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Postoperative pain impairs subsequent performance on a spatial memory task via effects on *N*-methyl-D-aspartate receptor in aged rats

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ABSTRACT

Aims: Pain may be associated with postoperative cognitive dysfunction (POCD); however, this relationship remains underinvestigated. Therefore, we examined the impact of postoperative pain on cognitive functions in aged animals.

Main methods: Rats were allocated to the following groups: control (C), 1.2 % isoflurane for 2 hours alone (I), I with laparotomy (IL), IL with analgesia using local ropivacaine (IL + R), and IL with analgesia using systemic morphine (IL + M). Pain was assessed by rat grimace scale (RGS). Spatial memory was evaluated using a radial maze from postoperative days (POD) 3 to 14. NMDA receptor (NR) 2 subunits in hippocampus were measured by ELISA. Finally, effects of memantine, a low-affinity uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, on postoperative cognitive performance were tested.

Key findings: Postoperative RGS was increased in Group IL, but not in other groups. The number of memory errors in Group I were comparable to that in Group C, whereas errors in Group IL were increased. Importantly, in Group IL + R and IL + M, cognitive impairment was not found. The memory errors were positively correlated with the levels of NMDA receptor 2 subunits in hippocampus. Prophylactic treatment with memantine could prevent the development of memory deficits observed in Group IL without an analgesic effect.

Significance: Postoperative pain contributes to the development of memory deficits after anesthesia and surgery via up-regulation of hippocampal NMDA receptors. Our findings suggest that postoperative pain management may be important for the prevention of POCD in elderly patients.

Introduction

An increase in the aged population and advances in anesthetic and surgical techniques have increased the number of geriatric patients undergoing surgical operations under anesthesia (Chow et al., 2012). Consequently, postoperative cognitive dysfunction (POCD) has become a common problem in elderly patients (Monk et al., 2008). POCD is a temporary decline in cognitive functioning in the weeks or months following a surgical procedure. It is associated with long-term disability and even increased mortality (Steinmetz et al., 2009). Currently, however, there are few recognized intervention strategies for preventing POCD.

A relationship between postoperative pain and long-term memory impairment may exist, but the data on this relationship are limited (Fong et al., 2006). Most of these data focus on the choice of postoperative analgesic rather than the pain itself. Nevertheless, pain is closely linked to postoperative delirium (Morrison et al., 2003; Vaurio et al., 2006), a condition in which patients suffer an acute-onset of cognitive decline that is distinct from POCD (Deiner and Silverstein, 2009). Although the continuum between postoperative delirium and POCD remains controversial (Deiner and Silverstein, 2009), postoperative delirium can progress to POCD (Saczynski et al., 2012). Therefore, we hypothesized that postoperative pain mediates the long-lasting memory deficits in elderly patients.

The mechanisms that cause POCD in elderly patients are largely unknown. Several preclinical studies suggest that general anesthesia causes subsequent long-term memory impairment. For example, isoflurane anesthesia administered at clinically relevant doses causes long-term cognitive impairments in unoperated animals (Culley et al., 2004; Lin and Zuo, 2011; Cao et al., 2012). Other studies, however, indicate inhalation anesthesia can enhance cognitive functions after exposure (Komatsu et al., 1993; Rammes et al., 2009; Callaway et al., 2012). Cognitive improvement following isoflurane exposure was defined by a transient up-regulation of *N*-methyl-D-aspartate (NMDA) receptor (NR) in the hippocampus (Rammes et al., 2009). NRs play pivotal

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roles in learning, memory, and processing pain (Moriarty et al., 2011; Zhuo, 2009). Therefore, we further hypothesized that postoperative pain causes POCD by increasing NR expression.

We tested these hypotheses by first investigating in isoflurane-anesthetized aged rats the effects of postoperative pain on performance in working and reference memory tasks. Specifically, we tested 2 clinically relevant analgesics—a local anesthetic and a systemic opioid analgesic—for their ability to mitigate the effects of postoperative pain on cognitive dysfunction. Furthermore, to confirm NRs involvement in pain-related cognitive impairment, we examined whether the non-competitive NR antagonist memantine prevented the development of memory deficits after anesthesia and surgery.

Materials and methods

Animals

Male Wistar rats aged between 24 and 25 months, and weighing between 550 and 640 g, were used in this study. Subjects were maintained at approximately 90% of their free-feeding body weight during the entire radial arm maze procedure. All experiments were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School.

Anesthesia and surgery

Simple laparotomy was used as an acute postoperative pain model. Anesthesia was induced and maintained with 1.2% isoflurane in 100% oxygen. One and a half hours after anesthesia was induced, a 1.0 cm longitudinal midline incision was made through the skin, abdominal muscle, and the peritoneum. The muscle layers were then repaired with 5–0 Vicryl sutures, and the skin was closed with tissue adhesive glue. The surgery duration was fixed at 10 min for each procedure, and the incised muscles were retracted during this period. Isoflurane anesthesia was continued until the total exposure time reached 2 h. This duration was selected because previous studies with isoflurane indicate both positive and negative effects on cognitive function with this time frame (Komatsu et al., 1993; Culley et al., 2004; Rammes et al., 2009; Lin and Zuo, 2011; Cao et al., 2012). Animals emerged from anesthesia 20 min after the end of surgery, which was due to the onset-time of systemic analgesia with postoperative morphine doses. In a pilot study in our laboratory, the minimum alveolar concentrations (MAC) value of isoflurane in aged rats was 1.01 ± 0.08% ($n = 8$). Therefore, 1.2% isoflurane is equivalent to approximately 1.2 MAC.

Rats were allocated to 1 of 5 experimental groups ($n = 8$ rats/group): (1) 100% oxygen inhalation for 2 h without surgery (Group C); (2) isoflurane anesthesia without surgery (Group I); (3) isoflurane anesthesia with laparotomy (Group IL); (4) isoflurane anesthesia with laparotomy plus single-dose surgical wound infiltration with 0.2% ropivacaine (300 μl) after surgery (Group IL + R); and (5) isoflurane anesthesia with laparotomy plus a single subcutaneous dose administration with 0.8 mg/kg morphine after surgery (Group IL + M). The doses of the 2 analgesic regimens used in this study were determined based on our preliminary findings. During the inhalation period, noninvasive recordings of pulse rate, arterial oxygen saturation, and MAP were measured by tail-cuff plethysmography. In a separate experiment, we tested whether the 2 analgesic methods used in this study show direct effects on memory function by treating the rats in Group I with either the analgesic or an identical volume of physiological saline ($n = 8$ rats/group).

Measurement of pain

Postoperative pain intensity was measured using a rat grimace scale (RGS), as previously reported (Sotocinal et al., 2011). Briefly, rats were placed individually in a clear plastic cage and allowed to acclimate to the environment for 10 min. Facial expressions of rats were recorded by digital video cameras (HandyCam HDR-CX560, Sony, Japan) for 10 min before surgery (baseline), at 2, 4, 6, 8, 12, 24, and 48 h after inhalation period. Still-frames (front-view) were captured and cropped to display each rat's head to a non-participating assistant. Randomized and unlabeled facial images were presented on a high-resolution computer monitor, and the RGS was assigned by the experienced evaluator who was blinded to the study treatment using a 3-point scale (0 = no pain, 1 = moderate pain, 2 = intense pain) for each of the 4 RGS action units: orbital tightening, nose/cheek fluttering, ear position, and whisker change. The final RGS score is calculated by taking the average score of all 4 RGS action units.

Single and repeated open-field test

A repeated open-field habituation test was conducted 1 week before the start of experiments to screen for baseline memory function in all rats. Rats were exposed to the same open field for 3 consecutive 5-min trials, with an interval of 30 min between each trial. The activity counts at the third exposure were compared to those after the first exposure. Single open-field tests were subsequently performed to evaluate locomotor activity on POD 3, 7, and 14. The total accumulated counts of horizontal beam crosses were recorded for 60 min.

Radial arm maze

A 12-arm radial maze was used to evaluate spatial working and reference memory performance, with some modifications to a method described previously (Culley et al., 2004). Briefly, rats were habituated to the maze daily for 10 min, for 5 consecutive days. During this habituation phase, rats were allowed to freely explore and eat randomly scattered food rewards in the maze.

The maze consisted of a circular center platform (34-cm diameter) surrounded by 12 equally spaced radial arms (50-cm-long, 11-cm-wide, and 20-cm-high transparent walls). A plastic food cup intended to contain 45 mg of reward pellet was located at the end of each arm. Six arms were randomly assigned to be baited, and the remaining 6 arms were never baited. The locations of baited and unbaited arms were varied between animals, but remained consistent throughout testing for each rat. The maze was surrounded by multiple visual cues: posters, a door and windows, a chair, and some desks. All experimental trials were conducted during the dark phase of the light/dark cycle.

Formal radial arm testing was performed for 12 consecutive days (POD 3 to 14). During each trial, rats were individually placed at the center of the maze and allowed to freely explore the maze until all 6 reward pellets in the food cups were consumed. Each session was terminated either when the rat had retrieved all reward pellets or after 15 min had elapsed. The numbers of errors were recorded, and the following 2 parameters of memory function were determined: (1) working memory errors, defined as repeat entries into the arms that had already been visited within a trial, and (2) reference memory errors, defined as first entries into non-baited arms. The data obtained were analyzed in 2-day blocks. The averaged latency per arm choice (s/arm entry) was also calculated as a measure of motivation and motor ability. All trials were monitored by an overhead video camera, and analysis of behavior was always scored by an investigator who was blind to experimental group assignment. After the completion of the last trial, all subjects were killed by rapid decapitation under terminal anesthesia with inhaled isoflurane. Three different brain regions, the hippocampus, prefrontal cortex (PFC), and amygdala were dissected according to the dissection method described by Glowinski and Iversen (Glowinski and Iversen, 1966). Brain samples were stored at −80 °C until required for ELISA.