

Ca²⁺-Independent Phospholipase A₂ Inhibitor Impairs Spatial Memory of Mice

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ABSTRACT—Pharmacological blockade of Ca²⁺-independent phospholipase A₂ (PLA₂) is reported to disintegrate hippocampal synaptic plasticity, which is thought to be the cellular mechanism underlying learning and memory. Therefore, we investigated the effect of the Ca²⁺-independent PLA₂ inhibitor bromoenol lactone (BEL) on spontaneous alternation behaviors of mice. When 3 nmol BEL was intracerebroventricularly injected 30 min prior to the test, the mice showed a poor alternation ratio, compared with control animals. The data suggest that Ca²⁺-independent PLA₂ activity is required for spatial memory.

Keywords: Phospholipase A₂, Learning and memory, Spontaneous alternation behavior

The activity of phospholipase A₂ (PLA₂), a key enzyme to release fatty acids from membrane glycerophospholipids, is dramatically reduced in the brain of Alzheimer's disease, compared with non-demented controls (1). Therefore, we assumed that PLA₂ is possibly associated with cognitive functions of mammals. Indeed, one of the PLA₂ products, arachidonic acid (AA), is thought to contribute to hippocampal synaptic plasticity, which may be the cellular basis of learning and memory in the vertebrates (2). Of three subclasses of PLA₂, i.e., Ca²⁺-dependent PLA₂, Ca²⁺-independent PLA₂ (iPLA₂) and secretory PLA₂, the iPLA₂ isozyme predominantly renders PLA₂ activities in the hippocampus (3). Recently, Wolf et al. (4) indicated that the selective iPLA₂ inhibitor bromoenol lactone (BEL) prevents the induction of hippocampal long-term potentiation (LTP), but inhibitors of the other isozymes did not. However, there is no information about the involvement of iPLA₂ in learning and memory. Therefore, we evaluated the effect of BEL on spatial memory using the spontaneous alternation behavior test of mice.

BEL (a gift from Maruha Co., Ltd., Tokyo) was dissolved in artificial cerebrospinal fluid and administered into the lateral cerebroventricle (AP: -0.3 mm, M: 1.1 mm, VD: 2.0 mm) of a male C57/black 6J mouse (9-week-old) (SLC, Shizuoka) 30 min before the behavior experiment. The animal was placed in one arm of a Y-shaped maze (Fig. 1) and allowed to explore the maze for a period of 8 min. Arm choices were recorded, and any three consecutive

choices of three different arms were counted as an alternation. Thus, the percentage of alternation was determined by dividing the total number of alternations by the total number of choices minus 2. Our previous paper shows that this procedure can accurately assess spatial working memory of mice (5).

Intact animals or the mice treated with the vehicle (0 nmol BEL) displayed a significant alternation ratio in arm

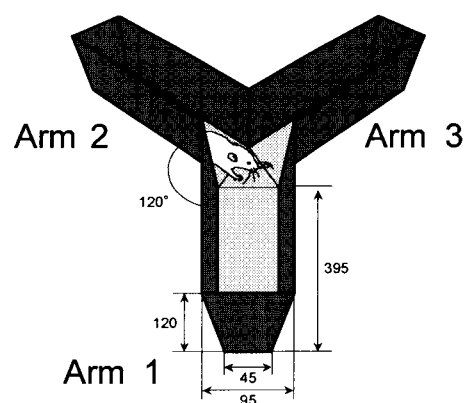


Fig. 1. Schematic drawing of the apparatus used for spontaneous alternation behavior test. The apparatus is a Y-shaped maze consisting of the three trough-shaped arms (95 mm in width, 395 mm in length, 120 mm in depth) that are separated by angles of 120° (Arm 1, 2 and 3). A mouse was placed in Arm 1 and allowed to explore the maze for a period of 8 min. The memory component in this task is that the mouse must remember which arm has been more recently visited in order to alternate; i.e., spatial working memory. The dimensions of the maze are given as mm.

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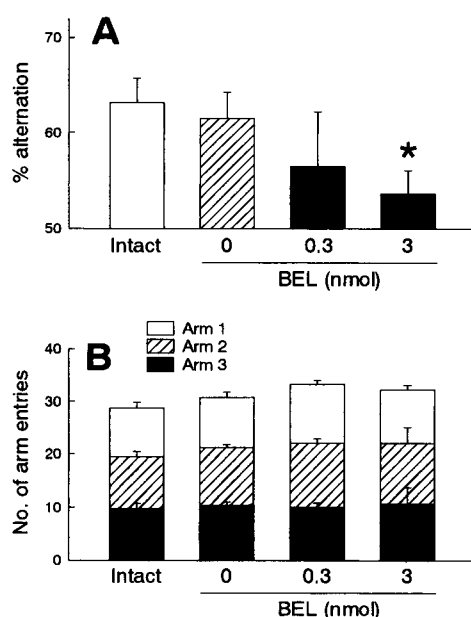


Fig. 2. Inhibitory effect of BEL on spontaneous alternation behaviors of mice. **A:** alteration ratio of intact animals ($N=8$) or the mice treated with 0 ($N=8$), 0.3 ($N=9$) or 3 ($N=8$) nmol BEL 30 min prior to the test was measured for 8 min. The chance level of alternation ratio is assumed to be 50%. * $P<0.05$ vs Intact: LSD-type multiple range comparison following one-way ANOVA. **B:** The number of entries into each arm was also measured during the test. Data show the means \pm S.E.M. of N animals.

choices (>60%) (Fig. 2A). However, BEL decreased the alternation ratio in a dose-dependent manner, and the alternation behaviors in 3 nmol BEL-treated mice were almost eliminated, the ratio of which was close to a putative chance level of 50% ($P=0.025$, $F(1,29)=5.58$, LSD-type multiple range comparison; $F(3,29)=3.01$, one-way ANOVA). Because it is also possible that changes in locomotor activities or preference for arms alter the task performance of mice, we simultaneously monitored the number of entries into each arm (Fig. 2B). However, none of the groups displayed a preference for a particular arm, ($P=0.443$, $F(2,58)=0.83$, two-way ANOVA) and the numbers of arm choices were not different among the groups ($P=0.267$, $F(3,58)=1.35$, two-way ANOVA), which suggest that BEL impaired spontaneous alternation behaviors through inhibiting spatial memory ability rather than changing locomotor characteristics or activities.

Although previous reports indicated that the relatively low specificity and nonselective PLA₂ inhibitor nordihydroguaiaretic acid produces amnesia in the water maze task of rats (6) or passive avoidance task of chicks (7), the present study is the first indication that iPLA₂ isozyme is obligatory for the spatial task performance. BEL is known as an excellently selective and potent inhibitor of iPLA₂ in a concentration ranging from 0.3 to 30 μ M (8). The final

concentration of 3 nmol BEL injected into the cerebroventricle is calculated to be about 10 μ M, which is comparable to the dose used in the in vitro study showing that BEL prevented hippocampal LTP induction but other isozyme inhibitors did not (4). Therefore, BEL utilized in the present study is thought to selectively inhibit activation of iPLA₂. However, this study did not address possible involvement of other subtypes of PLA₂. Investigations using specific and identifiable inhibitors against other isozymes would clarify this point. Also, it remained to be determined here whether BEL-induced cognitive deterioration resulted from inhibition of hippocampal LTP. Indeed, the spontaneous alteration behavior is well associated with neural processes in the prefrontal cortex (9). Thus, it is also possible that intracerebroventricularly applied BEL may alter physiological functions of other regions including the prefrontal cortex. Nevertheless, our present study using the selective inhibitor might provide a heuristic framework for therapeutic and preventive treatment for cognitive deficient or amnesia such as Alzheimer's diseases.

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