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Post-training intrahippocampal infusion of nicotine–bucladesine combination causes a synergistic enhancement effect on spatial memory retention in rats

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Abstract

We previously had shown that bilateral intrahippocampal infusion of 1 μg nicotine (but not 0.5 μg dose) led to an improvement in spatial memory retention in the Morris water maze task in male rats. We also reported that a similar type of bilateral infusion of H89, a protein kinase AII (PKA II) inhibitor, caused a deficit in spatial memory retention. In the present study, we wished to test the hypothesis that intrahippocampal infusion of dibutyryl cyclic AMP (DB-cAMP also called bucladesine), a membrane permeable selective activator of PKA, into the CA1 region can cause an improvement in spatial memory in this maze task. Indeed, bilateral infusion of 10 and 100 μM bucladesine (but not 1 and 5 μM doses) led to a significant reduction in escape latency and travel distance (showing an improvement in spatial memory) compared to the control. Also, bilateral infusion of 0.5 μg nicotine or 1 μM bucladesine alone did not lead to an improvement in spatial memory. However, such bilateral infusion of bucladesine at 1 and 5 μM concentrations infused within minutes after 0.5 μg nicotine infusion improved spatial memory retention. Taken together, our data suggest that intrahippocampal bucladesine infusions improve spatial memory retention in male rats and that bucladesine can interact synergistically with nicotine to improve spatial memory.

Keywords: Dorsal hippocampus; DB-cAMP; Spatial memory; Morris water maze; cAMP-dependent protein kinase A

1. Introduction

The hippocampus, a part of the limbic system, is thought to be necessary for several types of learning and memory formation in rats and other mammals (Baulieu and Robel, 1990; Abel et al., 1997). Formation of memory is considered a continuous process that requires the involvement of certain neural networks and multiple pre- and post-synaptic events (Sharifzadeh et al., 2005a; Friedrich et al., 2004). It has been shown that memory formation consists of several phases including a short-term memory (STM) and a mid-term memory (MTM) based on existing proteins as well as a long-term memory (LTM) that necessitates a new round of translation and transcription (DeZazzo and Tully, 1995; Huang and Kandel, 1998; Friedrich et al., 2004). These different phases are induced and maintained by numerous molecular pathways. A large number of chemical neurotransmitters, hormones, and other signaling substances use cyclic adenosine 3',5'-monophosphate (cAMP) as an intracellular second messenger. For example, cAMP activates the cAMP-dependent protein kinase A (PKA) and plays a key role in the induction of different phases of memory formation and neuronal changes (Davis, 1996; Frey et al., 1993; Abel et al., 1997; Fiala et al., 1999; Muller, 2000; Friedrich et al., 2004). Also, many studies of the molecular mechanisms underlying long term memory as well as long-term potentiation (LTP) have

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focused on the regulation of gene expression at the transcriptional level (Banko et al., 2004). Numerous forms of signaling cascades such as extracellular signal-regulated kinase (ERK) and cAMP response-element binding protein (CREB) have proven to be important signaling molecules in the synaptic plasticity and memory formation (Viola et al., 2000; Sweatt, 2001; Thiels and Klann, 2001; Friedrich et al., 2004). Some effector systems that are modulated by ERK during synaptic plasticity include regulation of activity-dependent changes in neuronal morphology (Wu et al., 2001; Segal et al., 2003), regulation of transcription via phosphorylation of transcription factors such as CREB (Adams et al., 2000; Thiels et al., 2002) and regulation of general protein synthesis (Kelleher et al., 2004).

A considerable body of evidence indicates that neuronal nicotinic acetylcholine receptor is involved in memory and cognition (Kim and Levin, 1996; Puma et al., 1999; Hiramatsu et al., 2000; Marti Barros et al., 2004). Our recent work showed an important role for nicotine in spatial memory retention (Sharifzadeh et al., 2005c). Moreover, based on other published data, nicotine improves cognitive function, including memory and attention, in a variety of experimental animal and human studies (Whitehouse and Kalaria, 1995; Decker et al., 1995; Brioni et al., 1997; Levin and Simon, 1998; Bancroft and Levin, 2000; Marti Barros et al., 2004). Also, experimental studies with rodents have shown a protective effect by nicotine on memory deficit induced by different brain lesions (Decker et al., 1992; Grigoryan et al., 1994) or induced by nicotine antagonists (Zarrindast et al., 1996), β-amyloid protein (Itoh et al., 1996; Decker et al., 1997), or a COX-2 (cyclooxygenase-2) inhibitor (Sharifzadeh et al., 2005c). Central nicotinic mechanisms appear to cause these memory-improving effects of nicotine. This is based on the observation that systemic administration of the peripheral nicotinic antagonist, hexamethonium, does not impair radial arm maze choice accuracy (Levin et al., 1987; Kim and Levin, 1996), whereas systemic administration of mecamylamine as central nicotinic receptor antagonist significantly impairs the maze choice accuracy (Kim and Levin, 1996).

Some studies provide evidence for a novel signaling route coupling the stimulation of nicotinic acetylcholine receptor to the activation of ERK in Ca^{2+}- and PKA-dependent manner (Dajas-Bailador et al., 2001). Nicotine has also been shown to activate a variety of kinases (TerBush and Holz, 1986; Messing et al., 1989; Tuominen et al., 1992; Tsutsui et al., 1994; Cox and Parsons, 1997). In hippocampal neurons, nicotine has been reported to activate (extracellular signal-regulated kinase/mitogen activated protein kinase) ERK/MAPK (Cattaneo et al., 1997; Heusch and Maneckjee, 1998; Tang et al., 1998; Dineley et al., 2001) through a PKC (protein kinase C)-dependent (Tang et al., 1998) or PKC-independent pathway (Heusch and Maneckjee, 1998).

Our present study examined the effects of intradorsohippocampal infusion of bucladesine, a membrane permeable selective activator of PKA, within minutes after the infusion of nicotine, on spatial memory retention in male rats. Memory retention in our experiments was evaluated 48 h after the drug infusion.

2. Materials and methods

2.1. Animals

Male Albino-Wistar rats (200–250 g) were subjects in this study. Three-month-old rats bred in the faculty of pharmacy at the Tehran University of Medical Sciences, were selected and placed in individual stainless-steel cages, handled daily, and given food and water ad libitum. A 12/12-h light/dark cycle was maintained, and the animals were trained and tested during the light cycle. All the procedures involving the use of animals were performed in accordance with the guidelines of the Helsinki on animal care.

2.2. Drugs

All drugs including Bucladesine (DB-cAMP), nicotine, ketamine and xylazine were purchased from Sigma (St. Louis, MO). Bucladesine was dissolved in dimethyl sulfoxide (DMSO) to final concentrations of 1, 5, 10, 100 μM. Nicotine was dissolved in normal saline to a final concentration of 0.5 μg.

2.3. Behavioral training and testing

In our studies, 4-day training trials of animals were conducted. Spatial memory retention was tested 48 h after the drug infusions.

Training of all groups of rats was conducted in the Morris water maze task. The water maze was a black circular tank (136 cm in diameter and 60 cm in height). The tank was filled with water (20±2 °C) to a depth of 25 cm and was located in a room containing several extra maze cues. The Plexiglas escape platform used for the spatial task was submerged at a depth of 1 cm from water surface. Rats received one training session consisting of four trials in a day and were tested on four consecutive days. A trial was started by placing the rat in the pool facing the wall in one of the four quadrants delineated by marks at the four cardinal directions. Rats were allowed to swim to the hidden platform and the escape latency (time to find the hidden platform) and path length (distance traveled to the hidden platform) were recorded. If an animal did not escape within 90 s, it was manually guided to the escape platform by the experimenter. Rats were allowed to rest on the platform for 20 s between each trial. This procedure was repeated with each rat from starting positions in all four quadrants. The submerged platform was located in the same quadrant on every trial. The interval between the last training trial and the first testing trial was 48 h. For evaluation of the performance in the visually guided platform, the platform position remained stable (northwest) over first 4 days of training and acquisition of this task was assessed. On testing day, the platform was elevated above water level, covered with a piece of aluminum foil and placed in the center of the opposite position (southeast quadrant) of water maze. This assessed motivation and sensorimotor coordination towards a visible platform. The testing included 1 block of 4 trials.

2.4. Nicotine and bucladesine infusions

The rats were injected intraperitoneally with 90–95 mg/kg Ketamine in combination with 20–25 mg/kg Xylazine to anesthetize them for stereotaxic surgery. The rats were then cannulated in the CA1 region of the dorsal hippocampus and were targeted using coordinates of 3.0 mm posterior and 3.0 mm lateral to bregma and 3.0 mm ventral to the surface of the skull according to the atlas of Paxinos and Watson (1997). Six days after recovery from surgery, the training of the animals were started in Morris water maze. Bucladesine (1 μl), nicotine (1 μl), or a combination of both compounds was infused into both sides of the dorsal hippocampus using a 5 μl Hamilton syringe immediately after the last trial of training as follows: Four groups of animals received 1 μM, 5 μM, 10 μM, 100 μM bucladesine, another group received 0.5 μg nicotine and an additional three groups received a combination of 0.5 μg nicotine immediately followed by an infusion of either 1 μM, 5 μM, or 10 μM of bucladesine. The animals' memory retention was tested 48 h later in the Morris water maze by measuring escape latency, traveled distance, and swimming speed, as described above.

2.5. Statistical analysis

A one-way ANOVA was used in most cases and a two-way ANOVA when specified. A Newman–Keulz multiple comparison post hoc test was performed to assess differences in behavioral scores. A P-value of 0.05 or less was considered statistically significant.

3. Results

3.1. Effects of training on escape latency, traveled distance, and swimming speed in the Morris water maze

Following four days of training in the Morris water maze in our experiments all groups of animals including saline-treated, Sham-operated (a group that had surgery and guide cannulas implantation without injection) and animals selected to receive bilateral infusion of nicotine or bucladesine learned how to find the hidden platform as indicated by reductions in escape latencies and traveled distance (Fig. 1A and B). There was significant differences (***, $P<0.001$) between the fourth day and first day of training for finding the hidden platform in terms of escape latency and traveled distance. The swimming speed did not change significantly due to the training trials in any of the animal groups (Fig. 1C).

Nicotine and bucladesine were dissolved in saline and DMSO, respectively. Post-training bilateral intrahippocampal infusion of saline did not cause any significant differences in escape latency, traveled distance and swimming speed compared to DMSO-treated and sham-operated groups (Fig. 2A,B and C). These results are similar to the findings in our previous studies (Sharifzadeh et al., 2005a).

Also, post-training bilateral infusion of a low concentration of nicotine (0.5 μg/side) into the CA1 region of the

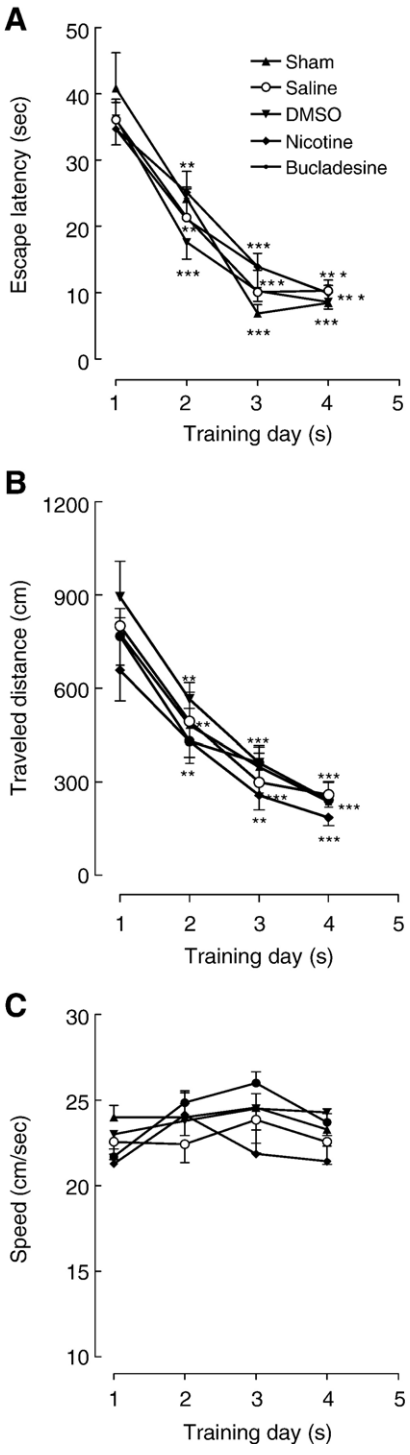

Fig. 1. Comparing the effects of 4-day training trials on spatial memory retention. In our experiments, all groups of animals including Sham-operated and those infused with saline, DMSO, nicotine, or bucladesine learned how to find the hidden platform. A significant difference (***, $P<0.001$) between the first and fourth days of training was observed in escape latency (A) and traveled distance (B). There was no significant difference in swimming speed between the first, second, third, and fourth days of training (C). (**, $P<0.01$) and (***, $P<0.001$) show statistical differences between second, third and fourth days with first day of training. Values are presented as mean ± S.E.M for at least 6 animals per group.

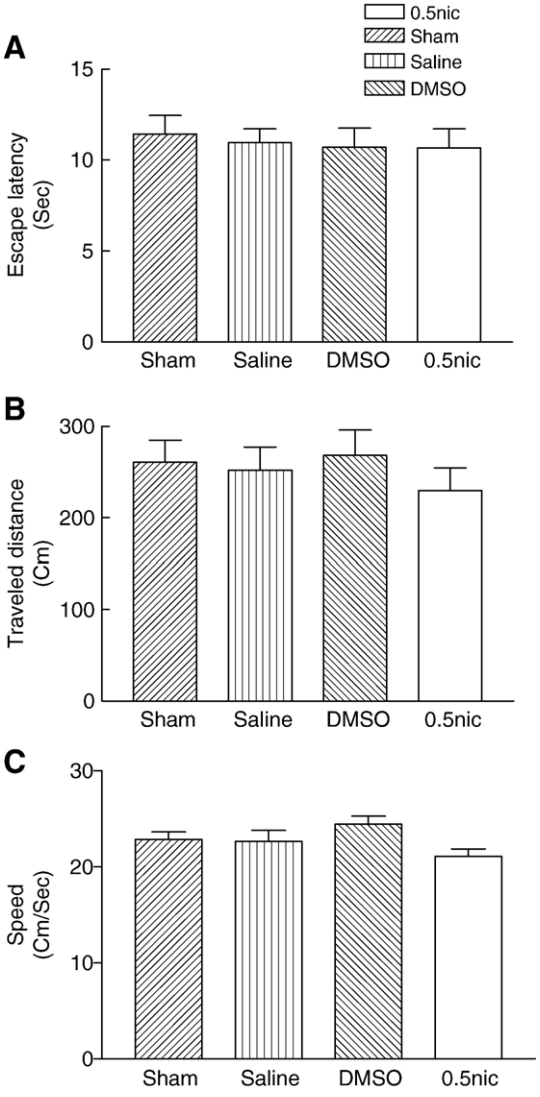

Fig. 2. Comparing effects of infusions of DMSO, Saline, and 0.5 μg nicotine and sham-operation on spatial memory retention. In all experiments, the testing trials were performed 48 h after any infusion. There was no significant difference in escape latency (A), traveled distance (B), or swimming speed (C) during testing between sham-operated animals and animals infused with saline or DMSO. Also, the 0.5 μg nicotine-infused rats showed no significant difference in the above parameters. Values are Mean±S.E.M for at least 6 animals.

hippocampus did not significantly alter the time, distance, and speed of finding the hidden platform compared to control (saline-treated animals) groups (Fig. 2A,B and C).

3.2. Effects of bucladesine on time and distance of finding hidden and visible platforms during testing trials

Immediately after the last training trials, animals were infused bilaterally into the CA1 region of hippocampus with different concentrations of bucladesine (1, 5, 10 and 100 μM/side). Spatial memory retention was evaluated 48 h after the drug infusions. The bilateral infusions of bucladesine led to significant improvements in escape latencies (*, $P<0.05$ for 5 μM, and ***, $P<0.001$ for 10, and 100 μM) and traveled distances (**, $P<0.01$ for 5 μM, and ***, $P<0.001$ for 10, and 100 μM) compared to DMSO-infused animals as a control group (Fig. 3A and B). The maximum enhancements in spatial memory retention were obtained with 10 and 100 μM/side of bucladesine (Fig. 3A, and B). In separate experiments, bilateral post-training intrahippocampal infusion of bucladesine did not affect performance in the visually guided platform (Fig. 3A and B). Statistical analysis showed a significant difference between control (DMSO-treated) and visible animals (***, $P<0.001$). The swimming speed was similar in all groups, indicating no motor disturbances in the treated animals (Fig. 3C).

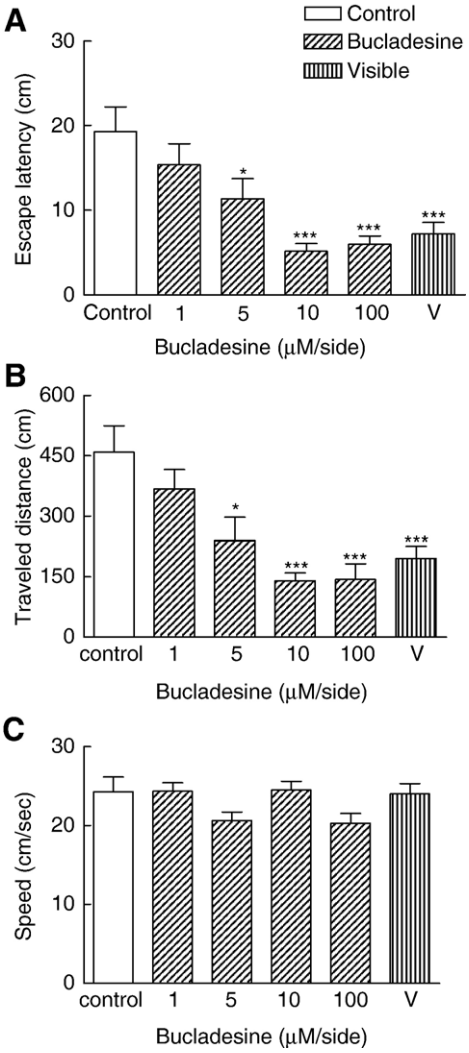

Fig. 3. Bucladesine-infused rats showed spatial memory improvement during the first test trials. There were significant reductions in escape latency (A), and traveled distance (B) when the animals were infused with 5, 10 and 100 μM concentrations of bucladesine, compared with the control (DMSO-treated) animals (*, $P<0.05$ for 5 μM, and ***, $P<0.001$ for 10, and 100 μM). V shows the visible group (visually guided platform). A significant difference was observed between control and visually guided platform-tested animals (***, $P<0.001$). (*, $P<0.05$) and (***, $P<0.001$) show statistical differences in comparison with control animals (DMSO-treated). The swimming speed in the bucladesine-infused animals did not show any significant alternation (C). Values are mean±S.E.M for at least 6 animals.

3.3. Synergistic effects of bucladesine and nicotine on spatial memory retention in the Morris water maze

Bilateral post-training intrahippocampal infusion of either 0.5 μg of nicotine (0.5 μg/side) or 1 μM bucladesine (1 μM/side) did not cause any significant alteration in escape latency and traveled distance. However, administration of combinations of these two compounds at these concentrations improved spatial memory retention significantly (Fig. 4A and B).

More specifically, a bilateral infusion of bucladesine (1 μM/side) 5 min after nicotine infusion (0.5 μg/side) decreased the time (**, $P<0.01$ for 1 and 5 μM and ***, $P<0.001$ for 10 μM) and distance (**, $P<0.01$) of finding the hidden platform

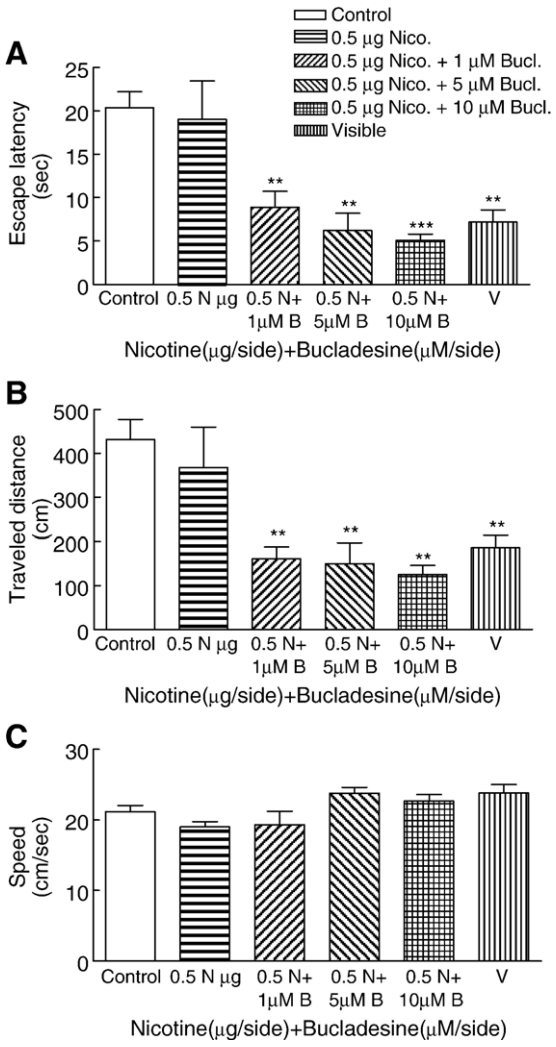

Fig. 4. Synergistic effects of infusion of bucladesine and 0.5 μg of nicotine. In all experiments, the testing trials were performed 48 h after the infusions. Escape latency (A) and traveled distance (B) were reduced when either 1, 5 or 10 μM of bucladesine was infused within minutes after infusion of 0.5 μg nicotine. Significant differences were observed between the control group and animals that received co-infusions of bucladesine and nicotine (**, $P<0.01$ and ***, $P<0.001$). V shows the visible group (visually guided platform). A significant difference was observed between control group and visually guided platform-tested animals (**, $P<0.01$). (**, $P<0.01$) and (***, $P<0.001$) show statistical differences in comparison with control animals. The swimming speed was similar in all groups (C). Values are mean±S.E.M for at least 6 animals.

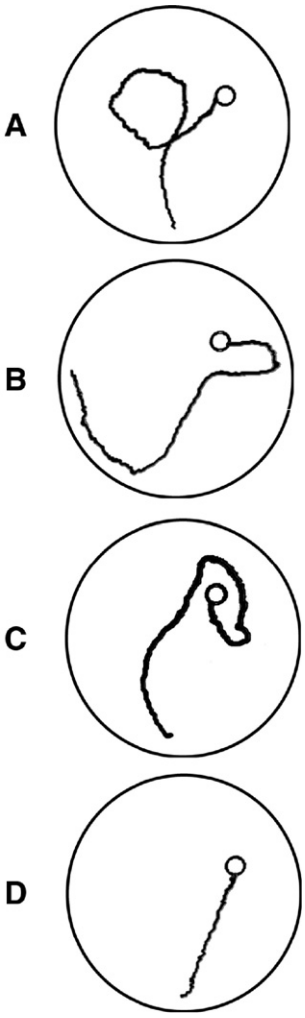

Fig. 5. Combined infusions of bucladesine and nicotine reduced travel path during the testing trials. A–D show representative traces of travel path following infusion of control (DMSO) (A), 0.5 μg nicotine (B), 1 μM bucladesine (C) and 0.5 μg nicotine combined with 1 μM bucladesine (D). The circles designate the position of the hidden platform. Compared with the control, travel path decreased when nicotine and bucladesine were co-infused.

significantly compared to the control group (Fig. 4A and B). Bilateral post-training intrahippocampal infusion of nicotine/bucladesine combination did not caused any significant alterations in the performance to the visually guided platform (Fig. 4A and B). No significant differences were observed in escape latency, traveled distance and swimming speed between nicotine–bucladesine-treated animals tested in the hidden platform task and those of the visible platform groups. Statistical analysis showed a significant difference between control (DMSO-treated) and visible groups (**, $P<0.01$) (Fig. 4A,B). Also, the swimming speed was very similar in the control, nicotine-infused, combination of nicotine with bucladesine and visible groups (Fig. 4C).

Fig. 5 shows changes in travel path in nicotine, bucladesine and nicotine–bucladesine combination groups during the testing trials of the hidden platform task compared to the control group (Fig. 5A). A reduction in travel path was observed following the infusion of nicotine (0.5 μg/side) (Fig. 5B), bucladesine

(1 μM/side) (Fig. 5C) as well as nicotine–bucladesine combinations (Fig. 5D).

4. Discussion

In humans damage to the hippocampus causes deficits in learning about people, places or objects (Vitolo et al., 2002). In our study, we employed the Morris water maze to test spatial memory, because performing the task in this maze requires a functional hippocampus (Brandeis et al., 1989).

A large number of chemical neurotransmitters, hormones, and other signaling substances use cAMP as an intracellular second messenger. A body of evidence shows that cAMP plays an important role in memory processes (Slack and Pockett, 1991; Nguyen and Woo, 2003; Banko et al., 2004; Sharifzadeh et al., 2005a; Lau et al., 2004). The principal target for cAMP in mammalian cells is cAMP-dependent protein kinase (PKA), which is ubiquitously expressed and mediates intracellular transduction and intracellular signal transmission in invertebrates and vertebrates (Nguyen and Woo, 2003). Moreover, protein phosphorylation is mediated by protein kinases, and it is a key regulatory mechanism in neurons, enabling and modulating many important cellular processes, including neuronal development, growth and plasticity (Walaas and Greengard, 1991; Nguyen and Woo, 2003).

cAMP-dependent PKA is a serine–threonine kinase that has been strongly implicated in the expression of long-term potentiation (LTP), long term depression (LTD), and hippocampal long-term memory (Ngyuen and Woo, 2003). Role of cAMP in activity-dependent forms of hippocampal synaptic plasticity was also reported previously (Ngyuen and Woo, 2003). Moreover, cAMP signaling in particular has been shown to be pivotal for synaptic plasticity and memory formation (Burrell and Sahley, 2001). To examine the role of dibutyryl cAMP in spatial memory, we used the drug bucladesine, a selective activator of PKA. The direct effect of bucladesine infusion into the CA1 region of hippocampus on spatial memory has not been previously reported. In our study, bucladesine infusion produced an improvement in spatial memory retention in the Morris water maze.

In this work, bucladesine was dissolved in DMSO (100%) for all our infusions. We found that bilateral intrahippocampal infusion of DMSO did not induce any significant differences in escape latency, traveled distances and swimming speed, compared with Sham-operated or saline-treated rats during testing trials. The effective use of DMSO as a vehicle has also been reported in our previous works (Sharifzadeh et al., 2005a,b,c). Thus, here it seemed that DMSO infusions also did not cause motor disturbances or have significant effects on spatial memory parameters.

In our study, bucladesine infusions also did not cause any significant differences in swimming speed compared with animals receiving saline or DMSO. These observations suggest that bucladesine infusions did not produce motor disturbances and provide evidence in support of our conclusion that the observed spatial memory retention improvement was induced by bucladesine infusion. Furthermore, the findings from control visible animals showed that the performance in the visually guided platform was not affected in the treated animals and confirmed the enhancing effect of bucladesine on spatial memory retention.

As stated earlier cAMP/PKA signaling has been shown to be pivotal for specific types of long-term synaptic plasticity and long-term memory (Burrell and Sahley, 2001). In addition, electrophysiological and behavioral studies, performed on the Aplysia Californica, have also confirmed the requirement for cAMP/PKA signaling in the establishment of short and long-lasting forms of synaptic plasticity, learning and memory (Kandel and Schwartz, 1982; Kandel, 2001; Nguyen and Woo, 2003). There is also evidence that in the absence of cAMP, PKA is an inactive tetrameric enzyme (Taylor et al., 1990). These findings demonstrated an important role for cAMP/PKA signaling in mediating both short-term and long-term memory (Hawkins et al., 1993; Bailey and Kandel, 1993; Kandel, 2001). Therefore, in the present work it is possible that bucladesine via activation of PKA and induction of cAMP/PKA pathway improved spatial memory retention.

A considerable body of evidence shows the important role of hippocampal cholinergic neurons in higher brain functions such as attention, learning and memory (Descarries et al., 2005; Hasselmo and McGaughy, 2004; Hiramatsu et al., 2000). Choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT) represent two well-known cholinergic markers (Roghani et al., 1994; Bejanin et al., 1994; Woolf et al., 2001; Sharifzadeh et al., 2005a,b,c). In cholinergic postsynaptic terminals, ChAT synthesizes acetylcholine (ACh) which is stored by VAChT into synaptic vesicles for regulated exocytotic release (Woolf et al., 2001; Sharifzadeh et al., 2005a). Also, there is some evidence that the expression of ChAT and VAChT genes is co-regulated (Berse and Blusztajn, 1995). It is proposed that through the cAMP/PKA/CREB pathway, CREB would increase transcription of ChAT and VAChT genes through cAMP-response elements (CREs), which should lead to an increase in ChAT and VAChT levels (Inoue et al., 1995; Berrard et al., 1995; Berse and Blusztajn, 1995). In our previous work, post-training intrahippocampal infusion of the PKAII inhibitor H89 impaired spatial memory retention and caused a qualitative reduction in the number of immunostained ChAT-containing nerve terminals in the CA1 region, as detected immunohistochemically. These infusions also caused a reduction in the number of immunostained ChAT-containing cell bodies in the medial septal region (Sharifzadeh et al., 2005a). Other investigators also showed that expression of ChAT and VAChT proteins in the PKA-deficient PC12 cell line A123.7 was greatly reduced (Shimojo et al., 1998). In addition, inhibition of PKA significantly reduced ChAT activity in PC12 cell line by inhibiting transcription of the cholinergic gene locus (Shimojo et al., 1998). Our previous and present findings along with those of others (Drain et al., 1991), suggest that activation of the cAMP/PKA/CREB signaling pathway in the hippocampus plays an important role in spatial memory formation. Thus, it is reasonable to assume that spatial memory retention improvement we observed in animals infused with bucladesine was caused by an increase in ChAT and VAChT protein levels. It is

also possible that the observed memory enhancement by bucladesine is mediated by the interaction of cAMP/PKA signaling cascade with different neurotransmitters such as NMDA (Chetkovich and Sweatt, 1993), dopamine (Monsma et al., 1990), serotonine (Pedarzani and Storm, 1993), or numerous signaling molecules including calcium-calmodulin, PKC, mitogen-activated protein kinases (MAPK) and tyrosine kinase (Schulman, 1995; Micheau and Riedel, 1999; Sanes and Lichtman, 1999; Soderling, 2000, Winder and Sweatt, 2001; Lisman et al., 2002).

Nicotinic acetylcholine receptor can modulate many cellular mechanisms, such as neurotransmitter release (Wonnacott, 1997), synaptic plasticity (Ji et al., 2001) and memory processing (Levin and Simon, 1998). The improvement effects of nicotine on memory in intact rats or mice have been reported in several studies (Sansone et al., 1991; Puma et al., 1999). Our previous work indicated that intrahippocampal infusion of nicotine (at 1 μg dose) enhanced spatial memory retention when the interval between infusion of the drug and testing trials was 48 h (Sharifzadeh et al., 2005c). In the present study to evaluate the interactive effects of nicotine and bucladesine on spatial memory, we used a dose of nicotine (0.5 μg) that we had found previously not to induce any significant alterations on escape latency, traveled distance, and swimming speed.

One of the major findings of the present study is that intrahippocampal infusion of bucladesine 5 min after nicotine caused a significant improvement on spatial memory retention compared with administration of nicotine or bucladesine alone. The results obtained from visible group showed that the administration of nicotine/bucladesine combination did not affect performance in the visually guided platform and thus confirmed the synergistic effects of these agents on spatial memory retention in the hidden platform task. There is evidence that nicotine activates the signal-regulated PKAI and PKAII via the nicotinic acetylcholine receptor in the hippocampal neurons (Dajas-Bailador et al., 2001). Previous published reports indicate that MAPK or extracellular signal-regulated protein kinase (ERK1/2) which are implicated in many of the cellular processes can be modulated by nicotinic acetylcholine receptor stimulation (Dajas-Bailador et al., 2001). These serine–threonine protein kinases have important roles in the differentiation and memory (Fukunaga and Miyamoto, 1998; Sweatt, 2001). In addition, generation of cAMP results in the activation of the ERK1/2 cascade (Vossler et al., 1997), which has been described as a novel link between Ca2+ entry through voltage operated calcium channel (VOCC) and subsequently activation of the ERK1/2 signaling pathway (Grewal et al., 2000a,b). Also, it has been demonstrated that the nicotinic acetylcholine receptor mediates the nicotine-evoked activation of ERK1/2 in a Ca2+-dependent manner that involves the activation of PKA in the hippocampal neurons (Dajas-Bailador et al., 2001). Therefore, the enhancing effects of the bucladesine/nicotine on spatial memory retention observed in our study may be in part due to a synergistic effect and activation of PKA and cAMP/PKA signaling pathways by both compounds.

Lastly, nicotine is also known to increase expression of VAChT (Prendergast and Buccafusco, 1998), and release of acetylcholine (Sullivan et al., 1997; Hiramatsu et al., 2000) both of which have been implicated in learning and memory enhancement. Thus, it is reasonable to deduce that the spatial memory retention enhancement we observed in animals infused with the combination of nicotine and bucladesine was caused, in part, by an increased activation of cholinergic pathways in the CA1 region of hippocampus.

In conclusion, our data and those of others, described above, provide evidence in support of the interacting effects of nicotine and bucladesine on the cAMP/PKA pathway in the brain. Understanding the exact cellular and molecular mechanism(s) of this interaction needs a more complete knowledge of bucladesine, nicotine and cAMP/PKA signaling roles in memory function, which should be realized in future studies.

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