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Brain IL-1 β was involved in reserpine-induced behavioral depression in rats

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ABSTRACT

AIM: To investigate the mechanism of brain interleukin-1 β (IL-1 β) in reserpine-induced behavioral depression in rats. **METHODS:** Porsult swim test was used in the measurement of depressive behavior and ELISA was used in measurement of brain IL-1 β . **RESULTS:** Intraperitoneal injection of reserpine (0, 4, 6, and 8 mg/kg, ip) increased floating time in the Porsult swim test in a dose- and time-dependent manner in rats. Intracerebroventricular injection (icv) of IL-1 β receptor antagonist (IL-1ra, 6 mg/kg) blocked the increment of floating time in Porsult swim test at 48 and 72 h after reserpine injection, but not at 1 and 24 h after injection. Brain IL-1 β increased after reserpine treatment in posterior cortex, hippocampus, and hypothalamus. The increase of IL-1 β concentration starts at 24 hours after injection of reserpine and reached the peak at 48 h. **CONCLUSION:** Reserpine induced behavioral depression partially via brain interleukin-1 β generation.

INTRODUCTION

Reserpine, one of the earliest drugs to be effective as a central tranquilizing agent, was employed mainly for its action in the treatment of hypertension. After chronic administration of this drug some patients complained of major depression in 1950's^[1]. Subsequent research in rats showed that reserpine-induced behavioral depression was correlated to the depletion of brain monoamines. However, the concentrations of noradrenaline (NA), adrenaline (A), dopamine (DA), and serotonin (5-HT) in brain remained low levels for more than a week after acute reserpine treatment (5 mg/kg), but the behavioral depression usually ended in 72 h^[2].

These data suggest that the brain monoamine is not the only or direct reason for reserpine-induced behavioral depression. Our previous work also showed that adenosine mediated reserpine-induced behavioral depression^[3]. It is well known that glial cells could synthesize IL-1 β in response to various physiological and pathological stimuli. The IL-1 β production has also been detected in both brain tissue damage and repair^[4]. The production of IL-1 β in brain causes 'sickness behavior' mediated by central nervous system, which includes suppression of location and exploration, suppression of food gathering and sexual behavior and others^[5]. It is possible that the outcome of reserpine-induced behavioral depression might be more directly related to brain adenosine signaling associated neuronal over-activation, or that the damage of nervous tissue induced by reserpine causes brain IL-1 β release. To investigate the role of IL-1 β in behavioral depression, we measured brain IL-1 β after reserpine treatment, and checked the effect

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of IL-1 β receptor antagonist on reserpine-induced behavioral depression in rats.

MATERIALS AND METHODS

Subjects Male Sprague-Dawley albino rats (240-260 g) were used for the study. The animals were procured from the Central Animal House, Psychology Department, the University of California, Los Angeles (Grade II, Certificate No ARC 00-099-01), and housed in individual cages with free food and water in a room maintained on 12:12 h light-dark cycle for a week before the experiment. Experiment occurred in the light portion of the cycle. All rats were handled daily.

Surgery Rats were anesthetized with pentobarbital (60 mg/kg). A 22-gauge stainless steel guide cannula was implanted to the right lateral cerebral ventricle. Coordinates for cannula placement were 0.5 mm posterior, 1.5 mm lateral to bregma, and 4.2 mm ventral to the skull surface. It was then fixed to the skull with steel screws and dental cement. Rat was then returned to its cage for a week before the experiment.

Porsult swim test The swim test was a modification of that initially described by Porsult and used by Weiss as a test of behavioral depression in rodents^[6]. The swim tank consisted of a plexiglass cylinder 65 cm tall and 30 cm in diameter. The tank was filled with water (at room temperature) to a height of 14 cm to the top. The swim test was conducted in a quiet, darkened testing room lighted by a 25 W red light. A camera system was used to observe and record the rat behavior. A set of "water wings" (made from light weight plastic bubble) were placed onto the animal. Then the rat dropped into the water. The rat was observed for a period of 15 min and timed the duration of two types of motor activity: 1) struggling, which is defined as vigorous movement of all paws with the forepaws breaking the surface of the water; 2) floating, which is defined as the animal remaining motionless with no movement of limbs.

Tissue collection Animals were anesthetized with a brief exposure to ether, and brains were quickly removed after decapitation. All dissections were performed on a frosted glass plate placed on top of crushed ice, and brain structures were quickly frozen on dry ice. Brain samples, which included hypothalamus, hippocampus, and front cortex were stored at -70 °C until the time of sonication.

Tissue processing Each tissue was added to 0.5-1.0 mL of Iscove's culture medium containing 5 %

fetal calf serum and a cocktail enzyme inhibitor (in mmol/L: amino-*n*-caproic acid 100, edetic acid 10, benzamidine-HCl 5, and phenylmethylsulfonyl fluoride 0.2). Total protein was mechanically dissociated from tissue using an ultrasonic cell disruptor. Sonicated samples were centrifuged at 10 000 \times g at 4 °C for 10 min. Supernatants were removed and stored at 4 °C until an ELISA was performed. Bradford protein assays were also performed to determine total protein concentrations in brain sonication samples.

ELISA IL-1 β ELISA kit was from R & D Systems. Ninety-six flat-bottom wells were coated with sheep anti-rat IL-1 β immunoaffinity-purified polyclonal antibody overnight at 4 °C. After washing the plates in assay buffer, 100 μ L of rat IL-1 β standards or samples were added to each well and incubated at room temperature for 4 h. After washing the plates, 100 μ L of biotinylated, immunoaffinity-purified polyclonal sheep anti-rat IL-1 β Ab (1:2000) with 1 % normal goat serum was added to each well and incubated at room temperature for 1 h. The color was developed by use of avidin-HRP and the chromogen orthophenylene diamine (Sigma). Plates were read at 490 nm, and results were expressed as picograms of IL-1/100 μ g of total protein. The detection limit of this assay was 5 ng/L.

Statistical analysis The statistical analysis of differences between each group was compared by single factor analysis of variance (ANOVA), followed by Newman-Keuls post-hoc contrasts. $P < 0.05$ was considered statistically significant.

RESULTS

Dose response and time course of reserpine on behavioral depression Rats were assigned randomly to one of four groups of ten rats each. One group (0) was injected vehicle of reserpine (Me₂SO) and the other three groups (4,6,8) were injected with 4, 6 and 8 mg/kg of reserpine (ip). Porsult swim testing occurred one hour later. Results showed that floating time increased as a direct function of drug dose (Fig 1). Reserpine 4 mg/kg had significant longer floating time compared with the vehicle control group ($P < 0.05$), and lesser than did reserpine 6 mg/kg ($P < 0.01$) and 8 mg/kg ($P < 0.01$). The floating time is not significantly different between reserpine 6 mg/kg and 8 mg/kg. In this case, we chose 6 mg/kg as a regular dose of reserpine in following experiment. Fig 2 shows the mean floating time when Porsult testing occurred at 1, 24, 48, and 72 h after injection of reserpine (6 mg/kg, ip), the

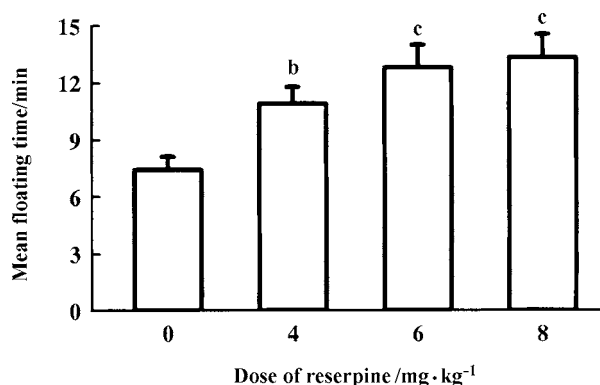


Fig 1. Injection of different doses of reserpine increase mean floating time. $n=6$. Mean±SD. ^b $P<0.05$, ^c $P<0.01$ vs reserpine 0 mg/kg,

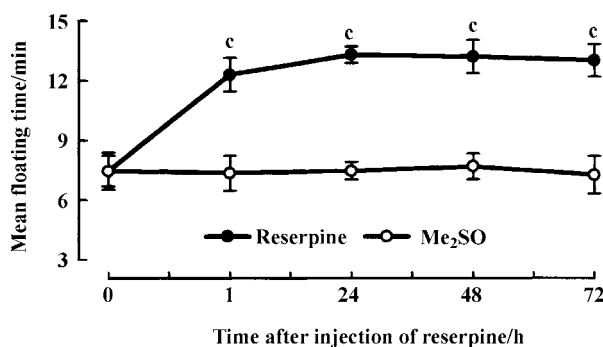


Fig 2. Time course of reserpine on behavioral depression. $n=6$. Mean±SD. ^c $P<0.01$ vs Me₂SO at 1, 24, 48, and 72 h,

floating time in reserpine injected groups significantly increase from 1 h to 72 h after injection compared with vehicle injected groups ($P<0.01$). This data provided evidence that acute administration of reserpine 6 mg/kg could produce behavioral depression at least from 1 h to 72 h.

Effect of IL-1ra on reserpine-induced behavioral depression Porsult swim test occurred at 1, 24, 48, and 72 h after injection of reserpine. IL-1ra 6 mg/kg was injected 15 min before test. IL-1ra did not have any effect on floating time at 1 and 24 h after injection of reserpine (Tab 1), but it decreased the floating time at 48 and 72 h after injection of reserpine. IL-1ra has no effect alone on floating time in swim test (Tab 1).

Effect of reserpine treatment on brain IL-1 β protein Rats was injected with reserpine 6 mg/kg and killed 1, 24, 48 h, and 72 h later. Rats in control group were injected with Me₂SO, the solvent of reserpine. Levels of IL-1 β protein in hypothalamus, front cortex, and hippocampus were examined. At 1 h and 24 h after

Tab 1. Effect of IL-1ra on reserpine-induced behavioral depression. $n=10$. Mean±SD. ^c $P<0.01$ vs group R+NS.

Time/h	Mean floating time /min			
	Me ₂ SO	R	R+NS	R+ILra
1	7.9±0.7	13.5±0.6	13.1±0.7	12.3±0.7
24	7.0±0.5	12.4±0.6	13.5±0.9	13.7±1.0
48	7.2±0.6	12.2±0.8	12.9±0.8	8.7±0.8 ^c
72	8.0±0.8	13.8±0.9	13.4±0.5	9.2±0.5 ^c

Me₂SO: vehicle of reserpine; R: reserpine; ILra: IL-1ra; NS: normal saline.

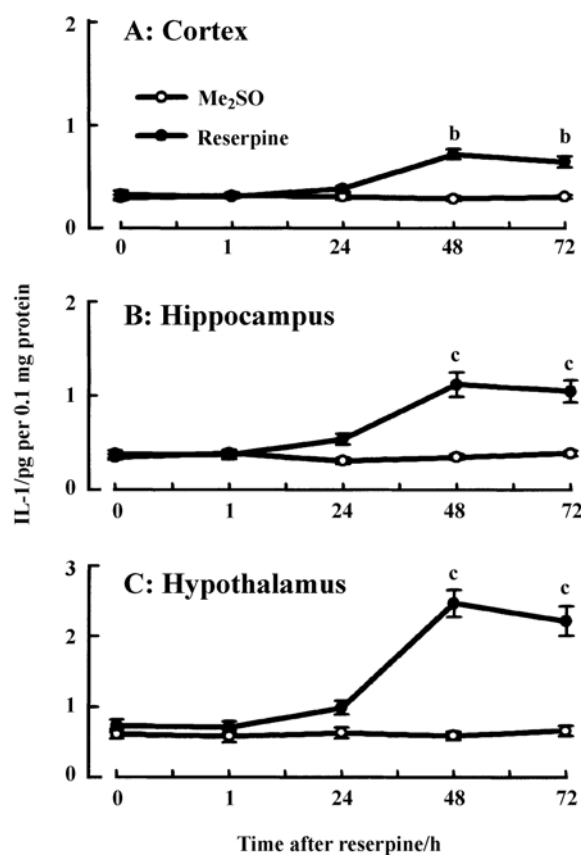


Fig 3. Effect of reserpine 6 mg/kg ip on brain IL-1 β concentration. Reserpine: peritoneal injection of reserpine 6 mg/kg; Me₂SO: solvent of reserpine. peritoneal injection of reserpine increased brain IL-1 β : in posterior cortex. $n=6$. Mean±SD. ^b $P<0.05$, ^c $P<0.01$ vs Me₂SO.

reserpine treatment, levels of IL-1 β protein in above regions were not different compared with that in control group. However, levels of IL-1 β protein significantly increased in hypothalamus, front cortex and hippocampus 48 h and 72 h after reserpine treatment (Fig 3). In the above three regions, concentration of IL-1 β

protein in hypothalamus increased most significantly.

DISCUSSION

It is well known that Interleukin-1 β is a polypeptide released by activated macrophages, monocytes and brain glial cells, which mediate a lot of host's responses to infection and inflammation. Increasing evidences suggest that it also has a role as an intrinsic neuromodulator in the central nervous system. Both peripheral and central administration of IL-1 β was induced a syndrome of physiological and behavioral changes including fever, hypersomnia, anorexia, adipsia, and a decreased interest in social interactions that has been referred to as "sickness behavior". We hypothesize that although reserpine-induced behavioral depression is usually attributed to drug-induced depletion of brain monoamines, IL-1 β might be involved in the process and the outcome might be more directly related to brain IL-1 signaling system.

This study provides evidence that IL-1ra blocked reserpine-induced depression at 48 and 72 h after reserpine but did not at 1 and 24 h after reserpine, which suggests that IL-1 β is generated in brain after reserpine treatment and mediates reserpine-induced depression. This result indicates that the mechanism of reserpine-induced behavioral depression is not only due to the depletion of brain monoamines, but also IL-1 β system. To further address the hypotheses that IL-1 β might release in brain after reserpine treatment because of the brain tissue damage and repair, we measured the levels of IL-1 β indifferent regions in brain. After 24 h of reserpine treatment, IL-1 β started increase, reached the peak at 48 h and lasted to 72 h. The time course of IL-1 β increase is parallel with the depressive behavior 24 h

after reserpine treatment, but not responsible to the depressive behavior within 24 h after reserpine treatment. This study demonstrates that reserpine stimulates brain IL-1 β express and partially mediates reserpine-induced depression.

Combined these results with our previous study that intracerebroventricular injection of IL-2 β induced behavior depression in rats^[7], we believe that brain IL-1 β plays an important role in depression, especially in stress-induced depression. Thus we hypothesize that anti-inflammatory drugs could be used for depression treatment via its effect on brain cytokines. This will bring out a clue to investigate new antidepressant drugs.

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