Rescue of cAMP Response Element-Binding Protein Signaling Reversed Spatial Memory Retention Impairments Induced by Subanesthetic Dose of Propofol

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SUMMARY

Aims: The intravenous anesthetic propofol caused episodic memory impairments in human. We hypothesized propofol caused episodic-like spatial memory retention but not acquisition impairments in rats and rescuing cAMP response element-binding protein (CREB) signaling using selective type IV phosphodiesterase (PDEIV) inhibitor rolipram reversed these effects. **Methods:** Male Sprague-Dawley rats were randomized into four groups: control; propofol (25 mg/kg, intraperitoneal); rolipram; and rolipram + propofol (pretreatment of rolipram 25 min before propofol, 0.3 mg/kg, intraperitoneal). Sedation and motor coordination were evaluated 5, 15, and 25 min after propofol injection. Invisible Morris water maze (MWM) acquisition and probe test (memory retention) were performed 5 min and 24 h after propofol injection. Visible MWM training was simultaneously performed to resist nonspatial effects. Hippocampal CREB signaling was detected 5 min, 50 min, and 24 h after propofol administration. **Results:** Rolipram did not change propofol-induced anesthetic/sedative states or impair motor skills. No difference was found on the latency to the platform during the visible MWM. Propofol impaired spatial memory retention but not acquisition. Rolipram reversed propofol-induced spatial memory impairments and suppression on cAMP levels, CaMKII α and CREB phosphorylation, brain-derived neurotrophic factor (BDNF) and Arc protein expression. **Conclusions:** Propofol caused spatial memory retention impairments but not acquisition inability possibly by inhibiting CREB signaling.

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Introduction

Understanding how general anesthetics produce amnesia is important as amnesia is the most basic need of patients undergoing general anesthesia [1]. Moreover, intraoperative awareness and postoperative cognitive deficits are undesirable and might be related with the amnesic effects of general anesthetics [2]. The intravenous anesthetic propofol selectively disrupts long-term episodic memory, which comprises three temporal stages: encoding (acquisition), consolidation, and retrieval [3]. Subanesthetic dose of propofol has no impact on memory encoding and retrieval in human and probably prevents hippocampal-dependent consolida-

tion of short-time memory into long-term memory [3–8]. However, few animal models have been tried to reproduce the selective inhibition of episodic-like memory consolidation by propofol.

The non-cued Morris water maze (MWM) is a test evaluating spatial learning and memory for rodents. It is a hippocampus-dependent episodic-like memory training task giving information on “what–where” elements [9,10]. Furthermore, this multiple-trial model can be used to simultaneously test memory acquisition and consolidation ability [11,12]. The current study aimed to use the non-cued MWM to test whether propofol selectively caused spatial memory consolidation impairments.

Figure 1 An overview of the “molecular switch” role of CREB in hippocampal-dependent memory consolidation. CREB phosphorylation can be caused by an increase in cAMP by excitatory neurotransmitters via GPCR (G protein coupled receptors); an increase in Ca^{2+} influx via activation of NMDA receptors and VDCC (voltage-dependent calcium channels); or activation of RTK (receptor tyrosine kinase) pathway by BDNF. Activation of CREB signaling increases the gene expression of BDNF, Arc and c-Fos which are then captured by active synapse to stabilize memory. Rolipram enhances CREB signaling through inhibition of type IV cyclic AMP phosphodiesterase (PDEIV).

Spatial memory consolidation but not encoding relies on CREB-mediated gene expression in the hippocampus [13–15]. As shown in Figure 1, CREB can be activated through phosphorylation by an increase in cAMP and Ca^{2+} , and by activation of ERK1/2, AKT, CaMKII α and CaMKIV pathway [13,16]. Phosphorylated CREB (pCREB) then activates cAMP response elements (CRE)-mediated transcription through transcription coactivator CREB-binding protein (CBP). c-Fos and brain-derived neurotrophic factor (BDNF) are known as two marker genes for CREB activation. Arc (activity-regulated cytoskeleton-associated protein) is also regulated by CREB [17]. These proteins are then captured by activated synapses to modify synapse structure and strength, which stabilizes memory [13,18]. We detected hippocampal CREB signaling changes and its relationship to propofol-induced memory consolidation defects during the non-cued MWM.

As a selective PDEIV inhibitor, rolipram can increase the intensity and duration of CREB signaling by elevating cAMP concentrations [19] (Figure 1). Moreover, rolipram facilitated memory consolidation-dependent late-phase long-term potentiation and improved context freezing memory [20]. Subchronic delivery of rolipram also enhanced fear memory retention [21]. Thus, rolipram is a useful tool to reverse CREB inhibition and following memory consolidation impairments. We further used rolipram to confirm that CREB signaling was involved in spatial memory retention impairments caused by propofol.

Methods and Materials

Animals

Male 8–12-week-old Sprague-Dawley rats (180–220 g; SIPPR/BK, Shanghai, China) were used in this study. The rats were housed in a temperature-controlled ($22 \pm 2^\circ\text{C}$) environment under a 12–12 h light-dark cycle (lights on at 7 am) with free access to food and water. All procedures involving animal care and treatments were approved by the Animal Care and Use Committee of Second Military Medical University (Shanghai, China) and performed

according to the Guide for the Care and Use of Laboratory Animals (NIH, 1996). Animals were adapted for at least 2 h before behavioral tests, which were all performed between 9 am and 4 pm.

Drugs

Propofol (DiprivanTM, 10 mg/mL; Corden Pharma S.p.A., Sermolona, Italy) was administered intraperitoneally at 25 mg/kg, the minimal dose for consistent anterograde amnesia [22]. Rolipram (Cayman, Ann Arbor, MI, USA) was dissolved in 0.9% saline/1.5% dimethyl sulfoxide (DMSO) with a final concentration of 0.15 mg/mL. An intraperitoneal dose of 0.3 mg/kg was chosen for rolipram because it was enough to reverse memory impairments by CREB signaling inhibition [23], did not improve spatial memory efficacy alone [24], and had no effect on sedation (results shown afterwards). Based on calculations from Krause [25] and Barad [20], the cerebrospinal fluid concentration for rolipram 30 min after injection would not exceed $3 \mu\text{M}$, which had no effect on basal synaptic transmission [20]. The injection volume was 2 mL/kg, 25 min before propofol administration. Isovolumetric 0.9% saline/1.5% DMSO was used as a vehicle for rolipram.

Experimental Schedule

Rats were randomly assigned into four groups (Figure 2): C (control); P (propofol); R (rolipram), and R + P (pretreatment of rolipram 25 min before propofol administration).

Experiment 1 Sedative/Anesthetic Scores

The sedative/anesthetic levels were evaluated in the manners by Laalou [26] and Gamou [27]: wakefulness (score 0): spontaneous locomotor activity in 1 minute's observation; light sedation (0.2): no spontaneous locomotion in 1 minute's observation; deep sedation (0.4): no motor response when placed on a grid inclined (45°) with the head down during a 30-s period of observation; light anesthesia (0.6): no righting

Figure 2 Experimental design for non-cued Morris Water Maze (MWM) and CREB signaling assay. Animals were randomized into four groups (C, P, R, R + P) according to their mean latency (time to find the platform) in Day 1 and received corresponding injections before Day 2's training. CREB signaling assay was done at time 0, 45 min and 24 h. Abbreviations: C, control; P, propofol; R, rolipram; R + P, rolipram + propofol.

reflex during a 30-s period of observation; moderate anesthesia (0.8): no paw withdrawal reflex and deep anesthesia (1.0): no eye-blink reflex. Rats were scored by blinded experimenters 5, 15, and 25 min after propofol injection.

Experiment 2 Accelerated Rotarod Test

The rotarod test was performed to resist the possibility that impaired memory was induced by motor incoordination. We used the DIGBehv-RRTG system (Jiliang Tech, Shanghai, China) for this experiment. It consisted of a smooth rod (diameter: 6.7 cm) separated in five compartments (width: 5 cm) situated 30 cm high above five planks. Rats were first allowed to habituate with the apparatus for 30 s with no rotation and for 2 min with a low speed rotation (4 rpm). Rats that were unable to stay on the rod for 2 min over two trials were excluded from further experiment. Drugs were then administered, and each rat was tested 5, 15, and 25 min after propofol injection. The parameters of rotarod system include start speed (4 rpm), acceleration (3 rpm/10 s), and maximal speed (40 rpm). The latency to fall for the three trials, measured both as time (second) and rod velocity (rpm) was recorded.

Experiment 3 Non-Cued Morris Water Maze (MWM)

The apparatus, tracking system, and software system (DigBehv-MG3.0) were provided by Jiliang Tech (Shanghai, China). A black circular tank (160 cm in diameter, 50 cm deep) was filled with water ($23 \pm 1^\circ\text{C}$) to a depth of 25 cm. The maze was arbitrarily divided into four equal spaced quadrants (north [N], east [E], south [S], and west [W]). A black rounded platform (12 cm in diameter) was hidden in the middle of the SW quadrant (target quadrant), 1.5 cm below the water surface.

Based on published articles [11,24,28], our experiments comprised 2-day spatial navigation training (Day 1 and Day 2) and 1-day probe test (Day 3). The animals received corresponding injections before Day 2's training (Figure 2). For acquisition training, the rat was submitted to eight consecutive trials. The sequential position for Day 1 was S-W-SE-NW-SE-NW-S-SE and for Day 2

was SE-NW-W-S-W-SE-S-NW. The time limit to locate the platform was 120 s and the inter-trial interval was 15 s. We chose this massed training strategy due to two considerations: (1) to rule out the role of memory consolidation during the training phase, which usually took effect about 30 min after learning [29] so that only memory acquisition was tested; (2) to shorten the duration of the training phase to less than 15 min so that propofol could affect more trials. The swimming velocity, latency, and path length of eight trials were recorded and analyzed. In Day 1's training, rats that could not find the platform in more than five trials or swim coordinately were excluded. The rest were then randomly assigned into four groups. In Day 2's training, rats that swam with difficulty due to over sedation were discarded as previous experiments [2,22].

Twenty-four hours after Day 2's training (Day 3), a single probe test was given. The platform was removed from the pool, and the rats were allowed to swim freely for a fixed amount of time (60 s). The average distance to the target platform and time in the target quadrant were collected as indicators for spatial memory retention levels.

Hippocampal tissues were freshly isolated, collected, and stored for CREB signaling assay at time 0 (5 min after propofol/vehicle injection), time 45 min (50 min after propofol injection and about 30 min after MWM learning), and time 24 h (immediately after probe test).

Experiment 4 Cued Morris Water Maze (MWM)

We further conducted cued MWM training to exclude the role of nonspatial factors on propofol-induced spatial memory impairments. The system and design were the same as non-cued MWM except that the platform was elevated above the water surface (1 cm). The training included eight trials on Day 1 and Day 2 and four trials on Day 3. The sequential start (platform) position for Day 1 was N(SE)-E(NE)-S(SW)-W(SE)-S(NE)-N(NW)-W(NE)-E(SE), for Day 2 was W(NW)-S(SE)-E(SW)-N(SW)-E(NE)-W(NE)-N(SW)-S(SW), and for Day 3 was N(NE)-S(NW)-E(NW)-W(SW). Swimming velocity and latency were recorded for analysis.

Experiment 5 CREB Signaling Assay

We randomly chose eight rats per group per time point to conduct CREB signaling assay. For cAMP radioimmunoassay, we used the left hippocampus ($N = 8$ per group). For protein assays, we randomly selected four right hippocampus tissues per group per time point from eight samples.

cAMP Radioimmunoassay

cAMP concentrations were measured using a commercial 1^{125} radioimmunoassay kit (Isotope Laboratory of Shanghai University of Traditional Chinese Medicine, Shanghai, China), according to the manufacturer's instructions. Every sample was tested for two times, and the mean value was used. Protein concentrations were detected using bicinchoninic acid protein assay kit (Beyotime, Haimen, China). cAMP concentration was expressed as pmol of cAMP/mg of protein.

Western Blotting

The hippocampus was homogenized in RIPA lysis buffer with 1% protease and phosphatase cocktail (Kangcheng, Shanghai, China). Lysates were separated in 12% SDS-polyacrylamide gel and electrotransferred to a nitrocellulose membrane. After blocking, the membranes were incubated overnight with anti-ERK1/2 (1:1000), anti-pERK1/2^{Thr202/Tyr204} (1:1000), anti-AKT (1:2000), anti-pAKT^{Ser473} (1:2000), anti-CaMKII α (1:2000),

anti-pCaMKII α ^{Thr286} (1:2000; Abcam, Cambridge, UK), anti-CaMKIV (1:1000), anti-CREB (1:1000), anti-pCREB^{Ser133} (1:300), anti-BDNF (1:2000; catalog No.: AB1534, mature BDNF only, Millipore, Billerica, MA, USA), anti-Arc (1:1000; Bioworld, Nanjing, China), or anti-c-Fos (1:1000; Bioworld, Nanjing, China). All antibodies were from Cell Signaling (Danvers, MA, USA) unless specified. A horseradish peroxidase-conjugated secondary antibody was used. The blots were visualized by an enhanced chemiluminescence reaction (ECL) system and photographed by ChemiDocTM XRS⁺ System (Bio-Rad, Hercules, CA, USA). GAPDH (1:10000; Kangcheng, Shanghai, China) was chosen as an internal control. Band densitometry analysis was performed by QuantityOne software (Bio-Rad).

Statistical Analysis

All analyses were performed with SPSS17.0 (SPSS Science, Chicago, IL, USA). For sedative/anesthetic scores, time and rod velocity from accelerated rotarod test and swimming velocity, latency, path length and cumulative distance from cued and non-cued MWM training, two-way ANOVA with repeated measures followed by *post hoc* Bonferroni's test were used. The average distance to the target platform and time in the target quadrant from MWM probe test, cAMP concentration and Western blotting data were analyzed using one-way ANOVA followed by *post hoc* Bonferroni's test. $P < 0.05$ (two-tailed) was considered statistically significant.

Figure 3 Sedation and motor coordination after injection of propofol with or without pretreatment of rolipram. (A) Temporal changes in sedative/anesthetic scores. The symbols $\blacktriangle$, $\blacksquare$, $\bullet$ and $\star$ indicate the score in each animal from different groups at different time points ($N = 6$ per group). Propofol (25 mg/kg) increased the sedation levels compared to the control group and rolipram had no effect on propofol-induced sedation (*post hoc* Bonferroni's test, $P = 0.006$ and 1 respectively). (B,C) Accelerated rotarod performances (B for rod velocity and C for time to fall) assessed in control, propofol, rolipram and rolipram + propofol treated rats 5, 15 and 25 min after propofol administration (rolipram was administered 25 min before propofol). Propofol and rolipram had no effect on motor function when injected alone or together. Data were shown as mean $\pm$ SD. $N = 8$ for each group at each time point.

Figure 4 Rolipram reversed propofol-induced spatial memory impairments. **(A)** Propofol had no effect on latency during Day 2's cued MWM training. $N = 8$ per group. **(B)** Propofol had no effect on latency during Day 3's cued MWM training. $N = 8$ per group. **(C)** Propofol with or without rolipram had no effect on latency during Day 2's spatial navigation learning ($F_{3,61} = 1.74$; $P = 0.168$). $N = 16$ for control, propofol and rolipram + propofol group. $N = 17$ for rolipram group. **(D)** Rolipram reversed larger distance to the target quadrant caused by propofol. $N = 8$ per group. **(E)** Rolipram reversed shorter time in the target quadrant caused by propofol. $N = 8$ per group. **(F)** Representative tracking paths for each group from a single rat. Data were shown as mean $\pm$ SD. * $P < 0.05$ versus control group.

Results

Rolipram had No Effect on Propofol-Induced Sedative/Anesthetic Levels

Four light sedative states (0.2) and two deep sedative states (0.4) were observed 5 min after propofol administration (Figure 3A). Pretreatment with rolipram (25 min before propofol) induced three light sedative state (0.2), two deep sedative states (0.4), and one light anesthetic state (0.6). Rolipram alone induced one light sedative state. Propofol significantly increased the sedative/anesthetic scores compared with the control group ($P = 0.006$). Rolipram pretreatment had no effect on sedation caused by propofol ($P = 1$).

Propofol Caused No Motor Function Impairments in Accelerated Rotarod Test

Two-way ANOVA with repeated measurements revealed a time effect ($F_{2,27} = 8.257$; $P = 0.001$), no group effect ($F_{3,28} = 0.178$; $P = 0.910$), and no group $\times$ time interaction ($F_{6,56} = 0.292$; $P = 0.938$) for rod velocity (RPM) (Figure 3B). There was a time effect ($F_{2,27} = 7.152$; $P = 0.002$) but no group effect ($F_{3,28} = 0.522$; $P = 0.671$) or no group $\times$ time interaction ($F_{6,56} = 0.162$; $P = 0.986$) for time to fall (Figure 3C). Propofol group and rolipram + propofol group showed similar motor coordination with control group at all three time points.

Propofol did not Impair Non-Cued MWM Learning Efficacy

Two-way repeated measures analysis revealed no difference in velocity ($F_{3,28} = 0.090$; $P = 0.965$) and latency ($F_{3,28} = 0.366$; $P = 0.778$, Figure 4A) among four groups in Day 2's training. No difference in velocity and latency was found for each trial among groups by *post hoc* Bonferroni's test. There was no difference in velocity ($F_{3,28} = 1.125$; $P = 0.356$) and latency ($F_{3,28} = 0.576$; $P = 0.636$, Figure 4B) during Day 3's 4-trial training.

Rolipram Reversed Propofol-Induced Spatial Memory Impairments

Five rats were excluded from further test before Day 2 due to inability to find the platform. 100 rats were then randomized by mean latency into four groups (Figure 2). In Day 2's training, two animals from group P and one animals from group R + P were discarded for failing to conduct or finish this experiment due to over sedation.

For Day 2' s spatial training, no significant differences were found in swimming velocity ($F_{3,61} = 0.134$; $P = 0.94$), latency ($F_{3,61} = 1.74$; $P = 0.168$), and path length ($F_{3,61} = 2.019$; $P = 0.121$) among groups. The latency for each trial was shown in Figure 4C.

For probe trial, *post hoc* analysis indicated that propofol treatment had significantly larger average distance to the target platform compared with the control group ($P = 0.02$, Figure 4D) and shorter time in the target quadrant ($P = 0.031$, Figure 4E). Pretreatment of rolipram rescued these impairments, and rolipram alone had no effect on spatial memory (Figure 4D,E). Representative tracking paths were shown in Figure 4F.

Figure 5 Rolipram rescued hippocampal cAMP levels decreased by propofol. cAMP levels was decreased 5 min (time 0) after propofol administration (*post hoc* $P = 0.03$ vs. control group). Pretreatment of rolipram significantly raised cAMP levels at time 0 (*post hoc*, $P = 0.03$ vs. control group). Data were shown as mean $\pm$ SD, $N = 8$ per group per time point. * $P < 0.05$ versus control group; ** $P < 0.01$ versus propofol group at the same time point.

CREB Signaling Impaired by Propofol Was Reversed by Rolipram

cAMP Levels Reduced by Propofol Were Reversed by Rolipram Pretreatment

Propofol reduced the hippocampal cAMP levels 5 min later (time 0, $P = 0.03$ vs. control), but not at other time points. This was reversed by pretreatment of rolipram (25 min before propofol injection, $P = 0.025$ vs. control). Rolipram alone also increased cAMP levels at time 0 ($P = 0.03$ vs. control) but not other time points (Figure 5). No difference was found among four groups at time 45 min and 24 h.

CaMKII α Phosphorylation was Reduced by Propofol and Rescued by Rolipram but not AKT or ERK1/2 Phosphorylation or CaMKIV Expression

No changes were found among four groups at each time point in AKT ($P = 0.615$, Figure 6A) or ERK1/2 phosphorylation ($P = 0.78$, Figure 6B). CaMKIV expression was not changed at each time point ($P = 0.929$, Figure 6E). CaMKII α phosphorylation was reduced by propofol ($P < 0.001$ vs. control) and rescued by rolipram pretreatment ($P < 0.001$ vs. propofol and $P = 1$ vs. control) at time 45 min (Figure 6C,F). Rolipram alone had no effect on CaMKII α phosphorylation at each time point.

CREB Phosphorylation was Reduced by Propofol And Rescued by Rolipram

No changes were found for CREB phosphorylation among four groups at time 0 and time 24 h. At time 45 min, CREB phosphorylation was significantly reduced by propofol alone compared with the control group ($P = 0.006$). Rolipram pretreatment completely reversed this effect ($P = 0.001$ vs. propofol and $P = 1$ vs. control). Rolipram alone did not change CREB phosphorylation levels (Figure 6D,G).

BDNF and Arc Expression were Reduced by Propofol and Rescued by Rolipram

c-Fos expression was not changed by either propofol or rolipram at each time point ($P = 0.202$, Figure 6E). BDNF levels were reduced by propofol at time 0 ($P = 0.025$ vs. control group) and time 45 min ($P = 0.004$). Preinjection of rolipram abolished these changes ($P = 1$ and 0.296 for time 0 and 45 min, respectively, versus control). Rolipram alone did not change BDNF levels (Figure 6E,H). Arc levels were reduced by propofol at time 45 min ($P = 0.008$ vs. control) and time 24 h ($P = 0.035$). Preinjection of rolipram completely counterbalanced this action ($P = 1$ for both time 45 min and 24 h versus control, Figure 6E,I).

Discussion

The study found: (1) Subanesthetic dose of propofol caused spatial memory consolidation but not acquisition impairments. (2) Propofol inhibited hippocampal CREB and CaMKII α activation, BDNF and Arc protein expression *in vivo*. (3) Rescuing CREB signaling

Figure 6 Rolipram reversed CREB signaling impaired by propofol. **(A)** Hippocampal AKT phosphorylation was not affected by propofol or rolipram. **(B)** Hippocampal ERK1/2 phosphorylation was not affected by propofol or rolipram. **(C,F)** CaMKIIα phosphorylation was reduced by propofol (time 45 min) and rescued by rolipram preconditioning. **(D,G)** CREB activation was reduced by propofol (time 45 min) and rescued by rolipram preconditioning. **(E)** CaMKIV and c-Fos protein expression was not affected by propofol or rolipram. **(E,H)** BDNF expression was reduced by propofol at time 0 and 45 min and rescued by rolipram. **(E,I)** Arc expression was reduced by propofol at time 45 min and 24 h and rescued by rolipram. Data were normalized to control group at time 0 and shown as mean ± SD, N = 4 per group per time point. **P* < 0.05 and ***P* < 0.01 versus control group at the same time point. #*P* < 0.05 and ##*P* < 0.01 versus propofol group at the same time point. Abbreviations: C, control; P, propofol; R, rolipram; R + P, rolipram + propofol.

using rolipram reversed propofol-induced amnesia. These results supported that CREB signaling was involved in spatial memory consolidation defects by propofol.

Sedation and Motor Coordination after Propofol Injection

Propofol caused sedation but no motor skill impairments (Figure 3). The discrepancy might be due to different natures and sensitivities of the tests [30]. The sedative/anesthetic scores focused more on attention and alertness, while rotarod tests focused more on motor skills. Stanley found mice showing sedation in the cage did not exhibit significantly impaired performance on the rotarod [30]. Moreover, there was no difference in the

latency of cued MWM and the swimming speed of non-cued MWM. Taken together, subanesthetic dose of propofol might sedate the mice without motor incoordination.

Selective Impairment of Spatial Memory Consolidation by Propofol

Propofol administration just before non-cued spatial navigation training had no effect on spatial learning and short memory, while decreased long-term memory retention levels (Figure 4C–F). Similarly, episodic-like fear memory consolidation was inhibited by propofol [2,22]. Our repeated rotarod tests involved procedural memory processes [31] and a time-dependent improvement in motor coordination was found (Figure 3B,C).

We observed no difference in motor skills among groups. Furthermore, there was no difference in cued MWM training testing habit memory (Figure 4A,B) [32]. These results supported clinical findings [3–7] that propofol preferred to interrupt the consolidation of episodic memory.

Hippocampal CREB Signaling in Spatial Memory Consolidation and Propofol Anesthesia

Hippocampal cAMP was decreased by intraperitoneal propofol before MWM training (Figure 5). Propofol did not affect steady-state cAMP production in isolated cardiomyocyte but attenuated β -adrenoreceptor agonist isoproterenol-stimulated cAMP increase [33]. If this effect was similar in neurons, the suppression of *in vivo* cAMP production before training should involve other signaling pathways. For example, propofol suppressed calcium influx in hippocampal neurons [34], which might act via adenylate cyclase to inhibit cAMP generation. Furthermore, propofol inhibited baseline presynaptic acetylcholine release *in vivo* [35], which might bind to postsynaptic receptors to regulate cAMP metabolism.

CREB phosphorylation was inhibited 50 min after propofol injection and returned to normal 24 h later (Figure 6D,G). Meanwhile, spatial memory was disturbed 24 h after propofol administration (Figure 4D–F). These results suggested that transient alteration of pCREB during the memory consolidation phase (first several hours after training) strongly affected long-term memory formation. Consistent with our findings, decreasing hippocampal CREB during the learning and the memory consolidation phase but not 24 h later had no effect on spatial acquisition but impaired memory retention [36]. Increased hippocampal pCREB levels 0–6 h after inhibitory avoidance training were involved in memory consolidation [37]. Transient repression of CREB in mice forebrain neurons (<4 h after training) blocked the consolidation of fear memory [38]. These studies suggested that CREB phosphorylation was crucial for initiation of memory consolidation but was not a marker of memory retention levels [13].

Reduced mature BDNF level 5 min after propofol administration (Figure 6E,H) was consistent with clinical reports that serum BDNF decreased rapidly during propofol anesthesia [39]. The immediate hippocampal BDNF decrease did not impair following MWM learning (Figure 4C). This was supported by observations that BDNF ablation resulted in impaired learning on days 4–7 but not on day 1 [40]. Heterozygous BDNF knockout mice with reduced hippocampal BDNF showed normal MWM learning ability [41]. Moreover, hippocampus-specific deletion of BDNF caused spatial learning inability on days 4–5 but not on day 1 [42]. Taken together, these studies suggested that endogenous BDNF was not essential for short-term spatial memory formation. BDNF was decreased by propofol at time 45 min but not 24 h. This might be caused by a biphasic BDNF expression for memory consolidation: one immediately after encoding responsible for memory lasting for 1–2 days, whereas the other at 12 h after memory formation [43]. Compared to BDNF, Arc protein expression started within minutes after learning, lasts for only 2–4 h and was quickly degraded [18,44]. Thus, propofol inhibited Arc expression at both time 45 min and 24 h (Figure 6E,I).

Rolipram and Rescue of Propofol-Induced Spatial Impairments

Elevating cAMP levels using rolipram (Figure 5) reversed propofol-induced memory retention impairments (Figure 4D,E). These results suggested the involvement of cAMP in propofol-induced amnesia. The finding should not be interpreted as conclusive. There was still a possibility that cAMP alteration was only a concomitant response without function. As shown in Figure 6, Ca^{2+} /CaMKII α signaling was also inhibited by propofol, which may regulate cAMP production through adenylate cyclase (AC). Furthermore, we found BDNF was inhibited immediately by propofol (Figure 6), which could also inhibit cAMP regeneration (Figure 1). However, as all pathways converged on CREB, propofol inhibited CREB phosphorylation and rescuing CREB signaling using rolipram reversed spatial memory consolidation impairments, we could conclude that CREB activation inhibition was involved in amnesia by propofol.

Limitation

Besides CREB signaling, rolipram could regulate several other signaling pathways, which might affect spatial memory consolidation. For example, increased cAMP levels by rolipram could activate Epac1 and HCN channels, which might play a role in spatial learning and memory [45,46]. Thus, the reversal effects of rolipram on propofol-induced amnesia might not be totally dependent on CREB signaling. Moreover, we intraperitoneally injected rolipram but not locally. As type IV phosphodiesterase (PDEIV) was ubiquitously expressed in the brain and in different cells [47,48], CREB signaling in other brain regions such as prefrontal cortex, amygdala, and basal forebrain might be also regulated by rolipram and contributed to the anti-amnesic effects of rolipram. These possibilities should be included for consideration when interpreting our findings.

Significance and Future Direction

Our results revealed that propofol disrupted episodic-like memory consolidation and caused anterograde amnesia by possibly inhibiting hippocampal CREB signaling. Early studies implied that subanesthetic dose of propofol hindered the transformation of short-term memory to long-term memory possibly by suppressing new protein expression [7]. However, direct inhibition of protein synthesis produced profound retrograde amnesia, which was not a trait of propofol. Herein, we provided an explanation that propofol might indirectly suppress new protein synthesis by transiently inhibiting CREB activation. Further studies using CREB knockout mice or preemptively inhibiting CREB activation were needed to support the finding.

Although deemed as a short-acting anesthetic, propofol could suppress cognition for several hours after cessation of administration [49,50]. We found that rolipram could reverse propofol-induced amnesia. This raised the possibility of drug interventions to help patients recover from propofol anesthesia. For example, diazepam was a nonspecific PDEIV inhibitor used for reducing perioperative tension and anxiety [51]. It would be interesting to

test whether co-use of diazepam with propofol will produce less memory disturbances after some procedures such as endoscopy.

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Conflict of Interest

The authors declare no conflict of interests.

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