The Kappa-Opioid Agonist U69,593 Blocks Cocaine-Induced Enhancement of Brain Stimulation Reward

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Background: Increasing evidence indicates that brain kappa-opioid receptors (KORs) are involved in regulation of mood states. In animal models often used to study psychiatric illness, KOR agonists produce depressive-like effects (e.g., anhedonia), whereas KOR antagonists produce antidepressant- and anxiolytic-like effects. The ability of KOR agonists to produce anhedonia-like signs in laboratory animals raises the possibility that this class of drugs might be useful to ameliorate states characterized by excess reward or motivation, such as mania or stimulant intoxication.

Methods: We examined how the selective KOR agonist U69,593 affects cocaine-induced facilitation of intracranial self-stimulation (ICSS), a model of the abnormally increased reward function that characterizes mania and stimulant intoxication. Rats with stimulating electrodes implanted in the medial forebrain bundle (MFB) were tested with intraperitoneal injections of U69,593 (.063–.5 mg/kg) alone, cocaine (1.25–10 mg/kg) alone, and combinations of the drugs.

Results: Cocaine dose-dependently decreased ICSS thresholds, indicating that it enhanced the rewarding impact of MFB stimulation. In contrast, U69,593 dose-dependently increased ICSS thresholds, indicating that it decreased the rewarding impact of the stimulation. Pretreatment with U69,593 blocked cocaine-induced decreases in ICSS thresholds at doses that had negligible effects on their own.

Conclusions: Activation of KORs reduces the reward-related effects of cocaine. Inasmuch as cocaine-induced behavioral stimulation in rodents may model key aspects of enhanced mood in humans, these findings raise the possibility that KOR agonists might ameliorate symptoms of conditions characterized by increased motivation and hyperfunction of brain reward systems, such as mania and stimulant intoxication.

Key Words: Addiction, anti-manic, bipolar disorder, depression, ICSS, mania, model, rat

The biological basis of mood is not understood. Most research on mood and affective states focuses on brain systems containing monoamines, such as dopamine (DA), norepinephrine (NE), and serotonin (5HT). This focus is logical, because drugs with mood-elevating effects (including stimulants, antidepressants) have prominent interactions with these systems and tend to increase extracellular concentrations of monoamines and prolong their actions (1,2). However, there is accumulating evidence that brain opioids are also involved in the regulation of mood. As one example, we and others have found that kappa-opioid receptor (KOR) antagonists produce antidepressant-like (3–8) and anxioloytic-like (9) effects in animal models, whereas KOR agonists produce depressive-like effects (5,10,11). The molecular mechanisms by which these drugs alter mood are not understood, although KOR agonists decrease extracellular concentrations of DA within the nucleus accumbens (NAc) (1,11), a key component of the mesolimbic system. Dysregulation of the mesolimbic system is implicated in the pathophysiology of depressive conditions including bipolar disorder (12,13). Drugs that reduce the activity of brain reward systems may have utility in studying and altering the symptoms of mania, the defining state of bipolar disorder that is characterized by excessive involvement in rewarding or pleasurable activities (14).

Preclinical research on the biological basis of mania and bipolar disorder is complicated by an incomplete understanding of their pathophysiology. This has made it difficult to design models that recapitulate the behavioral symptoms of these conditions while ensuring construct validity. However, intracranial self-stimulation (ICSS) may be a useful paradigm with which to model certain aspects of mania. ICSS is an operant paradigm in which rodents respond at high rates for self-administered rewarding electrical stimulation through electrodes implanted into brain areas including the medial forebrain bundle (MFB) (15). The ICSS behavior fulfills several key diagnostic criteria used for mania in people (14). For example, rats show increases in a goal-directed activity (lever pressing for brain stimulation) and excessive involvement in this activity even under conditions in which there is a high potential for painful consequences: food-deprived rats choose to respond at a lever that produces stimulation rather than one that produces food (16), and rats tested in subfreezing conditions choose to respond at a lever that produces stimulation rather than one the produces heat (17). Drugs that reduce symptoms of mania (e.g., antipsychotics, mood stabilizers) attenuate ICSS (18,19), indicating that these agents produce anhedonia. This common effect raises the possibility that production of anhedonia-like states may contribute to (or at least predict) the efficacy of these drugs in treating mania. Drugs that trigger mania in humans or cause mania-like behaviors in laboratory animals (e.g., cocaine) produce a profound facilitation of ICSS, reflecting hyperfunction of brain reward systems (15,20). Genetic manipulations that cause mania-like signs in mice (including sleep disruptions) similarly facilitate ICSS (21). Thus even if ICSS does not produce mania-like behaviors in rodents through the same mechanisms that produce them in humans, it has predictive validity as a test with which to model aspects of bipolar disorder and identify new classes of agents that might ameliorate key symptoms of mania.

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The studies described here were designed to determine whether a prototypical KOR agonist (U69,593) affects the reward-related effects of cocaine in the ICSS test. Previous work indicates that interactions between KOR agonists and cocaine are complex and depend on the timing and context of the drug treatments. Although KOR agonists appear to block the development or expression of cocaine-induced conditioned place preferences (22,23), it has also been reported that exposure to KOR agonists can subsequently increase cocaine effects (24,25). ICSS offers several advantages that enable detailed analysis of acute interactions between KOR agonists and reward states. It is a highly trained behavior that is relatively impervious to treatments that might disrupt memory or cause anxiety, each of which could affect the outcome of place conditioning studies. It also enables real-time studies of drug interactions, whereas place conditioning studies test the memory of previously established associations. Finally, the "curve-shift" variant of the ICSS test enables distinctions between treatment effects on reward function and response capabilities (15,18). We report that the KOR agonist U69,593 blocks the reward-related effects of cocaine in the ICSS test at doses below those that have nonspecific effects on responding, providing support for the idea that this class of agents might ameliorate key symptoms of conditions characterized by heightened motivation (e.g., mania, stimulant intoxication).

Methods and Materials

Rats

Seven male Sprague-Dawley rats (Charles River Laboratories, Raleigh, North Carolina) were used. Rats were housed singly and maintained on a 12-hour light-dark (7 AM–7 PM) cycle with free access to food and water except during testing. Experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996) and McLean Hospital policies.

Drugs

Cocaine hydrochloride and U69,593 (5a,7a,8b)-N-methyl-N-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec8-yl)-benzenacetamide) were purchased from Sigma (St. Louis, Missouri). Cocaine was dissolved in 0.9% saline, whereas U69,593 was dissolved in .1 N acetic acid diluted with distilled water. Drugs were administered by intraperitoneal (IP) injection in a volume of 1 mL/kg. Dosages of the drugs were based on their salt form.

ICSS

Rats (350–375 g) were anesthetized with pentobarbital (65 mg/kg, IP), and given subcutaneous (SC) atropine sulfate (.25 mg/kg) to reduce bronchial secretions. Each rat was implanted with a monopolar, stainless steel electrode (.250-mm diameter; Plastics One, Roanoke, Virginia) aimed at the left medial forebrain bundle (MFB), at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral from the midsaggital suture and 7.8 mm below dura; 26). The electrodes were coated with polyamide insulation except at the flattened tip. Skull screws (one of which served as the ground) and the electrode were secured to the skull with dental acrylic.

After 1 week of recovery, the rats learned to respond for brain stimulation as described previously (15). Each lever press earned a .5-sec train of square-wave cathodal pulses (.1-msec pulse duration) at a frequency of 141 Hz. The stimulation current (100–300 μA) was adjusted gradually to the lowest value that would sustain reliable responding (at least 40 rewards/min). After the minimal effective current was found for each rat, it was held constant.

Each rat was then adapted to tests at its minimal effective current with a descending series of 15 stimulation frequencies. Each series comprised 1-min test trials at each frequency. For each frequency, there was an initial 5-sec "priming" phase during which noncontingent stimulation was given, followed by a 50-sec test phase during which the number of responses was counted, followed by a 5-sec timeout period during which no stimulation was available. The stimulation frequency was then lowered by 10% (.05 $\log_{10}$ units), and another trial was started. After responding had been evaluated at each of the 15 frequencies, the procedure was repeated such that each rat was given 6 such series per day (90 min of training). Minor adjustments were made to the current for each rat so that only the highest 7–8 frequencies would sustain responding. To characterize the functions relating response strength to reward magnitude, a least-squares line of best fit was plotted across the frequencies that sustained responding at 20%, 30%, 40%, 50%, and 60% of the maximum rate. The ICSS threshold was defined as the frequency at which the line intersected the x axis (theta-0) (27). Drug testing started when mean ICSS thresholds varied by less than 10% over three consecutive sessions.

For drug testing, three rate-frequency functions ("curves") were determined immediately before drug treatment. The second and third curves were averaged to obtain the baseline (threshold and maximal response rates) parameters. After obtaining baselines for each rat on each day, the rats received drug treatments, and four more 15-min rate-frequency curves were obtained (1 hour of testing). There were three phases of drug testing: a phase in which the rats were tested with U69,593 alone (.063–.5 mg/kg), a phase where they were tested with cocaine alone (1.25–10 mg/kg) alone, and a phase where they were tested with U69,593 (.063–.5 mg/kg) plus cocaine (5.0 mg/kg). Doses of U69,593 and cocaine are similar to those with effects in the forced-swim test (5). During the U69,593 alone phase, rats received an injection of the drug followed 10 min later by an injection of saline. Testing began immediately after the second injection. The doses were given in ascending and then descending order, such that each rat received vehicle and each dose of the drug twice. This experimental design was used to determine whether tolerance or sensitization would occur in response to repeated drug treatment. On alternate days rats were tested after injections of saline to ensure that they had recovered from prior treatment and to minimize the possibility of conditioned drug effects. During the cocaine-alone phase, rats received an injection of acid vehicle followed 10 min later by cocaine. As was the case with the U69,593-alone studies, the doses were given in ascending and then descending order, with saline treatment on alternating days. Approximately half of the rats were tested first with U69,593 (n = 4), whereas the others were tested first with cocaine (n = 3). During the U69,593-plus-cocaine phase, rats received an injection of U69,593 (.063, .125, .25, and .5 mg/kg) followed 10 min later by cocaine (5.0 mg/kg). This phase occurred last for all rats, and each dose of U69,593 was tested once.

Statistics

To determine whether there were differences between the first and second test with each treatment, the effects of U69,593 and cocaine on ICSS thresholds and maximal response rates over the entire 1-hour test period were evaluated in separate two-way