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Intrahippocampal infusion of ebselen impairs retention of an inhibitory avoidance task in rats

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Abstract

Ebselen is a seleno-compound used in the treatment of neurological disorders involving the glutamatergic system. Although ebselen is currently used in clinical trials, the physiological effects of this seleno-compound are poorly known. In this study, we investigated the effects of intrahippocampal infusion of ebselen (0.1–3 nmol) in rats submitted to an inhibitory avoidance task. Ebselen (1–3 nmol) infused after the training session impaired retention of inhibitory avoidance, tested 90 min or 24 h after the training session. Moreover, ebselen also impaired the retention when infused 30 min prior to training or 10 min prior to test sessions. In summary, ebselen impaired memory consolidation, acquisition and retrieval. This amnesic effect of ebselen could be related to oxidant activity at *N*-methyl-D-aspartate (NMDA) receptors. Our results indicate that more studies must be performed to investigate the mechanisms of this amnesic effect and whether ebselen has a cognition-impairing effect when administered chronically.

Keywords: Ebselen; Learning; Memory; Avoidance inhibitory

1. Introduction

Ebselen, 2-phenyl-1,2-benzisoselenazol-3[2*H*]-one, is a seleno-organic compound which catalyzes the reduction of peroxides to water or to the corresponding alcohol using thiols (including reduced glutathione) as substrate (Müller et al., 1984). In addition to its glutathione peroxidase activity, ebselen has antioxidant and anti-inflammatory actions that are not directly related to peroxide decomposition (Schewe et al., 1994). As a result of its antioxidant actions, ebselen has neuroprotective effects in some models of neurotoxicity such as cellular injury induced by glutamate or 4-hydroxy-nonenal, lipid peroxidation induced by quinolinic acid and in a model of Parkinson's disease (Malecki et al., 2000; Moussaoui et al., 2000; Porciúncula et al., 2001; Rossato et al., 2002). Ebselen is currently undergoing clinical trials for the treatment of human neuropathological conditions that are associated with over-stimulation of the glutamatergic system and reactive oxygen species, such as acute ischemic stroke and aneurysmal subarachnoid hemorrhage (Saito et al., 1998; Yamaguchi et al., 1998); in animal models ebselen was also neuroprotective (Imai et al., 2001; Namura et al., 2001; Noiri et al., 2001; Parnham and Sies, 2000).

Glutamate is the main excitatory neurotransmitter in the mammalian brain, acting through ionotropic and metabotropic receptors (Ozawa et al., 1998). As pointed out above, glutamate receptors are involved in acute brain injury and chronic neurodegenerative diseases, such as ischemia, brain trauma, and stroke, Alzheimer's and Parkinson's disease (Lee et al., 1999; Lipton and Rosenberg, 1994; Ozawa et al., 1998). Moreover, glutamate receptors have a fundamental role in neurophysiological processes such as learning and memory (Izquierdo and McGaugh, 2000; Morris and Davis,

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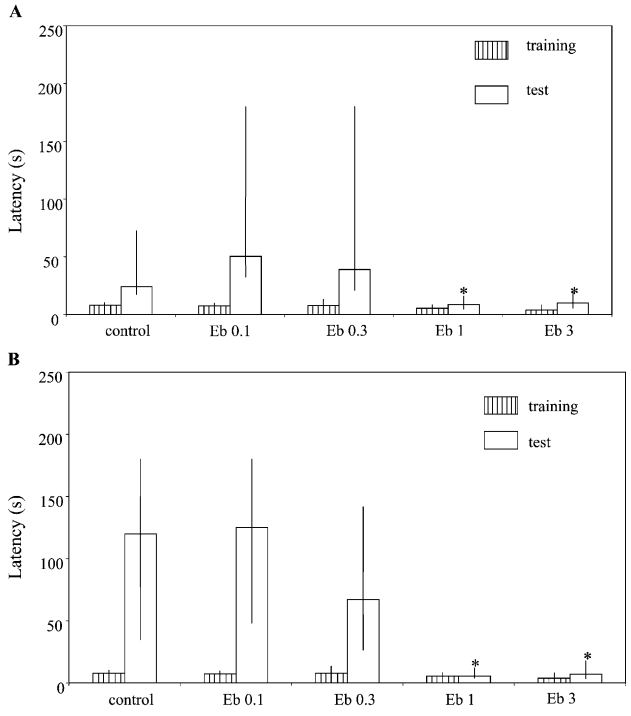

Fig. 1. Effect of intrahippocampal infusion of vehicle or ebselen (0.1–3 nmol) immediately after inhibitory avoidance training sessions. (A) Test session 1.5 h after the training session (short-term memory). (B) Testing session 24 h after the training session (long-term memory). Data are medians (interquartile ranges) of the step-down latencies in training and test sessions; $n = 10–16$ animals per group. Statistical comparison between groups was performed with Kruskal–Wallis test followed by Mann–Whitney test. * Significantly different from control group ($P < 0.01$).

1994). The involvement of glutamate receptors in learning was established using a variety of animal models (Castellano et al., 2001). In fact, after intrahippocampal infusion, ionotropic or metabotropic glutamate receptors antagonists are amnesic in different behavioral tasks (Izquierdo et al., 1992). Accordingly, infusion of AP5 (2-amino-5-phosphono-valeric acid), a *N*-methyl-D-aspartate (NMDA) receptor antagonist, into specific brain areas prior to or immediately after training impairs consolidation of one-trial inhibitory avoidance (Roesler et al., 1998).

Different components of glutamate neurotransmission are sensitive to redox agents (Nogueira et al., 2000, 2001). Indeed, reducing agents such as dithiothreitol enhance, whereas oxidant agents such as dithionitrobenzoate diminish, NMDA receptor activation in cultured neurons (Tang and Aizenman, 1993a,b). Recently, Herin et al. (2001) have demonstrated that ebselen counteracts the NMDA-activating effect of reducing agents and protects neurons from NMDA-induced cell death.

Experimental evidence showing that ebselen could modulate neuropsychological processes after its in vivo administration is lacking in the literature. Considering that NMDA receptor activation is involved in memory formation and that ebselen can modulate NMDA-mediated currents in cultured neurons, we investigated the effect of intrahippocampal administration of this seleno-compound on inhibitory avoidance task in adult rats.

2. Material and methods

2.1. Materials

Ebselen was purchased from Sigma (St. Louis, MO, USA) and dissolved in saline plus Tween 80, whose maximal concentration was 0.5% (v/v).

2.2. Animals

Male adult Wistar rats (250–300 g) were kept under a 12:12 h light: dark cycle at a temperature of 22 ± 1 °C. They were housed in plastic cages (five animals per cage) with tap water and commercial food ad libitum.

2.3. Surgical procedure

Three days before the experiments, animals were implanted bilaterally under deep sodium thiopental anesthesia (40 mg/kg) with a 27-gauge cannula aimed 1.0 mm above the pyramidal cell layer of the CA1 sub area of the dorsal hippocampus. Coordinates were: A, −4.3; L, +4.0; V, +3.4. The cannula was fixed to the skull with dental acrylic. Behavioral procedures were conducted between 8:00 a.m. and 12:00 a.m., 3 days after surgery.

2.4. Inhibitory avoidance

The IA apparatus was a 50 × 25 × 25 cm acrylic box whose floor consisted of parallel stainless steel bars (1 mm

Table 1
Effect of intrahippocampal infusion of vehicle or ebselen (1–3 nmol) on locomotor activity, measured during 5 min of exploration in an open-field session

Open-field performance				
	Number of rearings (0–2.5 min)	Number of rearings (2.5–5 min)	Number of crossings (0–2.5 min)	Number of crossings (2.5–5 min)
Control	9.1 ± 1.4	5.6 ± 1.3	30.1 ± 3.8	19.8 ± 3.7
Ebselen (1 nmol)	12.0 ± 2.4	8.3 ± 1.9	33.5 ± 3.9	19.5 ± 2.6
Ebselen (3 nmol)	11.4 ± 1.7	6.6 ± 1.5	42.6 ± 5.5	24.7 ± 3.4

Animals received vehicle or ebselen 10 min before exploratory sessions (crossing and rearing). Data are means ± S.E.M. of the number of crossings and rearings. $n = 6–11$ animals per group. Statistical comparison between groups was performed by ANOVA. Groups were not significantly different.

Table 2
Effect of intrahippocampal infusion of vehicle or ebselen (1–3 nmol) on plus-maze performance

Plus-maze performance				
	Number of entries in the open arms	Number of entries in the closed arms	Time (min) in the open arms	Time (min) in the closed arms
Control	5.6 ± 1.4	3.9 ± 1.3	1.30 ± 0.38	3.10 ± 0.37
Ebselen (1 nmol)	6.4 ± 2.4	3.5 ± 1.9	1.80 ± 0.50	2.50 ± 0.57
Ebselen (3 nmol)	6.7 ± 1.7	4.6 ± 1.5	1.40 ± 0.24	3.10 ± 0.25

Animals received vehicle or ebselen 5 min before sessions. Data are means ± S.E.M. of the number of entries to open and closed arms during a 5-min observation period. $n = 7–9$ animals per group. Statistical comparison between groups was performed by ANOVA. Groups were not significantly different.

diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed against the left wall of the box. In training sessions, animals were placed on the platform and the latency to step-down onto the floor with the four paws was measured with an automatic device; immediately after stepping-down animals received a 0.5 mA, 2 s footshock. In test sessions, carried out 1.5 h (short-term memory) and 24 h (long-term memory) after training, no footschock was given and the step-down latency (180 s ceiling) was taken as a measure of retention. Intrahippocampal infusion of either vehicle or ebselen was performed 30 min before training, immediately after training or 10 min prior to the long-term memory test. Cannula placement was verified after decapitation of the animals by infusing 0.5 μl of a solution of 4% methylene blue in saline through the cannula. Brains were stored in formalin for at least 72 h and cannula placement was verified by histological examination. Only data from the animals with correct cannula placement were analyzed. Data for inhibitory avoidance are shown as median values (interquartile ranges) of training and test latencies to step-down on the grid. Comparisons between groups were performed using a Kruskal–Wallis analysis of variance followed by a Mann–Whitney *U*-test if necessary.

2.5. Exploration of an open-field

The effect of ebselen on general locomotor activity was evaluated. Ebselen or vehicle was infused 30 min before the exploration session. Animals were placed in a 50 × 25 × 50 chamber made of brown polywood with a frontal glass wall. The floor of the open-field was divided into 12 equal squares by black lines. Animals explored the arena for 5 min. The number of crossing of the black lines and the number of rearings were measured. The number of crossings was used as a measure of locomotor activity and the number of rearings as a measure of exploratory behavior. Data are presented as means ± S.E.M. of crossings and rearings. Statistical analysis was performed by one-way analysis of variance (ANOVA).

2.6. Plus-maze

The plus-maze consisted of two 30 × 5 cm^2 open arms and two 30 × 5 × 15 cm^3 closed arms, with an open roof, arranged such that the two arms of each type were opposite each other. An observer sitting in the same room recorded the behavioural measures. All sessions were conducted under dim red light. During a 5-min session period, the following indexes of anxiety were measured: the number of entries into, and the time spent in open and closed arms. Data are shown as means ± S.E.M. of entries into and time spent (time in minutes) in the open and closed arms. Statistical analysis was performed by one-way analysis of variance (ANOVA).

The same animals underwent the three behavioral tasks (inhibitory avoidance, open field and plus maze). They were firstly submitted to the open field session, then (2 days later), to an elevated plus maze session, and finally (2 days after the plus maze test) to inhibitory avoidance training.

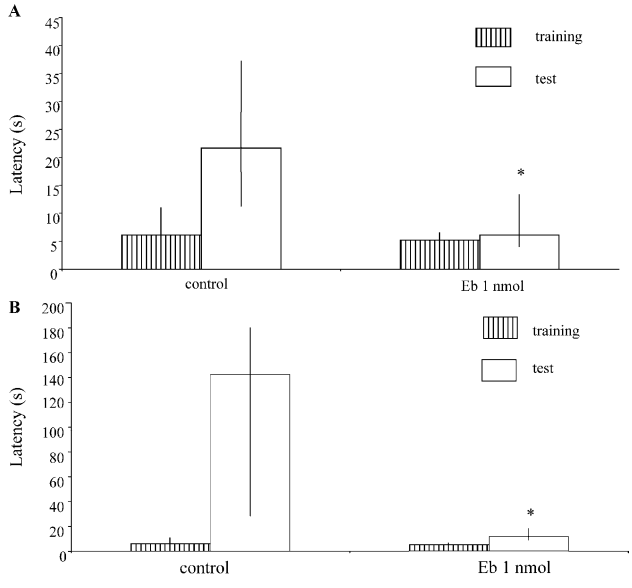

Fig. 2. Effect of intrahippocampal infusion of vehicle or 1 nmol of ebselen 30 min before inhibitory avoidance training sessions. (A) Test session 1.5 h after the training session (short-term memory). (B) Testing session 24 h after the training session (long-term memory). Data are medians (interquartile ranges) of the step-down latencies in training and test sessions; $n = 15$ animals per group. Statistical comparison between groups was performed with Kruskal–Wallis. * Significantly different from control group ($P < 0.01$).

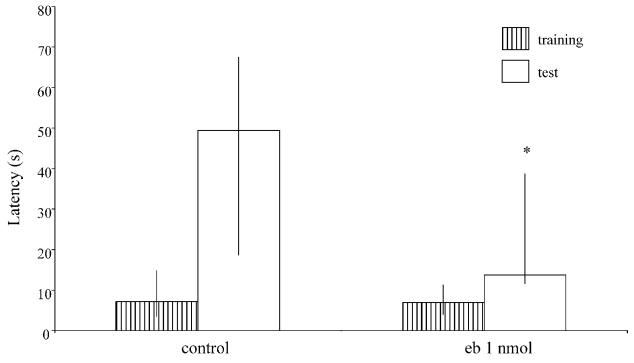

Fig. 3. Effect of intrahippocampal infusion of vehicle or 1 nmol of ebselen 10 min before the inhibitory avoidance test session 24 h after the training session. Data are medians (interquartile ranges) of the step-down latencies in training and test sessions; $n=9-11$ animals per group. Statistical comparison between groups was performed with Kruskal–Wallis. * $P=0.012$.

However, in the inhibitory avoidance task, different groups were injected 30 min before or immediately after training or 10 min prior long-term memory test.

3. Results

Intrahippocampal administration of ebselen immediately after the training session impaired the retention of inhibitory avoidance, evaluated 90 min (short-term memory, Fig. 1A) and 24 h (long-term memory, Fig. 1B) after training. Due the powerful amnesic effects of 1 and 3 nmol of ebselen, we evaluated the effect of these doses on anxiety and locomotor activity. Ebselen did not change the number of rearing or crossing responses in the open field (Table 1). In the plus-maze (Table 2), ebselen did not modify any parameter evaluated: the number of entries into the open or into the closed arms, and the time spent in the open or in the closed arms.

The effect of 1 nmol of ebselen on memory acquisition and retrieval was investigated. For studies involving memory acquisition (Fig. 2), ebselen was infused 30 min prior to training and inhibitory avoidance retention was evaluated 1.5 h (Fig. 2A) and 24 h (Fig. 2B) after training, for short- and long-term memory, respectively. The amnesic effect of ebselen on short- and long-term memory was confirmed, although the performance of the rats in the training session was not affected. Additionally, when infused 10 min prior to the test session, ebselen 1 nmol impaired memory retrieval 24 h after the training session (Fig. 3).

4. Discussion

In this study, the behavioral effects of a seleno-organic compound, ebselen, were investigated. Ebselen infused immediately after an inhibitory avoidance training session impaired both short- and long-term memory. The amnesic effect could not be attributed to influences on locomotor activity or anxiety levels, as assessed by open field or plus-maze tasks.

The influence of the lowest amnesic dose of ebselen (1 nmol) was further investigated on memory acquisition and retrieval, when infused 30 min prior to training sessions or 10 min before long-term memory test sessions, respectively. Ebselen also impaired inhibitory avoidance retention when infused before training and long-term memory test sessions. These results suggest that ebselen interfered not only with processes related to memory formation but also with retrieval. Acquisition, consolidation and retrieval share many of the same molecular mechanisms. Many of the molecular mechanisms involved in learning and memory are coincidence detectors—that is, they respond to appropriate stimuli synergistically. In the case of NMDA receptors, these stimuli are glutamate binding and depolarization of the postsynaptic cell; for activating the cAMP/protein kinase A (PKA) pathway, these stimuli include Ca^{2+} and G-protein activation of adenylyl cyclase. Retrieval depends on glutamate AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate) receptors and metabotropic glutamate receptors but not on NMDA receptors (Izquierdo et al., 1997). Moreover, retrieval depends on mitogen-activated protein kinase (MAPK) and on PKA in the hippocampus. The molecular events underlying consolidation are an extension of processes that begin during acquisition. Retrieval may share similar mechanisms with acquisition and memory performance, being enhanced if acquisition conditions match those of retrieval. Imaging experiments have suggested that similar brain regions are activated during retrieval and consolidation (Izquierdo and McGaugh, 2000).

It has been shown that the redox status of NMDA receptors modulates their functionality (Récasens et al., 1992; Tang and Aizenman, 1993a,b). Long-term potentiation, a plastic event triggered by NMDA receptors, has been shown to be impaired in glutathione-depleted animals and by dithionitrobenzoate, which is a well-known oxidant agent (Almaguer-Melian et al., 2000; Bernard et al., 1997). Surprisingly, different from its postulated antioxidant and consequent neuroprotective activities, it was recently reported that ebselen might also act as an oxidant of NMDA receptors through the NR1 modulatory site (Herin et al., 2001). Consequently, the amnesic effects observed in this study might be explained by its oxidant activity at critical sulfhydryl groups of NMDA receptors, and it was previously shown that the behavioral performance of mice is sensitive to the levels of in vivo oxidative stress (Dubey et al., 1996). Moreover, ebselen did not prevent the convulsions induced by a classical NMDA receptor agonist, quinolinic acid (Rossato et al., 2002), suggesting that ebselen is not an antagonist of glutamate receptors.

Recently, ebselen has been used with success for the treatment of ischemia in animal models (Imai et al., 2001; Namura et al., 2001; Noiri et al., 2001; Parnham and Sies,

2000), and for the treatment of stroke and aneurysmal subarachnoid hemorrhage in humans (Saito et al., 1998; Yamaguchi et al., 1998). The protective effect of ebselen against glutamate neurotoxicity indicates that ebselen could be considered in the near future for the management of acute and chronic brain diseases (Lee et al., 1999). The results of the present study clearly indicate that ebselen has amnesic effects, which is important to take into consideration in the perspective of its chronic use in humans. In conclusion, the results of the present investigation suggest that more detailed studies must be conducted to analyze whether ebselen has a cognition-impairing effect when administered chronically.

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