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Kappa Opioid Regulation of Stress-Related Behavior

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Kappa Opioid Regulation of Stress-Related Behavior

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Abstract

Anxiety disorders affect roughly 40 million American adults in a given year. Those suffering from anxiety disorders often experience additional stress-linked illnesses, such as depression. Previous research has shown that stress exposure increases levels of the endogenous neuropeptide dynorphin, which the kappa opioid system is selectively activated by. This study examined the role of the kappa opioid system in regulating stress-related behavior using the elevated plus-maze. Behavioral stress responses were examined in male Wistar rats following i.p. administration of opioid agonist U-50,488 (0 or 10 mg/kg). Subjects were pretreated with the kappa opioid antagonist nor-binaltorphimine (nor-BNI) 24 hours prior to testing in the elevated plus-maze (0 or 20 mg/kg). Injections of 10 mg/kg U-50,488 significantly decreased percent open arm time compared to controls, an effect reversed by pretreatment with 20 mg/kg nor-BNI ($F(1,44) = 6.10$, $p < 0.05$). A main effect of nor-BNI was found on the total number of arm entries ($F(1,44) = 11.73$, $p < 0.05$). Further analysis revealed that pretreatment with nor-BNI led to an increased number of arm entries in rats injected with U-50,488. The nor-BNI sensitivity of the behavioral responses suggests an activation of the kappa opioid receptors by a stress-induced release of dynorphin. The results indicate a relationship between kappa opioid receptors and stress-related behaviors and illustrate the potential therapeutic value of targeting the kappa opioid system in the treatment of anxiety and other stress-related disorders.

Introduction

Anxiety disorders affect roughly 40 million American adults (about 18% of the US population) in a given year (Kessler, 2005). In addition, those suffering from an anxiety disorder often experience comorbidity with other stress-linked mental illnesses such as depression and alcohol and drug abuse, suggesting that stress is a critical component in the development of these disorders.

A classic definition of stress is any response to demands, usually noxious, placed on the body (Selye, 1936). An alternate definition describes stress as any alterations in the psychological homeostatic process (Burchfield, 1979). Although exposure to certain levels of stress is a normal occurrence in everyday life, chronic stress can lead to the development of psychiatric disorders, such as anxiety and depression (Hennessy & Levine, 1979). Stress has been found to increase the release of several endogenous neurochemicals, including dynorphin (Nabeshima, et al., 1992), a neuropeptide that binds with high affinity to kappa opioid receptors (Chavkin, et al., 1982).

Recent evidence suggests the involvement of the dynorphin/kappa opioid receptor (KOR) system in mediating the stress responses. McLaughlin, et al. (2003) investigated the involvement of the kappa opioid system in the stress response by examining the behavior of mice subjected to forced swim stress. Mice lacking the prodynorphin gene showed a significant reduction in stress-induced immobility, an indication of depression-like behavior, in the forced swim test. Similar results were found in mice pretreated with the kappa opioid antagonist nor-binaltorphimine (nor-BNI). In addition, tests using social defeat stress (SDS) found prodynorphin knockout mice and those that received nor-BNI pretreatment to spend significantly less time in socially defeated, immobile postures (McLaughlin, et al., 2006). Taken together, these results implicate a role of the dynorphin/KOR system in behavioral responses to stress.

The dynorphin/KOR system has also been examined in stress-related reinstatement of drug seeking (Beardsley, et al., 2005; Valdez, et al., 2007). The KOR antagonist JDTic was found to block stress-induced reinstatement of cocaine seeking. It also decreased time spent immobile in the forced swim test, suggesting the antistress potential of KOR antagonists (Beardsley, et al., 2005). Another study found that the KOR agonists spiradoline and enadoline led to reinstatement of cocaine-seeking behavior in squirrel monkeys, but this effect was not reversed by the antagonist nor-BNI (Valdez, et al., 2007). These results suggest a possible mediation of stress-related behavior by a subpopulation of kappa opioid receptors. However, blockade of corticotropin releasing factor and norephinephrine, two brain systems also thought to be mediators of the stress response (Koob, 1999), prevented spiradoline-induced reinstatement. Although these findings suggest that brain kappa opioid system is involved in the regulation of stress-related behaviors, additional research is needed to understand the role of the KOR system.

The purpose of this study was to examine the role of the kappa opioid system in regulating behavioral responses to stress. The effects of U-50,488 on stress-related behavior were examined in the elevated plus-maze, a classic animal model of anxiety (Pellow & File, 1986). In addition, the effects of the selective antagonist nor-BNI administered as a pretreatment to U-50,488 were examined. The results of this experiment reveal the ability of nor-BNI to reduce stress-induced increases in anxiety-like behavior, suggesting that kappa opioid mechanisms are involved in the regulation of behaviors induced by external stressors.

Materials and Methods

Animals and Housing

Male Wistar rats (Charles River, Kingston, NY; n=45) weighing 150-200 g upon arrival were used in this experiment. Rats were habituated to colony housing 14 days prior to testing. Rats were housed in groups of 2-3 per cage with food and water available *ad libitum*. Rats were handled and weighed daily to minimize any stress associated with the experimental procedure. Animals were maintained on a 12-hr reverse light/dark cycle (lights on at 10:00 PM).

Apparatus

The elevated plus-maze (Med Associates, St. Albans, VT) measures unconditioned approach-avoidance behavior during exploration of a novel environment in rats (Pellow & File, 1986). The apparatus was made of dark Plexiglas and consisted of four arms (50 cm long X 10 cm wide). The two enclosed arms had 40 cm high dark walls, whereas the two open arms had 0.5 cm high ledges. The maze was elevated to a height of 50 cm.

Drugs

U50-488 [(*trans*)-3,4-dichloro-*N*-methyl-N-[2-(1-pyrrolidinyl)-cyclo-hexyl] benzeneacetamide; Tocris Biosciences, Ellisville, MO] and nor-binaltorphimine (nor-BNI; Tocris Biosciences, Ellisville, MO) were dissolved in 0.9% saline solution for i.p. injections.

Behavioral testing procedure

Rats received intraperitoneal (i.p.) injections of nor-BNI (0 or 20 mg/kg) at least 24 hours prior to testing in the elevated plus-maze. This pretreatment period was chosen because previous research has shown that nor-BNI is most selective for kappa opioid receptors at 24 hours as opposed to earlier time points (Endoh, et al., 1992). On the day of testing, rats were further divided into groups receiving different doses of U-50,488 (0 or 10 mg/kg). Injections of U-50,488 took place 10 minutes prior to testing in the elevated plus-maze.

To examine anxiety-like behavior and general motor activity, rats were placed individually onto the center of the apparatus facing a closed arm. The time spent on and entries onto each arm were recorded automatically by photocell beams and monitored by a computer for 5 minutes. The percentage of time spent exploring the open arms has been proposed to relate inversely to an anxiety-like state, whereas the number of arm entries measures general locomotor activity (Pellow, et al., 1985).

Data Analysis

Data were analyzed using two-way analysis of variance (ANOVA) with dose of U-50,488 and dose of nor-BNI as between subjects factors. Further analysis was performed using the Tukey's *post hoc* test as warranted.

Results

There was a significant interaction between U-50,488 dose and nor-BNI dose on the percentage of time spent exploring the open arms of the elevated plus-maze ($F(1,44) = 6.10$, $p < 0.05$, Figure 1).

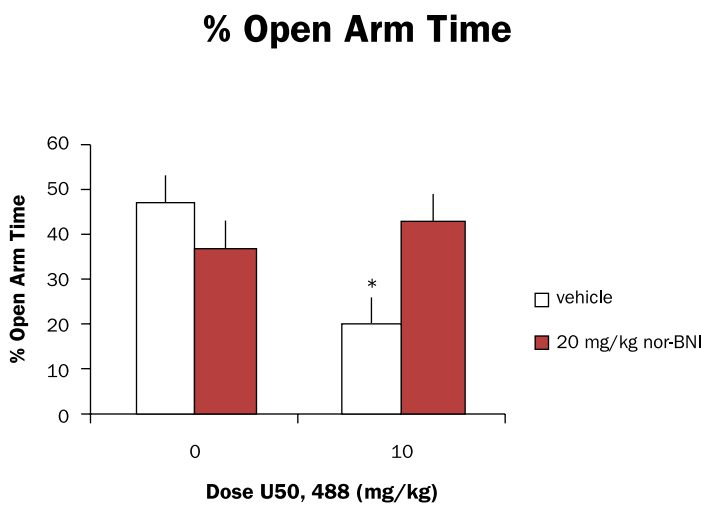

Figure 1. Effects of U-50,488 and nor-BNI on percent of time spent on the open arms of the elevated plus-maze. Rats were pretreated with nor-BNI or vehicle at least 24 hours prior to injection of U-50,488 (0 or 10 mg/kg). Injections of U-50,488 occurred 10 minutes before testing in the elevated plus-maze. Percent open arm time was calculated as the percent of open arm time / (open + closed) arm time. * $p < 0.05$ compared to vehicle, 0 mg/kg U-50,488 group, Tukey's test.

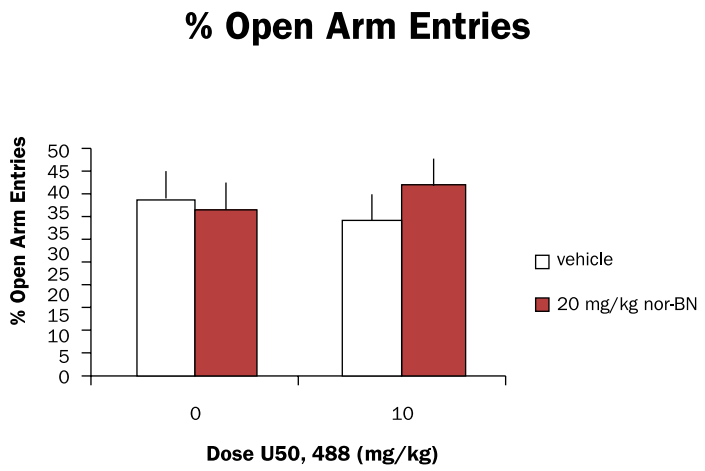

Figure 2. Effects of U-50,488 and nor-BNI on the percent of open arm entries in the elevated plus-maze. Rats were pretreated with nor-BNI or vehicle at least 24 hours prior to injection of U-50,488 (0 or 10 mg/kg). Injections of U-50,488 occurred 10 minutes before testing in the elevated plus-maze. Percent open arm entries was calculated as the number of open arm entries / (open + closed) arm entries. Neither U-50,488 or nor-BNI significantly altered the percentage of open arm entries. the elevated plus-maze. Percent open arm time was calculated as the percent of open arm time / (open + closed) arm time. * $p < 0.05$ compared to vehicle, 0 mg/kg U-50,488 group, Tukey's test.

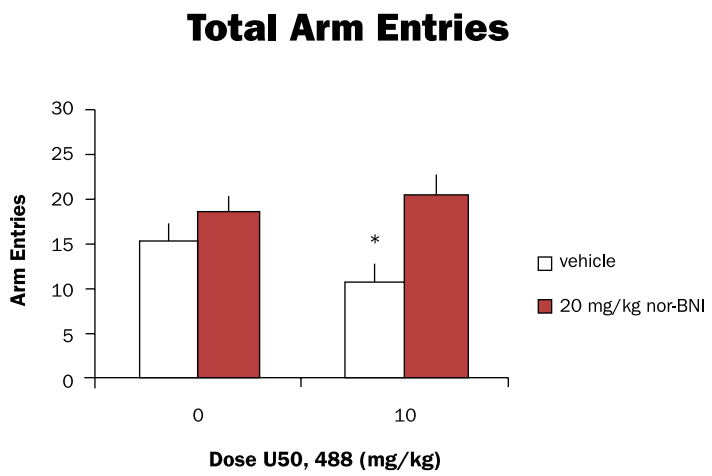

Figure 3. Effects of U-50,488 and nor-BNI on total number of arm entries. Rats were pretreated with nor-BNI or vehicle at least 24 hours prior to injection of U-50,488 (0 or 10 mg/kg). Injections of U-50,488 occurred 10 minutes before testing in the elevated plus-maze. * $p < 0.05$ compared to vehicle, 10 mg/kg U-50,488 group, Tukey's test.

Injections of 10 mg/kg U-50,488 significantly decreased percent open arm time compared to controls, an effect reversed by pretreatment with 20 mg/kg nor-BNI. No significant effects of U-50,488 or nor-BNI on the percentage of open arm entries were found (Figure 2).

A main effect of nor-BNI was found on the total number of arm entries ($F(1,44) = 11.73$, $p < 0.05$, Figure 3). Further analysis revealed that pretreatment with nor-BNI led to an increased number of arm entries in rats injected with U-50,488.

Discussion

The principle finding of this study is that the kappa opioid agonist U-50,488 decreased open arm exploration in the elevated plus-maze, and this effect was attenuated by the kappa opioid antagonist nor-BNI. These results suggest that stress-related behaviors are at least in part mediated by the activity of the endogenous kappa opioid system.

The time spent exploring the open arms of the elevated plus-maze can be considered a measure of emotionality. Rats naturally prefer the enclosed arms of the maze and spend more time in closed arms than in the open arms (Pellow, et al., 1985). Figure 1 illustrates a significant decrease in the amount of time spent on the open arms by rats treated with 10 m/kg of U-50,488 compared to those that received no treatment. This decrease in activity caused by U-50,488 indicates an increase in an anxiety-like state in the rat. We can conclude that the reversal of the effects by the kappa opioid antagonist nor-BNI is not due to a different opioid system, given that nor-BNI has been shown to exhibit maximum selectivity of kappa opioid receptors 24 hours after injection (Endoh, et al., 1992). This finding is consistent with previous studies that have found nor-BNI to reverse the effects of U-50,488 on the reinforcing efficacy of cocaine (Mclaughlin, et al., 2003, 2006a, 2006b; Negus, 2004).

The results failed to indicate a significant effect of U-50,488 or nor-BNI on percent of open arm entries. The percent of open arm entries is thought to be a measure of anxiety, given that anxiolytic compounds would be expected to increase the number of open arm entries without increasing the exploration of the enclosed arms (Pellow, et al., 1985). Although we did not achieve significant results in the percent of open arm entries, this measurement was not validated to correlate with other measurements of anxiety, like the number of arm entries or time spent on the open arms (Pellow, et al., 1985).

The total number of arm entries on the elevated plus-maze is a measurement of general locomotor activity of the rat (Pellow, et al., 1985). Previous research has suggested that altering kappa opioid activity can lead to general untoward motor effects (Negus, 2004). Exposure to a novel environment such as the elevated plus-maze is in itself anxiogenic and can result in an increase in time spent immobile (Lister, 1987). Typically, rats exposed to stress in a novel environment exhibit a decrease in motor activity and an increase in anxiety-related behavior, such as freezing or immobility (Pellow, et al., 1985). However, nor-BNI led to increased locomotion in rats injected with U-50,488, suggesting that this overall increase in activity may be a further indication of

the anxiolytic effects of nor-BNI. The results revealed a main effect of nor-BNI on the total number of arm entries in the elevated plus-maze (Figure 3). If the total number of arm entries is an indicator of anxiety, then an increase in number of arm entries would suggest a decrease in an anxiety-like state in the rat.

In summary, U-50,488 produced anxiogenic-like properties in rats tested on the elevated plus-maze. The effects of U-50,488 were reversed by the kappa opioid agonist nor-BNI. These results indicate that the kappa opioid system is involved in the regulation of anxiety-like behavior in animals. Understanding the role of the kappa opioid system in the regulation of behavioral stress responses may be crucial in understanding the mechanisms of stress-related disorders and provide insight into the potential therapeutic value of targeting the kappa opioid system for treatment of anxiety and other stress-linked disorders.

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