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Brief communication

Acute pentobarbital treatment impairs spatial learning and memory and hippocampal long-term potentiation in rats

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HIGHLIGHTS

- Intrahippocampal infusion of pentobarbital impairs spatial learning and memory.
- Bath application of pentobarbital impairs hippocampal LTP.
- Bath application of pentobarbital suppresses neuronal excitability.

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ABSTRACT

Reports of the effects of pentobarbital on learning and memory are contradictory. Some studies have not shown any interference with learning and memory, whereas others have shown that pentobarbital impairs memory and that these impairments can last for long periods. However, it is unclear whether acute local microinjections of pentobarbital affect learning and memory, and if so, the potential mechanisms are also unclear. Here, we reported that the intra-hippocampal infusion of pentobarbital (8.0 mM, 1 μl per side) significantly impaired hippocampus-dependent spatial learning and memory retrieval. Moreover, in vitro electrophysiological recordings revealed that these behavioral changes were accompanied by impaired hippocampal CA1 long-term potentiation (LTP) and suppressed neuronal excitability as reflected by a decrease in the number of action potentials (APs). These results suggest that acute pentobarbital application causes spatial learning and memory deficits that might be attributable to the suppression of synaptic plasticity and neuronal excitability.

1. Introduction

Pentobarbital is a barbiturate that is widely used to treat insomnia, for sedation and to treat status epilepticus. Additionally, pentobarbital has been used in high doses to induce comas for the management of cerebral ischemia and increased intracranial pressure associated with Reye's syndrome, stroke and traumatic brain injury [1]. Pentobarbital provides satisfactory control of seizures by acting on one or more targets in the brain that include ion channels, neurotransmitter metabolic enzymes, and neurotransmitter transporters [2].

However, in addition to their sedative and hypnotic effects, general anesthesia drugs, including pentobarbital, might also cause amnesia, which is one of the important causes of postoperative cognitive dysfunction. In recent years, the effects of general anesthetics on the cognitive functioning of patients undergoing surgery have received increasing attention. Unfortunately, reports of the effects of pentobarbital on learning and memory are contradictory. Some studies have shown no interference with learning and memory [3], whereas others have shown that pentobarbital application might impair memory [4–7]. For example, Kirk and colleagues reported that pentobarbital quantitatively impairs the memory acquisition processes involved in short-term recall performance [4]. The systemic administration of pentobarbital can produce memory dissociation in rats [5] and disrupt short-term memory and attention in monkeys performing an operant behavioral test battery [6]. A recent study also showed that neonatal treatment with pentobarbital leads to impairments of spatial memory that persists even into adulthood [7].

Because reports of the effects of systemic treatment with pentobarbital on learning and memory are not consistent, it is necessary to examine the influence of the direct application of pentobarbital to the hippocampus on spatial learning and memory. Thus, in the present study, we explored the effects of the intra-hippocampal infusion of pentobarbital on spatial learning and memory in the Morris water maze

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paradigm. Because hippocampal synaptic plasticity has been proposed as a cellular mechanism of learning and memory [8,9], we also tested the effects of pentobarbital on hippocampal CA1 LTP and neuronal excitability.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 200–250 g were obtained from the Chongqing Medical University Animal Care Center and housed in the laboratory colony of the Children's Hospital of Chongqing Medical University. The rats were housed in a room that was maintained at 21 °C on a 12-h dark/light cycle and were given free access to food and water. All experiment protocols were approved by the Chongqing Medical University Animal Care Center. All efforts were made to minimize the number of animals used.

2.2. Reagents

Pentobarbital was purchased from Sigma-Aldrich. For the intra-hippocampal infusions, the pentobarbital was dissolved in 0.9% sterile saline to 8.0 mM, and it was dissolved in artificial cerebrospinal fluid (ACSF) at different concentrations for the in vitro treatments.

2.3. Bilateral hippocampal microinjection

The rats were chronically implanted with cannulae above the dorsal hippocampus as previously described [10,11]. Briefly, under isoflurane anesthesia, the rats were placed in a stereotaxic apparatus (Stoelting, USA) and implanted with two 22-gauge stainless steel guide cannulae (10 mm; Plastics One Inc., Roanoke, VA) above the dorsal hippocampus (3.5 mm posterior to bregma, 2.5 mm lateral to the midline and 2.5 mm below the surface of the dura) that were fixed to the skull with four jeweler's screws and dental cement. Sterile dummy cannulae (30-Ga stainless steel rod, 10 mm, Plastics One Inc.) were inserted into the guide cannulae to prevent bacterial infection and cerebral spinal fluid leakage through the cannula. After surgery, all animals were allowed a 1-week post-operative recovery period before the initiation of the behavioral experiments.

On the day before the experiments, the animals were placed in the experiment room and given a sham intra-hippocampal injection to acclimate them to the injection procedure. The dummy cannulae were removed, and the rats were placed into a Plexiglas injection box ($25 \times 45 \times 25$ cm, the same size as the home cage) with 30-gauge injection cannulae in their guide cannulae. The injection cannulae (11 mm, Plastics One Inc., Roanoke, VA) were connected to a microsyringe pump (Harvard Apparatus) via PE-50 tubing and extended 1 mm beyond the tips of the guide cannulae.

The drugs were injected with 10-μl Hamilton syringes and a microsyringe pump at 0.5 μl/min for 2 min. After the injections, the injection cannulae were left in place for an additional minute to allow for the diffusion of the drug away from the cannula tips. The rats were then removed from the injection box, their dummy cannulae were replaced, and they were placed back in their home cages. The cannula placements were verified by histological examinations of the brains after methylene blue injection (1 μl per side), and only the data obtained from the rats with correctly inserted cannulae were included in statistical analyses.

2.4. Morris water maze test

Spatial learning and memory were examined with the Morris water maze using procedures similar to those described previously [12,13]. The Morris water maze consisted of a circular fiberglass pool (180-cm diameter) filled with water (25 ± 1 °C) that was made opaque with black non-toxic paint. The pool was surrounded by light blue curtains, and three distal visual cues were fixed to the curtains. Four floor light sources of equal power provided uniform illumination to the pool and testing room. A CCD camera suspended above the pool center recorded the swim paths of the animals, and the video output was digitized with an Any-maze tracking system (Stoelting, USA). The pool was artificially divided into four quadrants, i.e., N, E, S, and W. The Morris water maze test included spatial training and a probe test. Twenty-four hours before the spatial training, the animals were allowed to adapt to the maze via 60 s of free swimming. The animals were then trained in the spatial learning task for four trials per day for five consecutive days. In each trial, rats were placed in the water at one of four starting positions (N, E, S, or W) facing to the pool wall. The rats were then required to swim to find the hidden platform (13 cm in diameter, located in the SW quadrant), which was submerged 1 cm under the water. During each trial, the rats were allowed to swim until they found the hidden platform where they remained for 20 s before being returned to a holding cage. The rats that failed to find the hidden platform within 60 s were guided to the platform where they remained for 20 s. Twenty-four hours after the final training trial, the mice were returned to the pool at a novel drop point with the hidden platform absent, and their swim paths were recorded for 60 s.

To determine the effects of pentobarbital on spatial learning, the rats were divided into two groups, i.e., a vehicle and a pentobarbital group. The rats in the pentobarbital group were bilaterally infused with pentobarbital (8 mM, 1 μl per hippocampus) 20 min before the first training trial on each training day. The vehicle group was injected with the same volume of saline. To determine the effects of pentobarbital on spatial memory, the rats that were injected with saline on the training days were divided into two subgroups, i.e., a saline–saline and a saline–pentobarbital group. The animals in the saline–pentobarbital group were injected with pentobarbital bilaterally 20 min before the probe test, and the animals in the saline–saline group were injected with the same volume of saline. Additionally, the animals that were injected with pentobarbital on the training days were also injected with saline (pentobarbital–saline) 20 min before the probe test.

2.5. Electrophysiology

The rats were deeply anesthetized using urethane (1.5 g/kg, i.p.) and transcardially perfused with N-methyl-D-glucamine (NMDG) artificial cerebral spinal fluid (ACSF) prior to decapitation as described previously [14]. Next, acute coronal hippocampal slices were sectioned (400 μm thick) with a vibratome (VT1000S, Leica Microsystems) in ice-cold NMDG ACSF bubbled with 95% O_2 and 5% CO_2. The slices were then incubated in oxygenated HEPES ACSF for 1 h at 30 °C. Subsequently, the slices were gently transferred into a recording chamber filled with normal ACSF. The field excitatory postsynaptic potentials (fEPSPs) evoked by stimulation of the Schaffer collateral/commissural pathways were recorded in the hippocampus using pipettes (1–2 MΩ) filled with ACSF. Test fEPSPs were evoked at a frequency of 0.033 Hz and at a stimulus intensity that was adjusted to approximately 50% of the intensity that elicited the maximal response. After a 20-min stable baseline, LTP was induced by high-frequency stimulation (HFS, 100 pulses at 100 Hz). Pentobarbital and bicuculline were dissolved in ACSF at the required concentration and applied during incubation and recording. For the whole-cell patch-clamp recordings, the hippocampal CA1 pyramidal neurons were visualized with a microscope, and the recording electrodes (resistance: 3–5 MΩ) were filled with intracellular solution at a pH of 7.3 that contained the following (in mM): 117 potassium-gluconate, 13 KCl, 0.07 CaCl2, 4 NaCl, 10 HEPES, 0.1 EGTA, 2 Mg-ATP, and 0.4 Na-GTP [15]. To elicit spiking activity, depolarizing square wave current pulses (250 pA, 250 mS) were injected into the somas and followed by a 300 ms return to the holding membrane potential (−70 mV). Once stable APs were obtained, pentobarbital at different concentrations (0, 0.25, 0.5, 1, and 2 mM) was bath-applied. The APs were recorded for 5 min after drug application. Data acquisition (filtered