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Dual effects of bryostatin-1 on spatial memory and depression

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Abstract

Dementia and depression are clinical symptoms commonly associated in patients. Emerging evidence suggests that the two diseases share many profiles in their development and underlying neural/molecular mechanisms. Thus, interest is raised in developing new classes of antidepressant agents with activity of cognitive enhancement. Here, we show that bryostatin-1, a protein kinase C substrate activator, at bilateral intracerebroventricular doses of 0.64 or 2 pmol/site, significantly enhanced learning and memory of rats in a spatial water maze task. When applied at the doses at which it exhibits memory-enhancing activity, bryostatin-1 showed a significant antidepressant activity, as determined in an open space swim test. Both effects were not observed when a smaller dose was administered and were largely eliminated by co-administration of 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7), a protein kinase C inhibitor. These results support the hypothesis that memory processing and mood regulation share common neural mechanisms. Restoring impaired mood regulation with antidepressant agents that also exhibit memory-enhancing activity may represent one of the new strategies in the fight against depression associated with memory impairments.

Keywords: Cognition; Depression; Learning and memory; Mood; Open space swim test; Protein kinase C

1. Introduction

Depression is a serious disorder that affects the quality of human lives and has a dramatic impact on cognition and recovery from other mental disorders. Most adults will suffer one or more depressive episodes during lifetime. In the Unites States alone, about 2–5% of the population are depressed, while approximately 20% suffer from milder forms of the illness.

One factor that is recognized in human patients but not well studied experimentally is the impact of mood on the ability of cognition. Depressed patients exhibit diminished ability to think and concentrate. Furthermore, depression is an early symptom of Alzheimer's disease. Episodic memories, including spatial memory, are most obviously and severely impaired in Alzheimer's dementia. These memories are known to depend on the functional integrity of the hippocampus and its associated structures, which also exhibit severe atrophy in major depression, as measured with magnetic resonance imaging (Sheline et al., 1996; Bremner et al., 2000). The pathological mechanisms have not be defined but are most likely due to extreme sensitivity of the neural structures involved in mood regulation and memory, especially the hippocampal pyramidal cells and their synaptic interactions with neurons in the amygdala, to a variety of neurodegenerative damage and other diseases.

One important signaling molecule in learning and memory is the protein kinase C (PKC; Bank et al., 1988; Alkon et al., 1998). Reduced PKC activity is associated with Alzheimer's dementia (Cole et al., 1988; Favit et al., 1998) and suicide victims (Pandey et al., 2003), suggesting an involvement in cognition and mood regulation. Activation of PKC may represent a potential therapeutic strategy in improving memory and mood. The phorbol esters have been

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reported to be potent PKC stimulators. However, their potential as therapeutic drugs is limited by their action as tumor promoters (Blumberg, 1988). In this study, we examined the effects of bryostatin-1, an activator of the protein kinase C (PKC) substrates, on spatial memory and induced depressive behavior. Bryostatin-1 at appropriate doses improved rats' performance in spatial water maze task. When tested in an open space swim test, an animal model of induced depressive behavior (Sun and Alkon, 2003, 2004), bryostatin-1 significantly reduced non-searching immobility of rats, a response sensitive to 1-(5-isoquinolineulfonyl)-2-methylpiperazine (H-7) administration. The results support the hypothesis that episodic memory and mood regulation share some common neural targets/mechanisms (Sun and Alkon, 2002, 2004) and that bryostatin-1-like substances may have values as a new class of effective antidepressants with memory-enhancing profile.

2. Materials and methods

2.1. Chemicals

Bryostatin-1 was purchased from BioMol Research Laboratories, Inc. (Plymouth Meeting, PA, USA) and imipramine and H-7 (1-(5-isoquinolineulfonyl)-2-methylpiperazine) were from Sigma Chemicals (St. Louis, MO, USA). Agents were either injected intravenously (i.v.) or into the lateral cerebral ventricle (i.c.v.) through chronically placed cannulas. The vehicle for H-7 was artificial cerebrospinal fluid (aCFS). Bryostatin-1 was administered either i.c.v. or i.v. as detailed below. Bryostatin-1 was solubilized in dimethyl sulfoxide (DMSO; Sigma) at fivefold concentrated stock solutions, which was either diluted before the administration with saline for i.v. injection or aCSF for i.c.v. administration. The aCFS solution (pH 7.4) contains (mM): NaCl (124), KCl (3), $MgSO_4$ (1.3), $CaCl_2$ (2.4), $NaHCO_3$ (26), NaH_2PO_4 (1.25), and glucose (10). In the open space swim test, imipramine (10 mg/kg, i.p.) and vehicle were administered between the swim trial sessions, 23 h, 3 h, and 1 h before the second and the third trial sessions, respectively. The use of three doses between test trials produces more consistent predictive effects than those of a single dose on an acute test in induced depressive behavior (Porsolt et al., 1997).

2.2. Spatial water maze tasks

Effects of bryostatin-1 on spatial memory were evaluated in rats in vivo with the Morris water maze task. Male adult Wistar rats (200–250 g) were housed in a temperature-controlled (20–24 °C) room for a week, allowed free access to food and water, and kept on a 12-h light/dark cycle. All rats were randomly assigned to different groups (10 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 two-trials-per-day task for four 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 1–2 cm below the water surface. The navigation of the rats was viewed on-line by the investigators (Video Monitor BWM9, Javelin Electronics), 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) for a quantitative analysis. 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.

2.3. Visible platform test

A visible platform test was used to evaluate whether changes in rat performance on spatial learning and memory could be due to altered sensorimotor ability of the rats. The platform was placed at a new location, which differed from that used in spatial learning and memory, and was marked with a pole that protruded 9 in. above the water surface. The escape latency and the route of rats' swimming across the pool to the visible platform were recorded with the video-tracking system for a quantitative analysis.

2.4. Open space swim test: induction of depressive behavior

Rats were placed individually in a round pool, which has a diameter of 152 cm and a height of 60 cm and was filled with 40 cm H_2O (24±1 °C). The room and pool are part of the standard set-up used for spatial water maze task. No escape was provided in these trials during the test. Rats were free to swim (or not to swim) for 15 min, were then removed from the pool, and returned to their home cages after drying. The investigators were obscured from sight of the rats during the trials, but were able to observe the animals' behaviors on a video screen monitor (Video Monitor BWM9, Javelin Electronics) during the trials. The same procedure (15 min session/day) was followed 24 h later for two more days. The swimming/drifting path was recorded with a video-tracking system (Poly-Track Video Tracking System, San Diego Instruments). The distance moved (mobility) included all the distance moved during the entire 15 min. Active swimming was defined as when a rat was making active swimming motions as those to move around in the pool. We have previously reported that the swim activity measurements accurately reflect duration of mobility and that the maximum effect is induced on the third trial without antidepressant treatment (Sun and Alkon, 2003).

2.5. In vivo cannulation

In some experiments, agents and vehicle were administered i.c.v. through cannulation. Rats were anesthesized with sodium pentobarbital (60 mg/kg, i.p., and with supplemental doses if necessary) and placed in a stereotactic apparatus (Kopf Instruments, Tujunga, CA). The core temperature of rats was monitored and kept constant (38.0±0.5 °C) with a warming light and pad. Two stainless steel guide cannulas were placed with the tips positioned at the coordinates (anterior–posterior, 0.5 mm; lateral 1.5 mm; horizontal, 3.2 mm) under aseptic conditions. The guide cannulas were filled with stainless steel wires, which were replaced with stainless steel injection cannulas during injection procedure. At the end of surgery and under appropriate anesthesia, rats received (s.c.) banamine (1 mg/kg) and ketoprofen (5 mg/kg). A 7-day recovery period from surgery was allowed. For i.c.v. injection, the injection cannula was connected via PE20 tubing to a Hamilton microsyringe. The injection was performed when the animal was held manually (by hand, unanesthesized, a painless procedure). The injection was slow and in small volume (1 μl/2 min), using a microinjection pump. In our experience, the injection procedure did neither disrupt cerebrospinal fluid drainage nor cause any changes in arterial blood pressure or other behavior-related variables.

2.6. Data analysis

Statistical analyses were performed using the analysis of variance (ANOVA), whenever appropriate. The values are expressed as mean±S.E. of the mean.

All animals used in these experiments were treated under National Institutes of Health guidelines for the welfare of laboratory animals and the work conformed with the National Institutes of Health ethics committee guidelines.

3. Results

3.1. Bryostatin-1 enhanced rat water maze spatial learning and memory

Effects of bryostatin-1 on spatial learning and memory were evaluated in rats, using a hidden-platform water maze. As shown in Fig. 1A, the latency to escape to the hidden platform in the five groups of rats decreased gradually during the training sessions, indicating that all rats were able to learn the task through the training and that the learning was progressive. However, there was a significant group difference in escape latency ($F(4,45)=6.712$, $P<0.001$), indicating difference in learning between the treatments. The difference between rats that received bryostatin-1 or vehicle was significant ($F(2,27)=10.327$, $P<0.001$), indicating that spatial learning in rats that were injected with bryostatin-1 (0.64 or 2 pmol/1 μl/site, bilateral i.c.v.; Bryostatin-1 rats) was faster than that of the rats injected with vehicle (1 μl/site, bilateral i.c.v.; Control). Multiple comparison (Student–Newman–Keuls method) analysis revealed a significant difference from the third trials ($P<0.05$) between the control and the bryostatin-1 groups at the doses of 0.64 or 2 pmol/site. Co-administration of H-7 significantly reduced the effects of bryostatin-1 (2 pmol/1 μl/site, bilateral i.c.v.) on learning ($F(1,18)=10.343$, $P<0.001$; Fig. 1A), while the administration of H-7 alone produced a significant increase in escape latency ($F(1,18)=5.516$, $P<0.05$; Fig. 1A), compared with that of the control rats.

Quadrant tests 24 h after the last training trial showed that the bryostatin-1 rats (0.64 and 2 pmol/1 μl/site, bilateral i.c.v.), bryostatin-1/H-7, and vehicle rats all showed a target quadrant preference (bryostatin-1 at 0.64 pmol: $F(3,36)=42.329$, $P<0.001$; bryostatin-1 at 2 pmol: $F(3,36)=66.083$, $P<0.001$; Fig. 1C; bryostatin-1 at 2 pmol/H-7 rats: $F(3,36)=36.840$, $P<0.001$; Fig. 1D; the vehicle control rats: $F(3,36)=38.598$, $P<0.001$; Fig. 1B). The rats that received H-7 administration alone did not exhibit a target quadrant preference ($F(3,36)=2.579$, $P>0.05$; Fig. 1E). The difference in the target quadrant preference between the groups was significant. The target quadrant ratio, calculated by dividing the target quadrant distance by the average non-quadrant distance, showed a significant difference between the groups ($F(4,45)=12.121$, $P<0.001$; Fig. 1F), indicating that the bryostatin-1 rats spent significantly more time than the vehicle control rats, searching in the target quadrant (Quadrant 4) where the platform was previously placed. Thus, the bryostatin-1 groups showed a significant higher target quadrant ratio than that of the vehicle control (2 pmol/site: $F(1,18)=29.216$, $P<0.001$; 0.64 pmol/site: $F(1,18)=20.226$, $P<0.001$). The improved memory retention produced by bryostatin-1 at 2 pmol/site was sensitive to H-7, since co-administration of H-7 significantly reduced the target quadrant ratio as compared with that of the 2 pmol/site bryostatin-1 group ($F(1,18)=18.578$, $P<0.001$). H-7 alone impaired the memory retention (difference in the target quadrant ratio: $F(1,18)=8.997$, $P<0.01$, as compared with that of the vehicle control group). Thus, the rats injected with bryostatin-1 performed better than their controls in this spatial memory retention task.

3.2. Effects of bryostatin-1 on rat performance in a visible platform test

Better performance in the spatial learning and memory task could result from a better learning and recall, from better visibility and mobility, or both. We evaluated in a

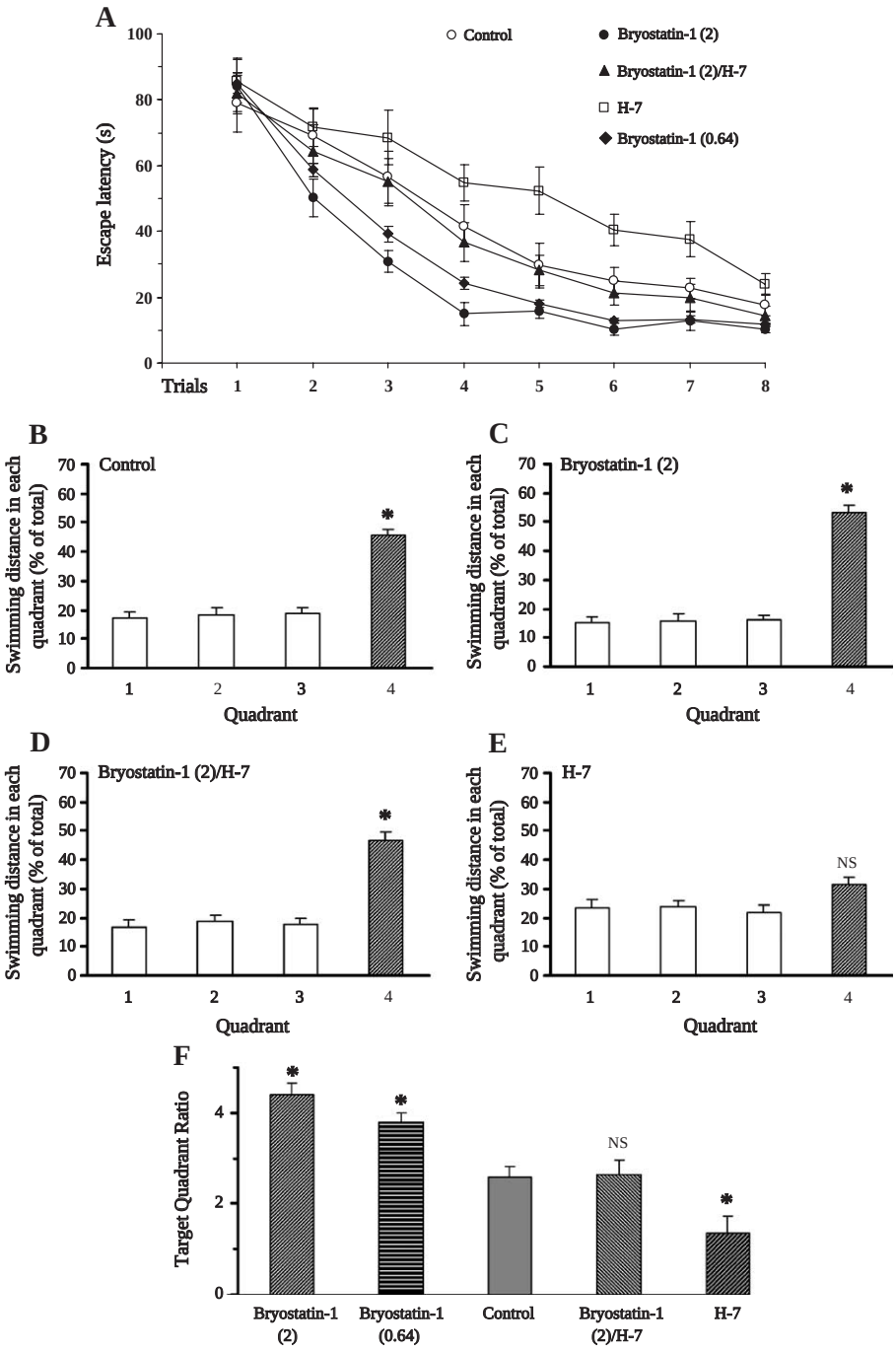

Fig. 1. Effects of bryostatin-1 on rat performance in the hidden platform water maze task. (A) Escape latency (means±S.E.M.) in water maze training across eight trials. (B–E) Results of the quadrant preference test, conducted at the end of the eighth training session. (F) The target ratio of quadrant preference test, calculated by dividing the target quadrant distance by the average distance in the other three non-target quadrants. Bilateral i.c.v. bryostatin-1 (1 μl/site of 0.64 or 2 μM solution; Bryostatin-1) was administered approximately 0.5 h before the first and fifth training trials. Bilateral i.c.v. H-7 (1 μl/site of 10 mM solution) or vehicle (1 μl/site; Control) was administered about 0.5 h before the first trial of the training days *p<0.01. NS: p>0.05.

visible platform task whether the difference in the spatial learning and memory task between groups could be due to effects of the administered agents on sensory and motor abilities of the animals. The visible platform test reflects rats' visual and motor abilities and does not require spatial map-based navigation. There was no significant difference (F(4,45)=0.315, P>0.05) in the escape latencies to the visible platform between the groups (not shown), indicating that the differences in rat performance in the spatial learning and memory task are specific, not due to differential effects of the agents/procedure on sensorimotor abilities of the rats. The test was performed on rats that had completed the invisible platform trials.

3.3. Effects of a smaller dose of bryostatin-1 on spatial learning and memory

When applied at 0.2 pmol/1 μl/site (bilateral i.c.v., 1/10 of the higher dose applied above) daily, bryostatin-1 produced no obvious effects on rat performance in the water maze task, compared with that of the vehicle group (1 μl/site, bilateral i.c.v.). Both groups of rats gradually learned the task and were able to find the hidden platform at a progressively shorter delay (Fig. 2). The difference between the two groups did not reach a significant level ($F(1,18)=0.635$, $P>0.05$), indicating that the bryostatin-1 dose had no effects on the spatial learning.

Quadrant tests 24 h after the last training trial showed that the bryostatin-1 rats (0.2 pmol/1 μl/site, bilateral i.c.v.) and the vehicle control rats (1 μl/site, bilateral i.c.v.) both exhibited a target quadrant preference (bryostatin-1 rats: $F(3,36)=71.221$, $P<0.001$; vehicle control rats: $F(3,36)=86.294$, $P<0.001$). There was no significant difference between the groups. The target quadrant ratio showed no significant difference between the groups ($F(1,18)=0.358$, $P>0.05$; Fig. 2), indicating that after the dose of bryostatin-1 (0.2 pmol/1 μl/site, bilateral i.c.v.), the rats spent no significantly different time in the target quadrant (Quadrant 4) than the vehicle control rats (1 μl/site, bilateral i.c.v.). Thus, the rats injected with the smallest doses of bryostatin-1 did not perform differently from their controls in this spatial memory retention test.

3.4. Effects of bryostatin-1 on non-searching immobility induced by an open space swim test

Separate groups of rats were used to determine effects of bryostatin-1 on induced non-searching immobility,

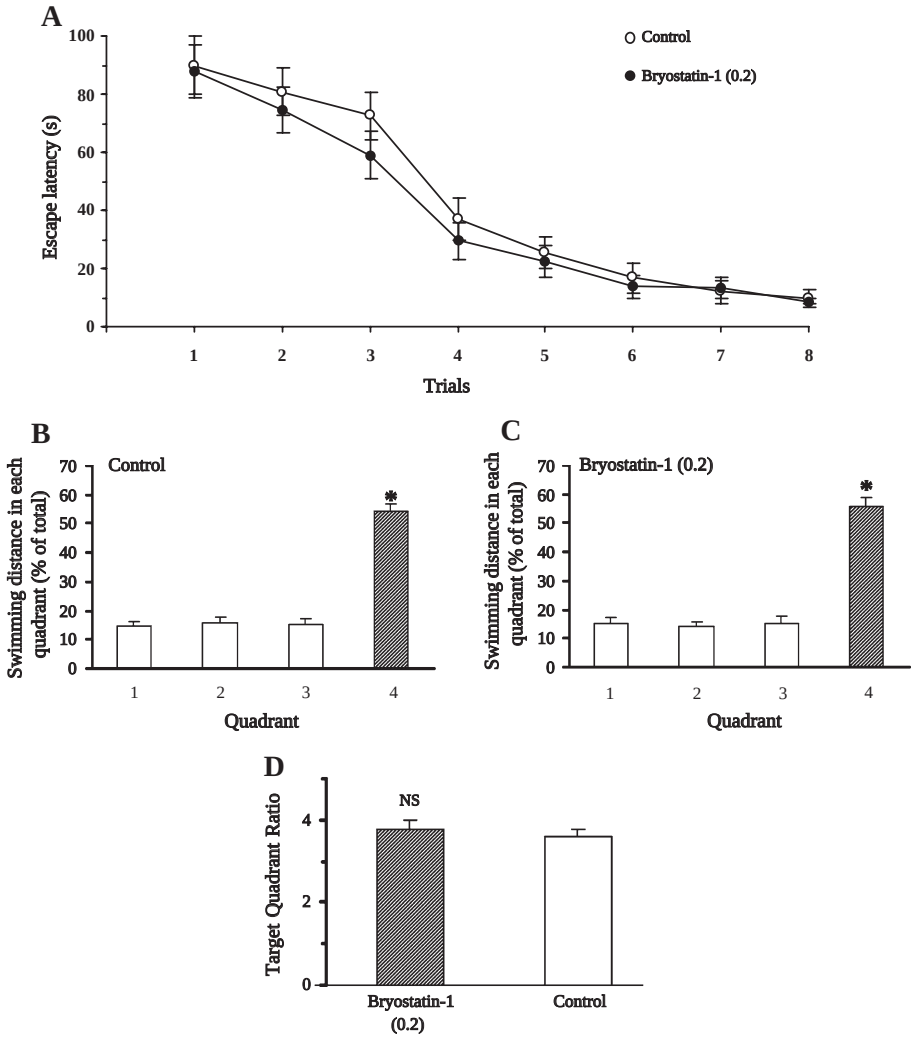

Fig. 2. Effects of lower doses of bryostatin-1 on rat performance in the hidden platform spatial water maze task. (A) Escape latency (mean±S.E.M.) in water maze training across eight trials. (B and C) Results of the quadrant preference test, conducted at the end of the eighth training session. (D) The target ratio of quadrant preference test, calculated by dividing the target quadrant distance by the average distance in the other three non-target quadrants. Bilateral i.c.v. bryostatin-1 (1 μl/site of 0.2 μM solution; Bryostatin-1) or vehicle (1 μl/site; Control) was administered approximately 0.5 h before the first trial of the training days (10 rats/group). *$p<0.01$. NS: $p>0.05$.

compared with those of an effective antidepressant imipramine and vehicle control. In the open space swim test, rats exhibited a gradual reduction in active swimming and more frequent and lasting periods of immobility (i.c.v. and i.v. vehicle control, $F(2,27)=25.533$, $P<0.001$ over three trials; Fig. 3), a non-searching status in which rats did not make additional movements other than respiration and those keeping their heads above the water surface. The immobility is one of the core depressive behaviors, indexing impaired mood regulation (Cryan et al., 2002; Nestler et al., 2002; Sun and Alkon, 2003). We have reported that the distance moved in the test reliably indexes the duration of mobility of the animals and that the induced immobility is sensitive to the treatments with the three prototypic classes of antidepressants and selective serotonin reuptake inhibitors (Sun and Alkon, 2003). There were significant effects of groups ($F(6,21)=5.265$, $P<0.01$), trials ($F(3,2388)=18.228$, $P<0.001$), and groups×trials ($F(18,2388=6.346$, $P<0.001$), indicating significant difference in the treatments and trials. Detailed analysis revealed that imipramine significantly reduced immobility, compared with vehicle control rats ($F(1,18)=12.968$, $P<0.005$; Fig. 3). A significant antidepressive effect was produced with a dose of bryostatin-1 (i.c.v. vehicle/100 nmol/kg bryostatin-1, i.v.) compared with the vehicle control group (i.c.v. and i.v. vehicle control, $F(1,18)=11.818$, $P<0.005$; Fig. 3). A similar antidepressive effect was also produced with a smaller dose of bryostatin-1 (i.c.v. vehicle/32 nmol/kg bryostatin-1, i.v.) compared with the vehicle control group (i.c.v. and i.v. vehicle control, $F(1,18)=9.306$, $P<0.01$; Fig. 3). Co-administration of H-7 (10 nmol/1 μl/site, i.c.v.), on the other hand, significantly reduced the effects of co-administered bryostatin-1 (100 nmol/kg, i.v.) on mobility of the rats, compared with those of the bryostatin-1 group at the same dose (i.c.v. vehicle and i.v. bryostatin-1; $F(1,18)=4.538$, $P<0.05$). The mobility of the co-administered groups was not significantly different from that of the control group ($F(1,18)=2.902$, $P>0.05$), indicating that the antidepressive effect of bryostatin-1 was sensitive to H-7, while H-7 alone (10 nmol/1 μl/site, i.c.v., 0.5 h before the second and third trials) did not appear to change the induced immobility in the test ($F(1,18)=0.0132$, $P>0.05$, compared with the control rats; Fig. 3).

One tenth of the highest effective i.v. bryostatin-1 dose, on the other hand, did not appear to produce any obvious effects on the mobility of the rats in the open space swim test. Thus, there was no significant mobility difference between the rats injected with the dose of bryostatin-1 (10 nmol/kg, i.v.) and the vehicle control ($F(1,18)=0.178$, $P>0.05$, Fig. 3), indicating that the bryostatin-1 effects on the induced non-searching immobility depended on the doses applied.

4. Discussion

Bryostatin-1 is a macrocyclic lactone and its main mechanism of biological action is modulation of PKC activity, acting as a partial agonist. Bryostatin-1 is distinct from the phorbol esters in several properties. For instance, 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, bryostatin-1 is reasonably well tolerated, having a maximum tolerated dose of 25 μg/m^2/week (Clamp et al., 2003). Its main dose-limiting toxicity is myalgia. Effects of bryostatin-1 on neural functions have not been well studied. As far as we know, this study is the first to report that it has effects on spatial memory and induced depressive behavior when administered at appropriate doses.

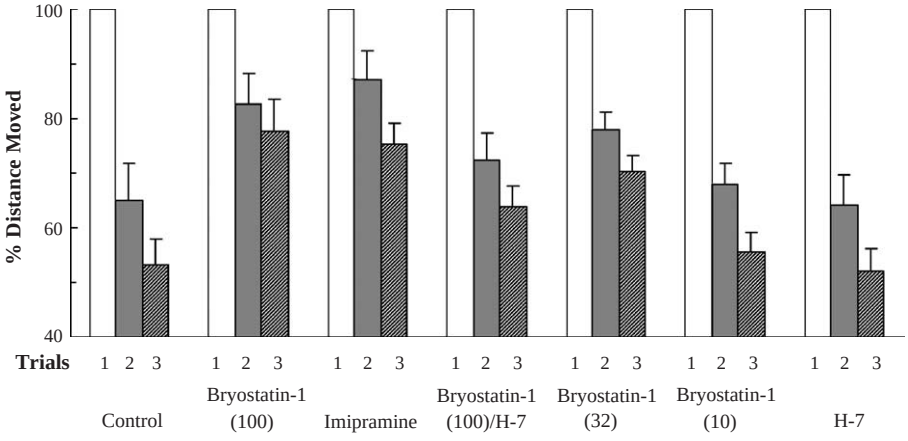

Fig. 3. Effects of bryostatin-1 on rat mobility in the open space swim test. Bryostatin-1 was i.v. (via a tail vein) administered either at a dose of 88.7 μg/kg (approximately 3 h before the second trial; the 100 nmol/kg group), 28.4 μg/kg (the 32 nmol/kg group; approximately 3 h before the second trials), or 8.87 μg/kg (the 10 nmol/kg group, approximately 3 h before the second and third trials). In the groups in which H-7 was co-administered or administered alone, H-7 (1 μl/site of 10 mM solution) was bilaterally i.c.v. administered approximately 0.5 h before the second and third trials. Imipramine (10 mg/kg/dose, i.p.) and vehicle were administered between the swimming trial sessions at 23 h, 3 h, and 1 h before the second and third trial sessions, respectively.

The hippocampal formation plays an important role in episodic, declarative, and spatial learning and memory (Riedel et al., 1999), and is an especially plastic and vulnerable brain structure that is damaged by a variety of injury and major depression. The involvement of PKC in brain functions and disorders, including cognition, mood regulation, and neurodegeneration, is implicated by several factors. First, the brain has the highest concentration of PKC of any organ in the body (Saito et al., 1988). Second, in the neural networks, PKC activation enhances calcium influx, increases neurotransmitter release, decreases a calcium-activated current in the hippocampus, and produces a potentiation of synaptic responses (Alkon et al., 1986, 1998; Kaczmarek, 1987; Alkon and Rasmussen, 1988; Bank et al., 1988; LoTurco et al., 1988). Phorbol esters have been shown to increase the size of the readily releasable pool at glutamatergic hippocampal synapses in culture through a PKC-dependent mechanism and to increase the rate at which the pool refills (Stevens and Sullivan, 1998). The PKC pathway may therefore regulate synaptic strength by modulating the readily releasable pool of vesicles. Third, distribution of PKC in the hippocampus is associated with associative learning (Olds et al., 1989). Long-term depression in the hippocampal CA1 field is associated with a transient decrease in pre- and/or postsynaptic PKC substrate phosphorylation (Ramakers et al., 2000; van Dam et al., 2002). Mice deficient in PKCβ showed normal brain anatomy and normal hippocampal synaptic transmission, paired facilitation, and long-term potentiation of synaptic responses, but a loss of learning in both cued and contextual fear conditioning (Weeber et al., 2000).

PKC activity, with the active site usually located at the C-terminus to phosphorylate serine and threonine residues, is mediated by a family of three subgroups based on their molecular structures and co-factor requirements: cPKC, nPKC, and aPKC. The cPKC (or classical PKCs) requires Ca^{2+} as a co-factor for activation and consists of α, β_I, β_{II}, and γ isoforms, all of which share a C_2 region corresponding to the Ca^{2+} binding site (Nishizuka, 1988). The nPKC (novel PKCs) does not require Ca^{2+} as a co-factor for activation and includes δ, ε, ε′, η, θ, and μ isoforms, in which the C_2 region is absent (Nishizuka, 1988). The cPKC and nPKC are activated by diacylglycerol, phorbol esters, and bryostatins. The third group (aPKC) is comprised of atypical isozymes, ζ and λ/ι, which do not bind phorbol esters or bryostatins and contain no C_2 region and one of the repeated cycteine-rich zinc finger binding motifs within the C1 domain (Yang and Kazanietz, 2003). The biological significance of the heterogeneity of the PKC family has not been really established. We do not know which PKC isozyme(s) may mediate the observed effects. The PKC pharmacology is limited by the availability of water-soluble, specific inhibitors of PKC isozymes for an in vivo administration. The pharmacological antagonism and isozyme involvement remain to be studied in detail.

The binding of bryostatin-1 to PKC results in PKC activation, autophosphorylation, and translocation to the cell membrane. Bryostatin-1-bound PKC is then down-regulated by ubiquitination and degradation in proteasomes. However, the C1 domain structures (Yang and Kazanietz, 2003) also exist in other molecules, such as PKD kinases (Van Lint et al., 2002), chimaerin Rac GTPase-activating proteins (Caloca et al., 2003), Ras guanyl nucleotide-releasing protein (Ebinu et al., 1998), Ras and Rap1 exchange factors, MUNC13 scaffolding proteins (Rhee et al., 2002), and diacylglycerol kinases β and γ. Some of the effects that have been attributed to PKC isozymes in response to phorbol esters may therefore be mediated by PKC-independent pathways (Yang and Kazanietz, 2003). However, the effectiveness of H-7, although not specific, in reducing the spatial memory enhancement and antidepressive effect produced by bryostatin-1 strongly suggests that PKC isozyme(s) are most likely involved.

The involvement of PKC isozymes in synaptic plasticity and learning and memory has been the focus of many investigations. Activation of PKC leads to the phosphorylation of numerous proteins, including glutamate receptor 2/3 (at serine 880) in Purkinje cells. The glutamate receptor 2/3 phosphorylation appears to be the critical step for parallel fiber long-term depression (Chung et al., 2003). An involvement of PKC in depression and antidepressant treatments has also been implicated in several studies. PKC may be involved in 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptor desensitization (Rahimian and Hrdina, 1995). The human plasma membrane serotonin transporter is a substrate for PKC. PKC activation or phosphatase inhibition down-regulates 5-HT uptake, probably via a reduction of the transporter expression (Ramamoorthy et al., 1998).

The pharmacokinetic features of bryostatin-1 and distribution in the rat brain have not been reported, but such data are available in mice (Zhang et al., 1996). The doses used in the study are based on a small preliminary test in which an i.c.v. dose of bryostatin-1 at 2 pmol/site (1.77 μg/site) appears to produce a similar response at an i.v. dose of the agent at 88.7 μg/kg. Since we did not measure the actual concentrations reached in the targeted neural structure, this can be viewed only as suggestive. The hypothetical CSF concentration obtainable after the bilateral i.c.v. injection of the substance is about 8 nM, in the range of CFS concentrations obtainable through an i.v. injection of bryostatin-1 at 88.7 μg/kg, after considering the different body surface areas between the two species. Bryostatin-1 is relatively stable to metabolic breakdown in vivo and has a wide tissue distribution after an i.v. dose, concentrating in the lung, liver, gastrointestinal tract, and fatty tissue in mice (Zhang et al., 1996). An i.v. dose of 40 μg/kg in mice produced a measurable brain tissue concentration of about 3.4 nM initially, much lower than its concentration in the plasma of about 11.3 nM (Zhang et al., 1996). There is therefore a distribution ratio of brain versus plasma of at least one to three in mice.

In summary, we have found that bryostatin-1 at appropriate doses produced an enhancement of rat performance in spatial memory task and an antidepressive effect. Study of the interaction between mood and memory is currently limited to the behavioral domain. Little is known about the underlying neural processes, although numerous reports point to the involvement of the hippocampus. The ignorance, however, does not undermine the importance of antidementics and antidepressants to human health and their therapeutic potentials. Medicine history is replete with effective therapeutic drugs and means—obtained and practiced years, decades, and even centuries before their acting mechanisms (digitalis, aspirin, acupuncture, antidepressants, memory enhancers, etc.) were and are going to be understood.

Drugs with dual antidepressive and memory-enhancing effects are desired for clinical therapies. Several issues, however, need to be addressed before bryostatin-1 and its analogues might be developed as therapeutic drugs. PKC is involved in a variety of functions and neurological disorders. It remains to be demonstrated whether a relatively non-selective activator of this pathway would be clinically useful in the treatment of the psychiatric illness. Defining the underlying isozyme(s) and developing isozyme-specific agents are thus essential. For instance, PKCδ and PKCε are implicated in heart failure and myocardial hypertrophy. Inhibition of PKCδ and activation of PKCε appears to protect against myocardial ischemia, but a low level of PKCδ activation is essential to maintain normal cardiomyocyte cytoskeletal integrity (Hahn et al., 2002). PKC isozyme activation plays an important pathological role in anoxic long-term potentiation of synaptic responses in the CA1 pyramidal cells of the hippocampus (Hsu and Huang, 1998), glutamate excitotoxicity in cultured rat hippocampal neurons (Hasham et al., 1997), tau phosphorylation (Ekinci and Shea, 1997), caspases-mediated apoptosis (Yashida et al., 2003), and amyloid neurotoxicity (Kuperstein et al., 2001). On the other hand, bryostatin-1 activates α-secretase and thus reduces the possibility of neurotoxic amyloid accumulation (Zhu et al., 2001; Etcheberrigaray et al., 2004). PKCδ is also capable of activating the extracellular signal-regulated kinase signaling pathway through Ras-dependent and -independent cascades, regulating cell proliferation and enhancing cell survival (Yang and Kazanietz, 2003), opposite to its pro-apoptotic action. With a full understanding of the intracellular targets of individual PKC isozymes in different cell types, the use of pharmacological or molecular therapeutic approaches based on PKC modulation might be justified clinically in the future.

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