces increase of cytokines, such as IL-1α and –1β, at hour 1

and hour 2 during the active period (dark period). The increase of IL-1 might contribute to the subsequent enhancement of SWS because of blockade of this incremental SWS by IL-1ra. Conclusively, present results provide the possibility of protective action of central cytokines induced after amygdaloid kindling protocol. However, a more detail of electrophysiological study needs to be conducted to further investigate the possible cellular mechanisms of central cytokines.

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Figure 1. Alterations of cytokine mRNA expression during the dark period after amygdaloid kindling development.

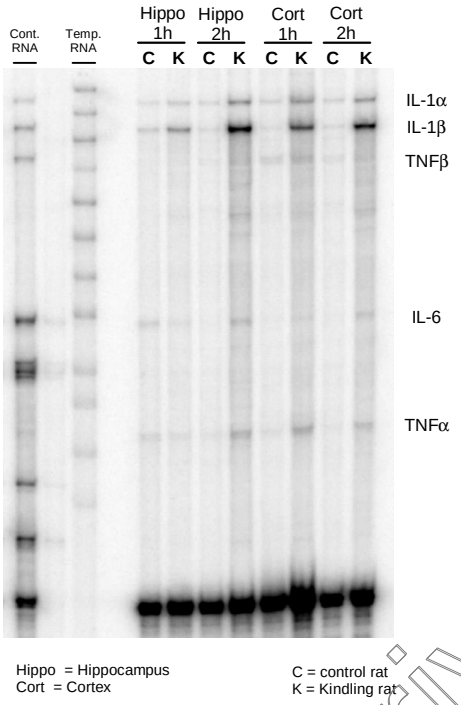


Table 1. Expression of cytokine mRNA in hippocampus and cortex during the dark period after amygdaloid kindling development.

	Hippo 1 h		Hippo 2 h		Cort 1h		Cort 2h	
	control	kindling	control	kindling	control	kindling	control	kindling
IL-1α	0.78±0.22 (8/8)	2.52±0.40* (8/8)	1.08±0.17 (8/8)	2.95±0.23* (8/8)	1.02±0.09 (8/8)	2.89±0.32* (8/8)	1.05±0.12 (8/8)	2.95±0.43* (8/8)
IL-1β	2.50±0.92 (8/8)	3.52±0.42 (8/8)	2.84±0.82 (8/8)	4.55±0.84 (8/8)	2.05±0.43 (7/7)	6.95±2.52* (7/7)	2.01±0.21 (7/7)	7.97±1.24* (7/7)
IL-6	1.63±0.45 (3/8)	2.03±0.16 (3/8)	1.69 (1/8)	3.10±1.05 (4/8)	Not detected	Not detected	Not detected	1.09 (1/8)
TNF-α	1.60±0.60 (4/8)	3.01±0.71 (4/8)	2.01±0.70 (3/8)	3.10±0.95 (3/8)	3.25±0.42 (4/7)	4.07±0.80 (4/7)	3.02 (1/7)	4.03±0.51 (3/7)

Figure 2. The effects of IL-1 receptor antagonist (IL-1ra) on the alteration of sleep-wake activity induced by amygdaloid kindling.

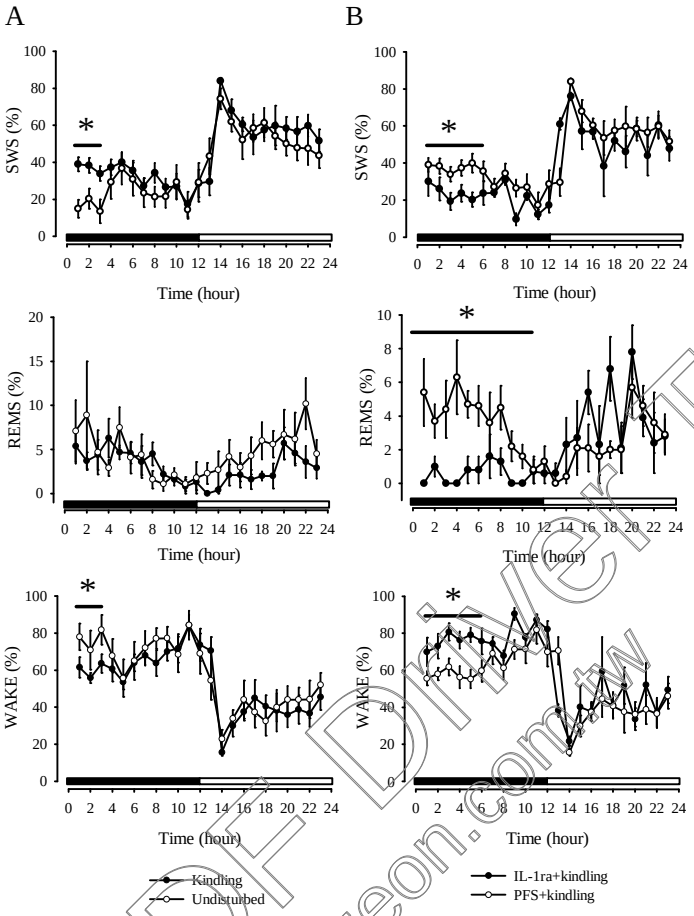
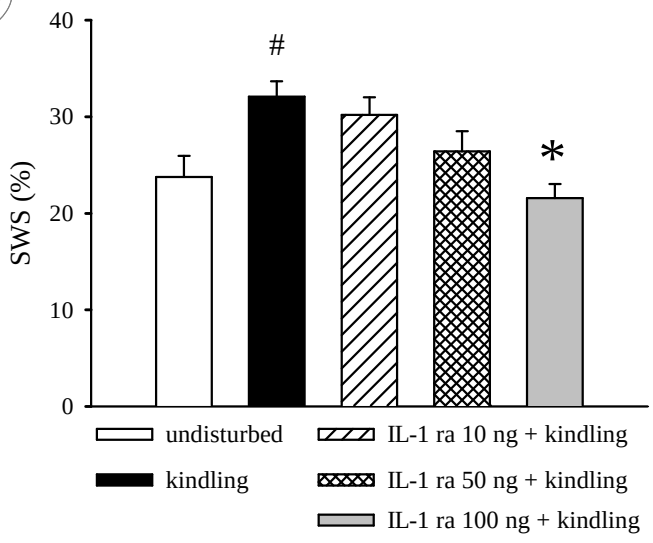


Figure 3. IL-1ra dose-dependently blocks Amygdaloid kindling-induced SWS enhancement during the dark period.



行政院國家科學委員會補助專題研究計畫成果報告

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