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European Journal of Pharmacology 584 (2008) 328–337
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Synergistic effects of chronic bryostatin-1 and α-tocopherol on spatial learning and memory in rats

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Received 15 November 2007; received in revised form 16 January 2008; accepted 6 February 2008
Available online 14 February 2008

Abstract

Evidence is emerging that protein kinase C (PKC) plays a crucial role in the neural processing of memory information and that PKC deficits underlie certain types of memory impairment, including Alzheimer's dementia. Chronic activation of PKC isozymes with bryostatin-1 induces synthesis of the proteins that are involved in memory consolidation and, therefore, may represent a pharmacological strategy for antidementic and memory therapies. PKC isozymes are, however, sensitive to oxidants, whose generation is also increased by PKC activation. Oxidants may be responsible for some adverse effects with PKC activators, potentially limiting their antidementic and memory-enhancing "benefit". We investigated the effects of intravenous bryostatin-1, a potent PKC activator, and of its co-administration with oral α-tocopherol, a potent antioxidant, on spatial learning and memory. Bryostatin-1 at a chronic and intravenous dose of 10 μg/m^2 (2 doses/week for 3 weeks) alone did not significantly affect the spatial learning and memory, but showed a synergistic effect when co-administered with α-tocopherol (60 IU/kg, orally and daily for 3 weeks), a potent lipid-soluble antioxidant and also a possible inhibitor of PKC in peripheral tissues. Acute administration of the same doses, however, did not have obvious influence on the learning and memory. These results provide support for the strategy of achieving memory-enhancing benefits with PKC activators and restricting their oxidant-related adverse effects with α-tocopherol co-administration. These agents, therefore, may hold significant potential as new, combined antidementic and memory therapeutics in the future.
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Keywords: Cognition; Learning and memory; Protein kinase C; Spatial memory; α-tocopherol; Vitamin E; (Rat)

1. Introduction

Emerging evidence suggests that protein kinase C (PKC) signaling may play a crucial role in many types of learning and memory (Alkon et al., 2007 for review). Abnormal PKC activity is also involved in the neurodegenerative pathophysiology of Alzheimer's disease and other types of dementia (Cole et al., 1988; Favit et al., 1998; Alkon et al., 2007), suggesting an involvement of dysfunctional PKC regulation in the pathogenesis of memory disorders. Activation of PKC thus represents a potential therapeutic strategy in antidementic and memory therapy. Supporting this strategy are the observations that PKC activation can reduce $A\beta_{1,42}$ in Alzheimer's disease transgenic mice (Etcheberrigaray et al., 2004) and induce synthesis of the proteins that are required for long-term memory consolidation (Alkon et al., 2005). In an earlier study, we observed that intracerebral ventricular administration of bryostatin-1, an activator of PKC substrates, at appropriate doses, improved rats' performance in the spatial water maze task (Sun and Alkon, 2005). The memory-enhancing effect was sensitive to 1-(5-isoquinolineulfonyl)-2-methylpiperazine, suggesting an involvement of PKC activation. One purpose of this study is to further examine the effects of bryostatin-1 on spatial memory when administered peripherally, since pharmacokinetic studies in mice have shown that its tissue distribution includes the brain with a transient peak level that was followed by a much lower steady-state level when administered intravenously (Zhang et al., 1996). With an intraperitoneal administration of the same

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doi:10.1016/j.ejphar.2008.02.014

dose, the peak level reached in the brain was much lower (Zhang et al., 1996). The second purpose of the study is to determine the necessity of chronic administration of the agents since such chronic PKC activation appears more effective in inducing the synthesis of some proteins that are involved in memory consolidation (Alkon et al., 2005).

Many of the adverse actions of PKC activators appear related to an interaction between PKC isozymes and oxidants. Activation of PKC facilitates memory-relevant information processing in neural networks but also leads to an increased generation of oxidants, or reactive oxygen species (Frey et al., 2006). Increased generation of oxidants may be responsible for bryostatin-1's dose-limiting impairment of muscular mitochondrial oxidative energy production (Hickman et al., 1995), a common condition associated with oxidative stress. Increased generation of oxidants in the brain is also well known to impair learning and memory (Fukui et al., 2005). In addition, PKC isozymes themselves are sensitive to reactive oxygen species (Jung et al., 2004; Pinton et al., 2007), in a manner independent of diacylglycerol, responsible for reactive oxygen species-triggered death of cortical neurons (Jung et al., 2004) and other tissue damage including apoptosis (Pinton et al., 2007). A sustained increase in the plasma levels of bryostatin-1 may also further sensitize PKC isozymes to oxidants. Furthermore, in some reports, PKC-induced cytokines may also contribute to toxic side effects such as myalgia (Li et al., 2007). It is therefore desirable to achieve the antidementic and memory-enhancing effects without the associated oxidants-related adverse actions of PKC activators. The effects of oxidants can be attenuated or eliminated by lipid-soluble antioxidants, such as vitamin E, which also exhibits a PKC-dependent and antioxidant-independent inhibition of platelet aggregation (Freedman and Keaney, 2001). In some cases, vitamin E has been found to inhibit PKC (Ferri et al., 2006) and therefore may potentially limit memory-enhancing benefits with PKC activators. Indeed, vitamin E-associated PKC inhibition has been suggested as a therapeutic strategy to reduce microvascular proliferation in diabetes (Gutterman, 2002). The third purpose of this study is thus to directly evaluate the possibility of co-administration of α-tocopherol, one of the eight isoforms of vitamin E and the most potent lipid-soluble antioxidant known in nature, in the bryostatin-1 treatment, particularly the possibility that that the co-administration of α-tocopherol might limit the memory-enhancing potential of the bryostatin-1.

2. Methods

2.1. Subjects

Adult male Wistar rats (250–300 gm; Charles River Labs, USA) were housed in a temperature-controlled (20–24 °C) room for at least a week prior to experimentation, allowed free access to food and water, and kept on a 12-h light/dark cycle.

2.2. Chemicals

Bryostatin-1 was purchased from BioMol Research Laboratories, Inc. (Plymouth Meeting, PA, USA) and (+)-a-tocopherol acetate, from Sigma Chemicals (St. Louis, WA, USA). Agents were either injected intravenously (i.v.) or orally via intra-gastric administration through ball-tipped animal feeding needles. Bryostatin-1 was solublized in dimethyl sulfoxide (Sigma) at 5-fold concentrated stock solutions, diluted before the administration with saline (final dimethyl sulfoxide concentration: 20%) and administered at 10 or 15 μg/m^2 (tail i.v., 2 doses/week, total 6 doses; Fig. 1), starting 2 weeks before the spatial learning training in a chronic study. The first training day began the next day after the 5th dose. The 6th dose was given on the 3rd training day, about 1 h before the first training trial of the day. (+)-α-Tocopherol acetate was administered daily at 60 IU/kg (in vegetable oil, about 46 mg/kg; daily, orally through ball-tipped animal feeding needles for the same 3 week period in a chronic study; On the training days, it was given about 1 h before the first trial of the day). The dose was chosen based on our preliminary studies of dose–response relationship in that a smaller dose, daily 30 IU/kg for 3 weeks, of (+)-α-tocopherol acetate had neither obvious effects on the learning and memory when administered alone nor on bryostatin-1-induced memory enhancement when co-administered (not shown).

To determine whether chronic pre-treatment is essential, we also evaluated the effects of acute administration of the agents during the training week. In the acute study, bryostatin-1 was administered on the 2nd and 4th training days (tail i.v., 2 doses/week, total 2 doses; Fig. 1) and (+)-α-tocopherol, daily starting on the 2nd training day, about 1 h before the first trial of the day.

Control rats received the same volumes of vehicle (1/5 dimethyl sulfoxide+4/5 saline by volume for tail i.v., 2 doses/week) and of vegetable oil (daily, intra-gastric administration) at the same schedule as those groups that received the bryostatin-1 and/or (+)-α-tocopherol treatments.

2.3. Spatial water maze tasks

Effects of bryostatin-1 and (+)-α-tocopherol on spatial memory were evaluated in rats *in vivo* with the Morris watermaze task. Rats were randomly assigned to different groups (8–10

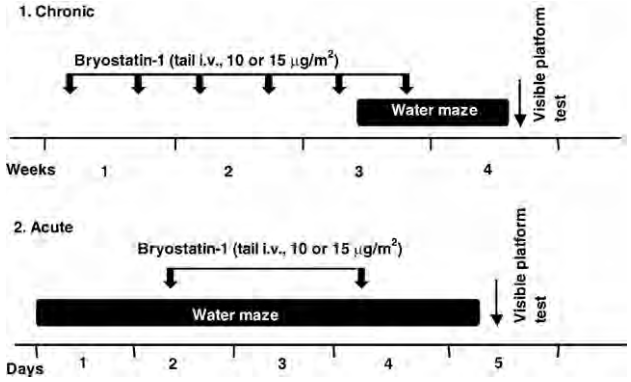

Fig. 1. Experimental procedures in the chronic and acute studies. Each short vertical arrow indicates the administration of an i.v. dose of bryostatin-1. Daily doses of α-tocopherol acetate were also given during the same periods in the rats that received α-tocopherol acetate.

each) and swam for 2 min in a 1.5 m (diameter)×0.6 m (depth) pool, filled with water to a depth of 40 cm (24±1 °C). On the following day, rats were trained in a 2-trials-per-day task for 4 consecutive days. Each training trial lasted for up to 2 min, during which rats learned to escape from the water by finding a hidden platform that was placed at a fixed location and submerged 2 cm below the water surface. The navigation of the rats was viewed on-line by the investigators (Video Monitor BWM9, Javelin Electronics Inc.), who were obscured from the rats' view, and tracked by a video-camera. The escape latency and the route of rats' swimming across the pool to the platform were recorded with a video-tracking system (Poly-Track Video Tracking System, San Diego Instruments, Inc.), for a quantitative analysis. A rat that failed to find the platform within 2 min was guided there, with the maximum latency of 120 s scored. A probe test was used to evaluate retention of the learned navigation experience. The probe test (1 min) was performed after removing the platform, 24 h after the last training trial, by monitoring the distance swum by each rat in the quadrants with the same video-tracking system.

2.4. Visible platform test

Immediately after the probe test, a visible platform test, a one-trial test, was used to evaluate whether there were significant alterations in sensorimotor ability of the rats with the different treatments, since performance in the water maze task could be influenced by alternations in the sensorimotor ability of the rats. The platform was placed at a new location, which differed from that used in spatial learning training trials, and was marked with a pole that protruded 9 in. above the water surface. The test involves neither learning nor a spatial map memory since the rats can see the marked platform location. The results thus illustrate whether changes in rat performance in the spatial learning and memory task could be due to an altered sensorimotor ability. The escape latency and the route of rats' swimming across the pool to the visible platform were recorded with a video-tracking system for a quantitative analysis.

2.5. Data analysis

Escape latency, i.e., distance swum, was used in the learning trials to index the rat's performance in the spatial learning. A target quadrant ratio was calculated (by dividing the target quadrant distance by the average of the non-target quadrant values during the probe test) to index the memory retention in the probe test. The ratio measures where the rat searched for the hidden platform, which has been removed in the test. Statistical analyses were performed using analysis of variance (ANOVA) whenever appropriate, with differences being determined by Tukey's *post hoc* tests. The values were expressed as means± S.E.M.

All animals used in these experiments were treated under the National Institutes of Health guidelines for the welfare of laboratory animals and the protocol, approved by the institutional ethics committee.

3. Results

3.1. Chronic intravenous bryostatin-1 enhanced rat water maze spatial learning and memory

We first evaluated chronic effects of bryostatin-1 at 15 μg/m^2, (+)-α-tocopherol acetate at 60 IU/kg, and a co-administration of 15 μg/m^2 bryostatin-1 and 60 IU/kg (+)-α-tocopherol acetate on spatial learning. There was a significant reduction in the escape latency over trials (Fig. 2; $F_{7,319}=46.062$, $P<0.001$; ANOVA), indicating that all rats learned the spatial maze task through the training trials. Overall, there was also a significant learning difference between the 4 groups ($F_{3,319}=6.765$, $P<0.001$). Detailed analysis revealed that the α-tocopherol, bryostatin-1 and α-tocopherol+bryostatin-1 groups all showed a significantly improved learning over the control (α-tocopherol over control: $F_{1,159}=12.317$, $P<0.001$; bryostatin-1 vs. control: $F_{1,159}=13.438$, $p<0.001$; α-tocopherol+bryostatin-1 vs. control: $F_{1,159}=17.681$, $P<0.001$). There was also a significant difference in learning between the α-tocopherol+Bryostatin-1 and α-tocopherol groups ($F_{1,159}=3.927$, $P<0.05$). The difference in learning between bryostatin-1+α-tocopherol group and bryostatin-1 group was, however, statistically insignificant (α-tocopherol+bryostatin-1 vs. bryostatin-1: $F_{1,159}=0.984$, $P>0.05$; Fig. 2). Thus, at the doses and duration administered, bryostatin-1 and α-tocopherol significantly improved learning, whilst chronic α-tocopherol administration did not negatively affect the learning effects of bryostatin-1. The average swim speeds for all eight trials did not differ between these groups ($P>0.05$; not shown).

The results of the probe memory test after the learning training trials revealed the same effects. All the rat groups showed a target quadrant preference in the probe test (Fig. 3). Data were analyzed using a target quadrant ratio (dividing the target quadrant distance by the average of the non-target quadrant values during the probe test; Fig. 3E). There were differences in the target quadrant ratios between the groups ($F_{3,39}=5.198$, $P<0.01$). The α-tocopherol, bryostatin-1 and bryostatin-1+α-tocopherol groups all showed a higher target quadrant ratio value than that of the control group (α-tocopherol vs. control: $F_{1,19}=4.392$, $P<0.05$; bryostatin-1 vs. control: $F_{1,19}=12.126$, $P<0.005$; α-tocopherol+bryostatin-1 vs. control: $F_{1,19}=12.214$, $P<0.005$), indicating enhanced memory

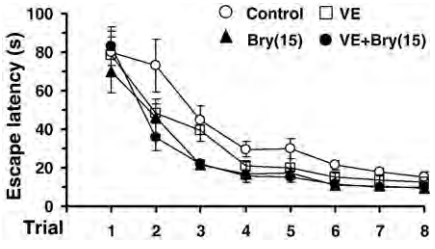

Fig. 2. Spatial water maze performance of rats over training trials. Data are shown as means±S.E.M. Bry(15), chronic bryostatin-1 at 15 μg/m^2; VE, chronic (+)-α-tocopherol acetate at 60 IU/kg; VE+Bry(15), a chronic co-administration of with bryostatin-1 at 15 μg/m^2 and (+)-α-tocopherol acetate at 60 IU/kg. 10 rats/group.

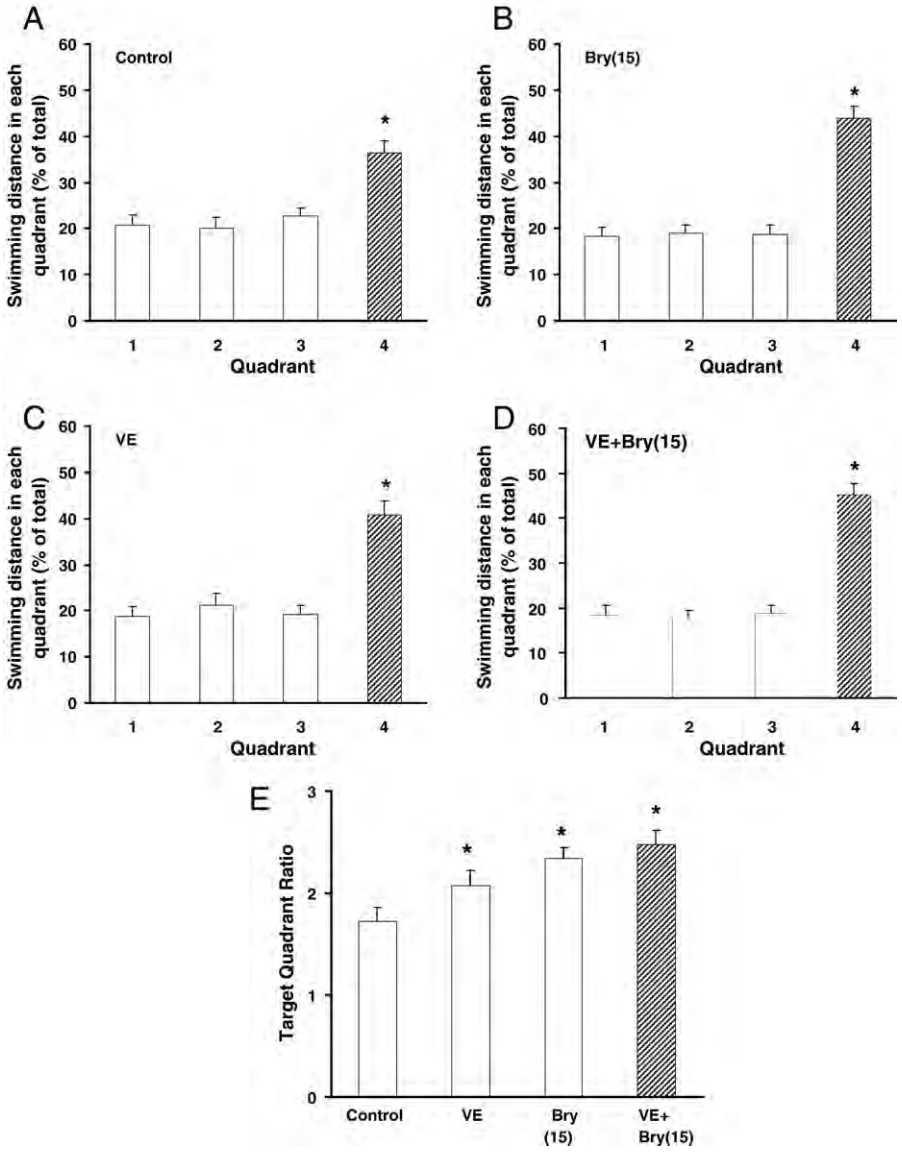

Fig. 3. Results of the probe tests after training trials. Quadrant 4 was the target quadrant where the hidden platform was placed during the training trials. Bry(15), chronic bryostatin-1 at 15 μg/m^2; VE, chronic (+)-α-tocopherol acetate at 60 IU/kg; VE+Bry(15), a chronic co-administration of with bryostatin-1 at 15 μg/m^2 and (+)-α-tocopherol acetate at 60 IU/kg. E shows the target quadrant ratio during probe test. 10 rats/group. *: $P<0.01$. NS: $P>0.05$.

with the treatments. The difference in the ratio values between the α-tocopherol+bryostatin-1 group and the bryostatin-1 group was, however, insignificant ($F_{1,19}=0.571$, $P>0.05$), whereas there was a significant difference in the target quadrant ratio between the α-tocopherol+bryostatin-1 and α-tocopherol groups ($F_{1,19}=4.483$, $P<0.05$). Thus, chronic co-administration at least did not negatively affect the memory-enhancing effect of bryostatin-1.

3.2. Synergistic effects of a smaller chronic dose of bryostatin-1 and α-tocopherol on spatial learning and memory

The above results revealed that chronic co-administration of α-tocopherol did not reduce the enhancing effects of chronic bryostatin-1 on spatial learning and memory. On the other hand, it is likely that an enhanced learning and memory produced by bryostatin-1 would make a further enhancement much more difficult to achieve experimentally. We therefore examined the chronic effects of a lower bryostatin-1 dose, intravenous bryostatin-1 at 10 μg/m^2 (2 doses/week for 3 weeks), intragastric (+)-α-tocopherol acetate at 60 IU/kg (daily for 3 weeks), and a co-administration of bryostatin-1 at 10 μg/m^2 and (+)-α-tocopherol acetate at 60 IU/kg for the same 3 week period on spatial learning and memory.

All rats learned the spatial maze over trials as illustrated by fewer seconds required by the rats to find the hidden escape platform as the training trials progressed (Fig. 4; $F_{7,319}=111.477$, $P<0.001$; ANOVA). There was, however, a significant learning difference between the 4 groups ($F_{3,319}=11.831$, $P<0.001$), revealing an impact of different treatments on the learning. Detailed analysis revealed that the bryostatin-1 group at the dose did not reach a significant difference over the control

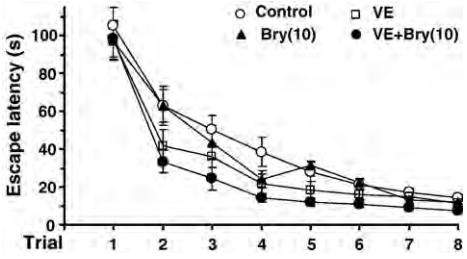

Fig. 4. Spatial water maze performance of rats over training trials. Data are shown as means ± S.E.M. Bry(10), chronic bryostatin-1 at 10 μg/m^2; VE, chronic (+)-α-tocopherol acetate at 60 IU/kg; VE + Bry(10), a chronic co-administration of with bryostatin-1 at 10 μg/m^2 and (+)-α-tocopherol acetate at 60 IU/kg. 10 rats/group.

($F_{1,159}=1.321$, $P>0.05$), while α-tocopherol and α-tocopherol + bryostatin-1 groups showed a significantly improved learning over the control (α-tocopherol vs. control: $F_{1,159}=9.626$, $P<0.005$; α-tocopherol + bryostatin-1 vs. control: $F_{1,159}=28.405$, $P<0.001$). Furthermore, the differences between α-tocopherol + bryostatin-1 group and either α-tocopherol or bryostatin-1 were also significant (α-tocopherol + bryostatin-1 vs. bryostatin-1: $F_{1,159}=20.217$, $P<0.001$; α-tocopherol + bryostatin-1 vs. α-tocopherol: $F_{1,159}=7.201$, $P<0.01$). Thus, at the smaller dose, bryostatin-1 did not significantly affect the learning in the test. However, the co-administration of α-tocopherol at 60 IU/kg significantly enhanced the learning since the learning improvement was significantly better than those obtained with α-tocopherol or bryostatin-1 alone, indicating a synergistic effect of bryostatin-1 and α-tocopherol. The average swim speeds for all eight trials did not differ between these groups ($P>0.05$; not shown).

Similarly, all the rat groups showed a target quadrant preference in the probe test (Fig. 5). The target quadrant ratio

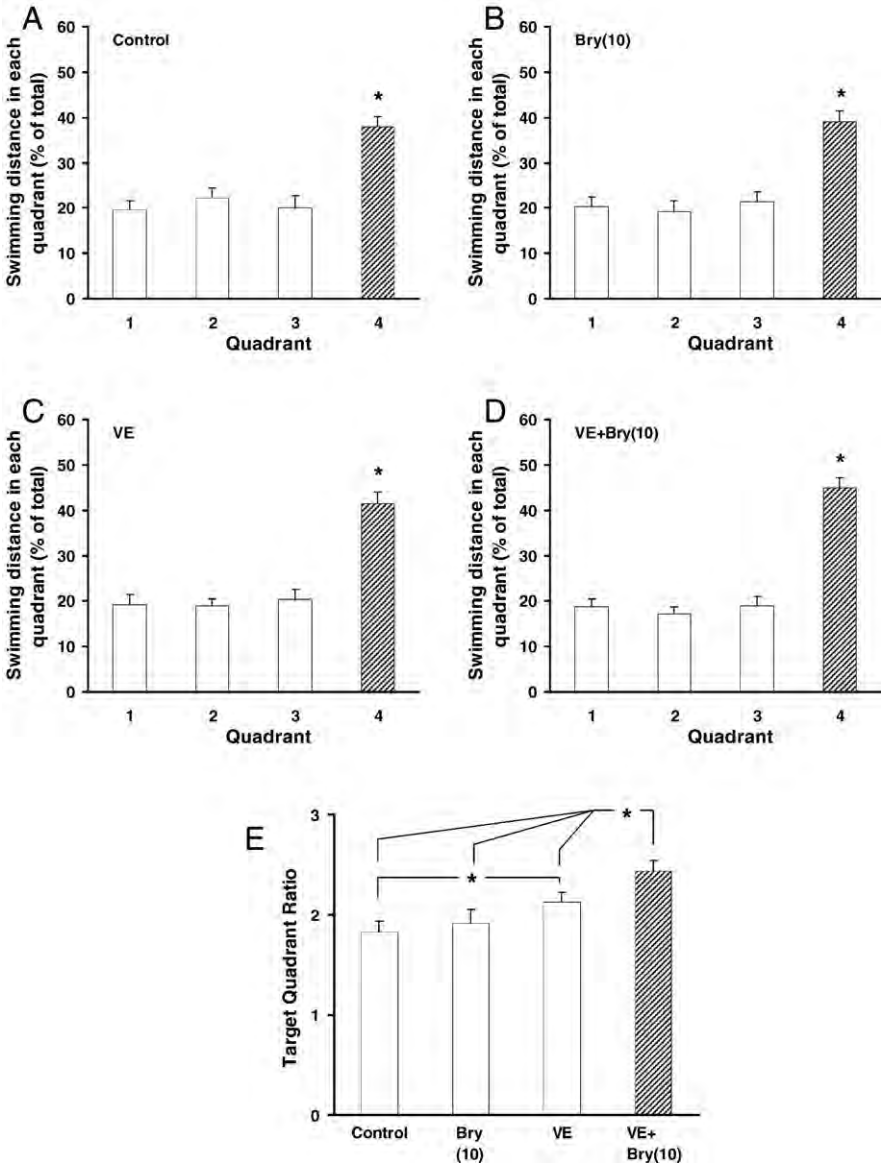

Fig. 5. Results of the probe tests after training trials. Quadrant 4 was the target quadrant where the hidden platform was placed during the training trials. Bry(10), chronic bryostatin-1 at 10 μg/m^2; VE, chronic (+)-α-tocopherol acetate at 60 IU/kg; VE + Bry(10), a chronic co-administration of with bryostatin-1 at 10 μg/m^2 and (+)-α-tocopherol acetate at 60 IU/kg. E shows the target quadrant ratio during probe test. 10 rats/group. *: $P<0.01$. NS: $P>0.05$.

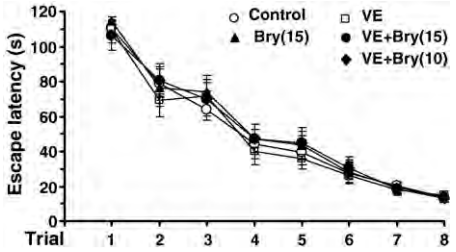

Fig. 6. Spatial water maze performance of rats over training trials. Data are shown as means±S.E.M. Bry(15), acute bryostatin-1 at 15 μg/m^2; VE, acute (+)-α-tocopherol acetate at 60 IU/kg; VE+Bry(15) or VE+Bry(10), acute co-administration of bryostatin-1 at 15 or 10 μg/m^2 with (+)-α-tocopherol acetate at 60 IU/kg. 8 rats/group.

analysis (Fig. 5E) revealed significant differences between the groups ($F_{3,39}=6.234$, $P<0.005$). In short, the bryostatin-1 group did not exhibit a significant difference over the control ($F_{1,19}=0.279$, $P>0.05$), whilst α-tocopherol and α-tocopherol+bryostatin-1 groups showed a higher target quadrant ratio value than that of the control (α-tocopherol vs. control: $F_{1,19}=4.455$, $P<0.05$; α-tocopherol+bryostatin-1 vs. control: $F_{1,19}=16.837$, $P<0.001$). The differences in the ratio values between the α-tocopherol+bryostatin-1 group and either the α-tocopherol or the bryostatin-1 were also significant (α-tocopherol+bryostatin-1 vs. bryostatin-1: $F_{1,19}=10.052$, $P<0.05$; α-tocopherol+bryostatin-1 vs. α-tocopherol: $F_{1,19}=5.309$, $P<0.05$), indicating enhanced memory. These results were consistent with the learning effects of the treatments.

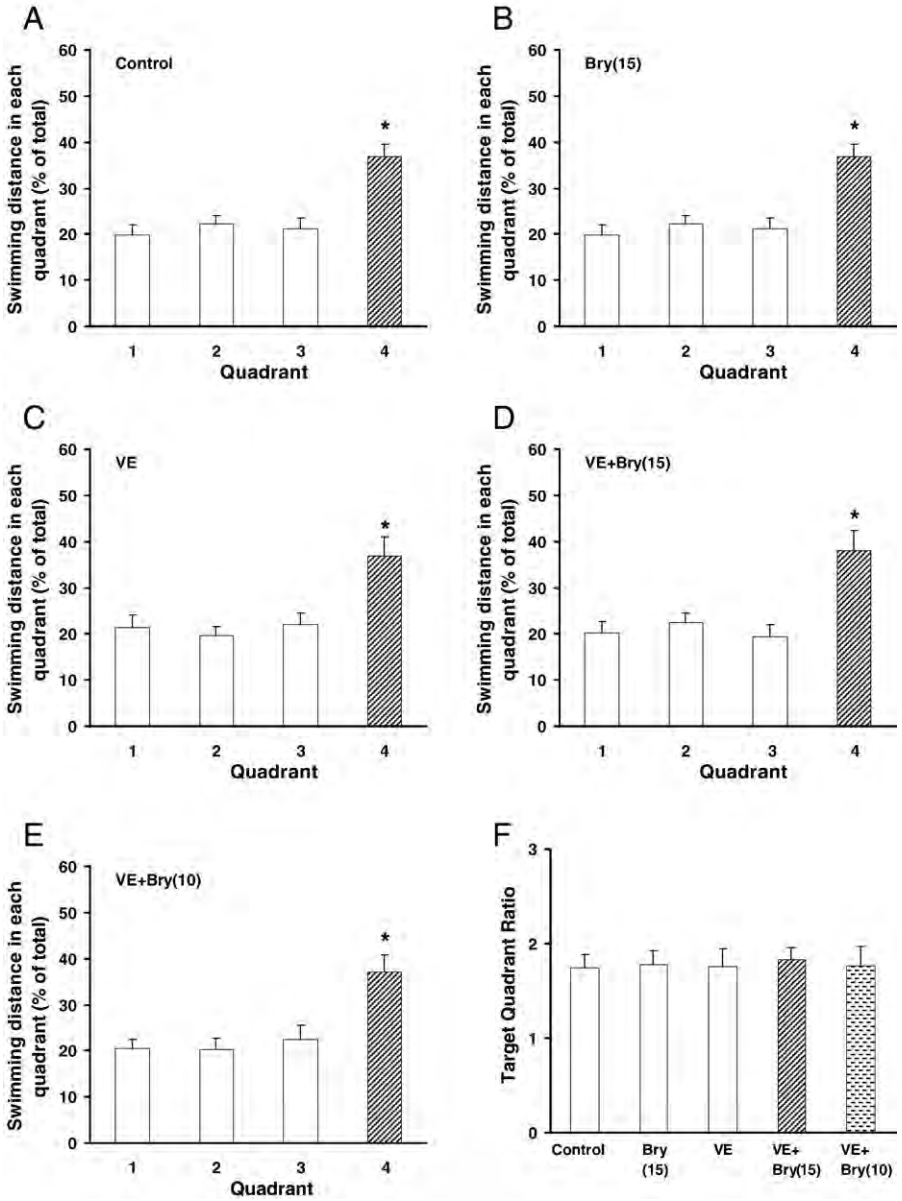

Fig. 7. Results of the probe tests after training trials. Quadrant 4 was the target quadrant where the hidden platform was placed during the training trials. Bry(15), acute bryostatin-1 at 15 μg/m^2; VE, acute (+)-α-tocopherol acetate at 60 IU/kg; VE+Bry(15) or VE+Bry(10), acute co-administration of bryostatin-1 at 15 or 10 μg/m^2 with (+)-α-tocopherol acetate at 60 IU/kg. E shows the target quadrant ratio during probe test. 8 rats/group.

3.3. No significant effects of acute bryostatin-1, α-tocopherol, and their co-administration on spatial learning and memory

We further evaluated whether the chronic administration of bryostatin-1 and α-tocopherol was essential to produce the learning and memory-enhancing effects. We examined the acute effects of intravenous bryostatin-1 at 15 μg/m^2 (2 doses/week), intra-gastric (+)-α-tocopherol acetate at 60 IU/kg (daily starting on the 2nd training day), and an acute co-administration of bryostatin-1 at 10 μg/m^2 or 15 μg/m^2 and (+)-α-tocopherol acetate at 60 IU/kg on spatial learning and memory.

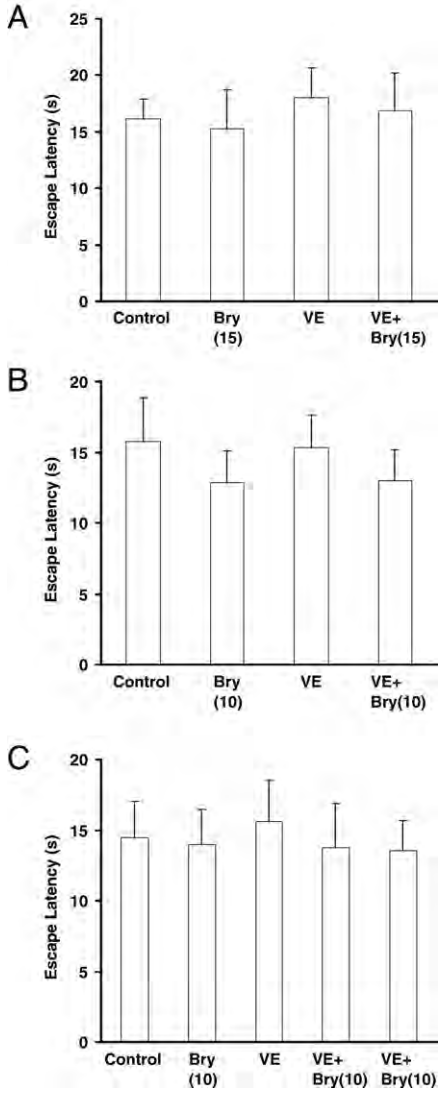

Fig. 8. Results of a visible platform test. A: Escape latencies of rats in a visible platform test of control rats, rats with chronic bryostatin-1 at 15 μg/m^2 [Bry (15)], (+)-α-Tocopherol acetate at 60 IU/kg (VE), or a chronic co-administration of with bryostatin-1 at 15 μg/m^2 and (+)-α-Tocopherol acetate at 60 IU/kg (VE+Bry). 10 rats/group. B: Escape latencies of rats in a visible platform test of control rats, rats with chronic bryostatin-1 at 10 μg/m^2 [Bry(10)], chronic (+)-α-Tocopherol acetate at 60 IU/kg (VE), or a chronic co-administration of with bryostatin-1 at 10 μg/m^2 and (+)-α-Tocopherol acetate at 60 IU/kg (VE+Bry). 10 rats/group. C: Escape latencies of rats in a visible platform test of control rats, rats with acute bryostatin-1 at 10 μg/m^2 [Bry(10)], 15 μg/m^2 [Bry(15)], (+)-α-Tocopherol acetate at 60 IU/kg (VE), or an acute co-administration of bryostatin-1 at either 10 μg/m^2 or 10 μg/m^2 and (+)-α-Tocopherol acetate at 60 IU/kg. 8 rats/group.

All rats learned the spatial maze over trials as the training trials progressed (Fig. 6; $F_{7,319}=43.468$, $P<0.001$). However, there was no significant learning difference between the 5 groups ($F_{4,319}=1.748$, $P>0.05$), revealing no significant impact of different treatments on the learning. The average swim speeds for all eight trials did not differ between these groups ($P>0.05$; not shown). Thus, chronic administration of the agents at the doses given is essential for obtaining the memory-enhancing effects in the test.

The probe test revealed the same type of responses in general. All the rat groups showed a target quadrant preference in the probe test (Fig. 7). The target quadrant ratio analysis (Fig. 7F), however, showed no significant differences between the groups ($F_{4,39}=0.0717$, $P>0.05$), consistent with the learning effects of the treatments.

3.4. No significant effects of bryostatin-1 and α-tocopherol on rat performance in a visible platform test

A visible platform test was used as a control, directly evaluating the sensorimotor ability of the rats after the different treatments. The test resulted in no significant difference between the groups ($F_{3,39}=0.223$, $P>0.05$; Fig. 8A) of the rats chronically treated with either vehicle, 15 μg/m^2 bryostatin-1, 60 IU/kg (+)-α-tocopherol acetate, or their co-administration. Similarly, no significant difference was found between the groups ($F_{3,39}=0.209$, $P>0.05$; Fig. 8B) of the rats chronically treated with either vehicle, 10 μg/m^2 bryostatin-1, 60 IU/kg (+)-α-tocopherol acetate, or their co-administration. Nor was there group difference ($F_{4,39}=0.264$, $P>0.05$; Fig. 8C) found in the rats acutely treated with either vehicle, bryostatin-1 at 15 μg/m^2, (+)-α-tocopherol acetate at 60 IU/kg, or a co-administration of bryostatin-1 at either 15 μg/m^2 or 10 μg/m^2 and (+)-α-tocopherol acetate at 60 IU/kg in the acute study. These results indicate that there were no significant group differences in sensorimotor ability of the rats after the different treatments so that differences in escape latency observed in the spatial learning and memory task were unlikely due to alterations in sensorimotor ability of the rats after the treatments.

4. Discussion

This study is the first to report that chronically and peripherally administered bryostatin1-1 can improve rat spatial learning and memory at an appropriate dose and that there is a synergistic action of the PKC activator with α-tocopherol. Their synergistic interaction is based on the effects produced by α-tocopherol at 60 IU/kg and bryostatin-1 at 10 μg/m^2. Without a synergistic interaction, the effect produced by α-tocopherol at 60 IU/kg and bryostatin-1 at 10 μg/m^2 would have not been significantly different from that produced by α-tocopherol at 60 IU/kg alone, since bryostatin-1 at 10 μg/m^2 was ineffective (i.e., a significant improvement better than an additive interaction). Learning and memory are delicate and very specific processes as illustrated by normal rats practicing in

the spatial learning and memory. Not surprisingly, further improvements in learning over a drug-enhanced learning are not easily achieved. In addition, because of its dose-limiting toxicity (see below), bryostatin-1 cannot be administered at much higher doses peripherally. Myalgia, for instance, associated with higher doses of bryostatin-1 would non-specifically affect rats' performance in the motor activity-dependent memory tasks. Thus, a dose–response curve with several widely-spread doses cannot be obtained in the type of study for an isobolographic analysis. Nevertheless, it is important to note that the potential PKC inhibiting action of α-tocopherol did not have negative impact on the learning and memory-enhancing effect of bryostatin-1, as defined in this study, so that α-tocopherol can be co-administered with the PKC activator to restrict the adverse effects associated with oxidants. Furthermore, the effects at the applied peripheral doses depend on a period of pre-treatments, consistent with the earlier observation that pre-treatment with bryostatin-1 before learning task induces the synthesis of proteins that are required for memory consolidation and is more efficient (Alkon et al., 2005), whereas an acute administration of the same doses of agents was not sufficient. The results, therefore, imply potential therapeutic values of developing these agents as antidementic and memory-enhancing therapeutics, but do not rule out the possibility that a higher dose of bryostatin-1 might improve spatial learning and memory acutely (Sun and Alkon, 2005).

PKC is a multigene family of phospholipid-dependent, serine-threonine kinases, central to many signal transduction pathways. It is ubiquitously and densely expressed in the brain and activated by Ca^{2+}, phospholipids and diacylglycerol, phorbol esters or other PKC activators. Its family members currently include cPKC (α, β_I, β_{II}, and γ), nPKC (δ, ε, ε', η, θ, and μ), and aPKC (ζ and λ/ι), often co-expressed in the same tissues and cells. The brain has the highest concentration of PKC of any organ in the body (Saito et al., 1988). PKC activation enhances Ca^{2+} influx, increases neurotransmitter refill rate and release, decreases a Ca^{2+}-activated current in the hippocampus, phosporylates numerous proteins, and produces a potentiation of synaptic responses (Alkon and Rasmussen, 1988; Alkon et al., 2007). Evidence is accumulating that PKC signaling may play a crucial role in many types of learning and memory, including Pavlovian conditioning of *Hermissenda* (Alkon et al., 2007 for review), rat spatial maze learning (Colombo et al., 1997; Sun and Alkon, 2005), memory of eye blink conditioning (Olds et al., 1989; Schreurs et al., 1996; Van der Zee et al., 1997), olfactory discrimination learning (Olds et al., 1994), conditioned taste aversion (Yasoshima and Yamamoto, 1997), contextual fear memory (Ahi et al., 2004), and conditioned avoidance (Jerusalinsky et al., 1994). PKC signaling is dysfunctional in the brains with Alzheimer's disease (see Alkon et al., 2007 for review). Consistent with the important role of PKC in learning and memory is also the evidence that PKC deficits and inhibition result in learning and memory impairments (Weeber et al., 2000; Sun and Alkon, 2005; Bonini et al., 2007).

Bryostatin-1 is a macrocyclic lactone and its main mechanism of biological action is modulation of PKC activity, acting as a partial activator. Bryostatin-1 activates the cPKC and nPKC isozymes and exhibits an obvious advantage for a therapeutic agent as, unlike the phorbol esters, it lacks tumor-promoting capabilities and actually counteracts tumor promotion induced by phorbol esters (Hennings et al., 1987). In clinical trials as an anti-tumor agent, it is reasonably well tolerated, having a tolerated dose of 25 μg m^{-2}/week (Clamp et al., 2003). In human clinical trials, its main dose-limiting toxicity is myalgia, probably resulting from mitochondrial dysfunction in the muscle (Hickman et al., 1995) or an interaction with cytokines (Li et al., 2007). Bryostatin-1 has been shown to distribute into the brain after peripheral administrations in mice (Zhang et al., 1996). In our study, dimethyl sulfoxide was used to solublize bryostatin-1. Its blood concentrations are estimated to be approximately 0.8%, assuming an immediate mixing with a blood volume of 5% body weight. It remains to be studied whether the level of dimethyl sulfoxide may alter the blood brain barrier.

With regard to bryostatin-1 and other PKC activators as antidementic and memory-enhancing agents, one major concern is their adverse effects related to oxidants. Not only are PKC isozymes activated by oxidants, leading to a variety of adverse effects and cellular damage. PKC activation is also associated with an increased generation of oxidants (Frey et al., 2006). Myalgia, for instance, may be due to muscular mitochondrial dysfunction (Hickman et al., 1995), a condition commonly contributing to an increased generation of oxidants. Reactive oxygen stress impairs learning and memory (Fukui et al., 2005). In terms of effects on learning and memory, bryostatin-1 thus has a two-side impact, an enhancement of the memory-related information processing in neural networks and an oxidant-induced impairment of the same processing. Under the 'normal' condition as shown in this study, the net impact of bryostatin-1 administration on learning and memory, produced in high concentrations, is an improvement. The results do not necessarily mean that the outcome could not be reversed under some conditions when sensitivity to oxidants is greatly enhanced or oxidants are enormously produced, such as in stroke, ischemia and reperfusion. By co-administering a powerful antioxidant, much of these oxidants-related adverse effects with PKC activators may be eliminated or attenuated.

The antioxidant effects of vitamin E are well established, including acting as a scavenger of reactive oxygen species and/or reducing the generation of reactive oxygen species (Schneider, 2005). Brain cells cannot make vitamin E and evidence has been provided that a daily peripheral dose of 2 mg/kg α-tocopherol for 2 weeks is sufficient to result in a significant increase in the α-tocopherol level in the rat brain (Ferri et al., 2003), more increase in the brain (2.17-fold of the control) than the increase in the plasma (1.4-fold of the control; Cuppini et al., 2002) with the same dose, consistent with the observation of tocopherol accumulation in the brain including the hippocampus observed by others (Joseph et al., 1998). The brain accumulation of tocopherols in animal studies does not contradict the report that no such increase was observed in the cerebrospinal fluid (CSF) with large doses of vitamin E in humans (e.g., Pappert et al., 1996), since it is well known that tocopherols are water-insoluble and

their pathway from the cerebral microcirculation to the neurons in the brain does not involve CSF, where one would thus not expect to see any changes actually reflecting the α-tocopherol level in the brain.

The interaction between PKC and vitamin E, however, is less clear. There are reports that vitamin E may inhibit (Ferri et al., 2006) or activate (Kogure et al., 2003) PKC. Diet vitamin E supplementation has been shown to protect hippocampal CA1 neurons through its antioxidant effect in rats (Fukui et al., 2005). Our results showed that at an appropriate dose, co-administered α-tocopherol did not inhibit the memory-enhancing action of bryostatin-1 but rather produced a synergistic effect on the learning and memory. It is interesting that at 60 IU/kg, α-tocopherol alone enhanced the learning and memory. It remains to be studied whether this effect results from blocking the interaction between the PKC isozymes and oxidants. The consideration of its antioxidant actions, however, does not mean that other mechanisms may not be involved since vitamin E has been shown to produce a variety of non-antioxidant effects, such as induction of target genes of hypoxia-inducible factor-1 (Zhang et al., 2004), the bcl 2 and gene-related expression, NF-κB transcriptional activity (Ekstrand-Hammarstrom et al., 2007), growth factors or cytokines (Singh et al., 2006), and the synthesis of proteins that may be involved in synaptic plasticity (Gohil et al., 2003).

In summary, we have found that chronic bryostatin-1 at an appropriate, intravenous dose produced an enhancement of spatial learning and memory in rats. Co-administration of α-tocopherol does not reduce bryostatin-1's memory-enhancing action but instead produced a synergistic effect on the learning and memory. For the treatment of dementic disorders including Alzheimer's disease, PKC activators not only increase PKC signaling activity but also reduce Aβ accumulation and tau protein hyperphosphorylation in the brain (Etcheberrigaray et al., 2004; Alkon et al., 2007). Memory disorders, such as Alzheimer's disease and vascular dementia, are often associated and may be, at least partly, caused by an increased generation of oxidants. These observations strongly suggest that by limiting oxidants-related adverse effects with co-administration of antioxidant agents such as vitamin E, PKC pharmacology may represent an attractive area for the development of new antidementic and cognitive therapeutics.

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