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Effect of saikosaponin A on maintenance of intravenous morphine self-administration

Seong Shoon Yoon[a], Hey Soo Kim[b], Hea-Young Cho[b], Jaesuk Yun[b], Eun Yong Chung[b], Choon-Gon Jang[c], Kwang Joong Kim[a], Chae Ha Yang[a,*]

[a] Department of Physiology, College of Oriental Medicine, Daegu Haany University, Daegu 706-828, Republic of Korea
[b] Division of Safety Pharmacology, National Institute of Toxicological Research, Korea Food & Drug Administration, Gangoe-myeon, Cheongwon-gun, Chungcheongbuk-do 363-951, Republic of Korea
[c] Department of Pharmacology, School of Pharmacy, Sungkyunkwan University, 300 Cheoncheon-dong, Jangan-gu, Suwon 440-746, Republic of Korea

HIGHLIGHTS

- Saikosaponin A dose-dependently reduced morphine self-administration.
- The effects of saikosaponin A were blocked by the GABA(B) receptor antagonist SCH 50911 but not the GABA(A) receptor antagonist bicuculline.
- Saikosaponin A did not affect food-reinforced responding.
- These results suggest that SSA may be effective in reducing the reinforcing effects of morphine.

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ABSTRACT

In this study, we investigated the effects of saikosaponin A (SSA), a major compound of *Bupleurum falcatum* L., on morphine self-administration behavior. Male Sprague–Dawley rats were trained to self-administer intravenous morphine (0.1 mg/kg per injection over 5 s) during daily 1-h sessions under a fixed-ratio 1 schedule. Rats were pretreated with SSA (0.25, 0.5, 1.0 mg/kg) by intraperitoneal injection 30 min prior to the start of the test session. Results demonstrated that pretreatment with SSA reduced morphine-maintained responding dose-dependently. Additionally, SSA inhibition of morphine-reinforced behavior was blocked by the selective $GABA_B$ receptor antagonist (2S)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50911), but not the selective $GABA_A$ receptor antagonist bicuculline. Together, these results suggest that SSA may effectively suppress morphine-reinforced behavior by activating $GABA_B$ receptors.

1. Introduction

The neurobiological substrate for opioid reinforcement in animals and opioid abuse in humans is believed to involve the mesolimbic dopamine system, which originates in the ventral tegmental area and projects to the nucleus accumbens. For example, dopamine-specific lesions of the nucleus accumbens with 6-hydroxydopamine suppress morphine self-administration [20]. Similarly, systemic dopamine D1 receptor blockade reduces the acquisition of conditioned place preference induced by morphine [16]. An *in vivo* fast cyclic voltammetry study showed a significant correlation between a dose-dependent increase in accumbal dopamine release and a dose-dependent decrease in self-administration rate during heroin self-administration. In this study, naloxone inhibited the dopamine release and self-administration behavior induced by heroin [24].

Activation of μ-opioid receptors expressed on γ-aminobutyric acid (GABA) neurons in the ventral tegmental area by opioids results in their hyperpolarization, causing disinhibition of the dopamine neurons. This leads to an increase in accumbal dopamine release [11,25]. Accordingly, it seems reasonable to propose that GABA agonists play a role in suppression of the reinforcing effects of opioids by modulating dopamine release in the nucleus accumbens. Specifically, systemic injection of the $GABA_B$ receptor agonist baclofen has been shown to decrease heroin self-administration behavior and heroin-induced dopamine release in the nucleus accumbens, which was suppressed by 2-hydroxysaclofen [25]. Similarly, activation of $GABA_B$ receptors by baclofen in the nucleus accumbens or the ventral tegmental area blocks morphine self-administration behavior [28] or morphine-induced conditioned

* Corresponding author at: Department of Physiology, College of Oriental Medicine, Daegu Haany University, 165 Sang-Dong, Suseong-Gu, Daegu 706-826, Republic of Korea. Tel.: +82 53 770 2254; fax: +82 53 768 6340.
E-mail address: chyang@dhu.ac.kr (C.H. Yang).

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place preference [22]. Although the role of $GABA_A$ receptors in the reinforcing effects of opioids remains complex and controversial, it has been suggested that $GABA_A$ receptors on ventral tegmental area dopamine neurons are involved in modulating dopamine release in the nucleus accumbens and opioid reinforcement [26]. Furthermore, behavioral studies reported that muscimol attenuated the expression and acquisition of morphine-induced conditioned place preference [30] and reduced morphine self-administration behavior [28,29].

Saikosaponin A (SSA) is a triterpine saponin isolated from *Bupleurum falcatum* L. (Umbelliferae) [7]. Results of some animal and clinical studies provide evidence for the involvement of the central nervous system (CNS) in the antidepressant and anxiolytic actions of *B. falcatum* L. [14,15]. Few experiments have investigated the effects of saikosaponins on the CNS. These CNS-related studies, using animal models, have provided evidence that saikosaponins can inhibit epileptic seizures in rats exposed to pentetrazole and regulate glutamate and GABA expression in the hippocampus of pentetrazole-induced kindling rats [27].

Here, we designed a morphine self-administration study to investigate the effect of SSA on the reinforcing effects of morphine and the possible mechanism of action of SSA on the GABA pathway. To our knowledge, this is the first report that SSA can attenuate the reinforcing effects of morphine.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Daehan Animal, Seoul, Korea), weighing 280–300 g at the beginning of the study, were individually housed in a temperature (21–23 °C) and humidity (55–65%)-controlled colony room with a 12/12-h light/dark cycle. Rats were kept on *ad libitum* food and water except during the food training period. The morphine self-administration study was conducted during their dark cycle. Experimental procedures and animal care were in accordance with the Institutional Animal Care and Use Committee at the Daegu Haany University. They met or exceeded the National Institutes of Health guidelines for the care and use of laboratory animals (NIH publication No. 80-23).

2.2. Drugs

All drugs used were purchased from Sigma Chemical Co. (St. Louis, MO, USA) except SSA, which was from Wako Chemical Ltd. (Sankyo, Tokyo, Japan) and (2S)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50911) from Tocris (Ellisville, MO, USA). SSA was dissolved in 5% Tween-80 and GABA antagonists were dissolved in sterile saline. The morphine solution was prepared daily and filtered through a syringe-mounted millex-HA filter (Millipore, Bedford, MA, USA). All administration of SSA and its vehicle (5% Tween-80) was counterbalanced according to a Latinized design across subjects.

2.3. Apparatus

Rats were tested in operant conditioning chambers (Med Associates, St. Albans, VT, USA). Each chamber was equipped with two response levers (4.8 × 1.9 cm), a cue light (3 W, 28 V), and a house light (3 W, 28 V). A cue light was located above each response lever. The floor of the chamber was lined with wood chip bedding and covered with a metal grid. The chambers were placed within a sound-attenuating wooden enclosure, equipped with a ventilation fan. A syringe in the infusion pump (Razel Scientific Instruments, Stamford, CT, USA) was connected to a fluid swivel (Med Associates, St. Albans, VT, USA) with Tygon tubing. A Tygon tubing shield with a metal spring connected the swivel to the animal's catheter cannula assembly.

2.4. Intravenous catheterization

For intravenous catheterization, a Silastic catheter (inner diameter, 0.02 in.; outer diameter, 0.037 in.; Dow Corning, Midland, MI, USA), treated with tridodecylmethyl ammonium chloride heparin (Polysciences Inc., Warrington, PA, USA), was introduced into the right external jugular vein under sodium pentobarbital anesthesia (50 mg/kg, i.p.). Catheters were secured to the jugular vein with Mersilene surgical mesh (Ethicon Inc., Somerville, NJ, USA) and exteriorized *via* a skin incision in the animal's back through a 22-gauge stainless-steel cannula (Plastics One, Roanoke, VA, USA), fixed to the head assembly with dental cement, and secured with a Prolene surgical mesh (Ethicon Inc.). Patency of the catheter was maintained by daily flushes of 0.2 ml saline containing heparin (20 U/ml) and gentamycin sulfate (0.33 mg/ml).

2.5. Morphine self-administration and food reinforcement studies

Rats were initially trained to press a lever for 45 mg food pellets (Bio-serv, Frenchtown, NJ, USA) during a daily 3-h session to facilitate the acquisition of morphine self-administration under food restriction. After acquisition criteria (100 food pellets for three consecutive sessions) were established, rats were kept on *ad libitum* food and water in their home cage for at least 1 day prior to surgery for the intravenous catheterization. Following a 1-week recovery period after the surgery, rats were allowed to acquire a lever press response for morphine (0.1 mg/kg per infusion over 5 s in a 0.1 ml volume) under a fixed-ratio (FR1) schedule. Each lever press resulted in illumination of a cue light located above the active lever and extinction of the house light for 5 s. Each infusion was followed by an additional 10 s time-out period in which the house light remained off. Presses on the inactive lever were recorded but had no programmed consequence. Baseline response levels were calculated as the mean absolute value of response in three consecutive sessions exhibiting less than 20% variation. Typically, this required 14–21 days following the initiation of morphine self-administration. Following the establishment of a stable baseline value, rats were pretreated with SSA (0.25, 0.5, 1.0 mg/kg) or vehicle by an intraperitoneal injection 30 min prior to the start of self-administration testing. Each pretreatment was separated by at least 3 days of stable basal responding. Doses of SSA and vehicle were presented in a counterbalanced order. To control for the possibility that SSA treatment caused non-specific motor impairment, the time to obtain 50 food pellets was measured in SSA-treated rats with a modified model of food self-administration, as described elsewhere [1]. Initially, drug-naïve rats were food-restricted to maintain 85% of the initial body weight and trained to press a lever for food pellets under a FR1 schedule. Following the achievement of acquisition criteria (100 food pellets for three consecutive sessions), rats were allowed to self-administer 50 food pellets in daily sessions. The baseline response level was defined as the average time to self-administer 50 pellets in three consecutive tests with less than 10% variation. After establishing stable baseline response rates, rats were given an intraperitoneal injection of SSA (1.0 mg/kg) or vehicle.

To pharmacologically characterize the effects of SSA on morphine self-administration, rats were given either the $GABA_B$ receptor antagonist SCH 50911 (2.0 mg/kg) by intravenous injection or the $GABA_A$ receptor antagonist bicuculline methiodide (1.0 mg/kg) by intraperitoneal injection immediately before injection of SSA (1.0 mg/kg, i.p.). These doses of GABA agonists were chosen, based on a previous report [29]. The previous results showed that the baclofen effect on morphine self-administration