Kappa Opioid Receptor Activation Disrupts Prepulse Inhibition of the Acoustic Startle in Rats

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Background: *Compelling evidence indicates that kappa opioid receptor (KOR) agonists produce perceptual distortions in animals and humans, yet the mechanism of action and clinical relevance of such effects remain unclear. Since abnormalities in preattentional functions and informational processing are hypothesized to underlie psychotic disorders, the present study has been designed to assess the role of KOR on sensorimotor gating.*

Methods: *The effects of the selective KOR agonist U50488 were evaluated on the behavioral paradigm of prepulse inhibition (PPI) of the acoustic startle reflex (ASR).*

Results: *U50488 (1.25, 2.5, and 5 mg/kg, subcutaneous [SC]) induced a dose-dependent reduction of PPI, which was efficiently prevented by the selective KOR antagonist norbinaltorphimine (nor-BNI, 10 mg/kg, SC), as well as by the atypical antipsychotic clozapine (5, 8 mg/kg, intraperitoneal [IP]) but not by the typical antipsychotic haloperidol (.1, .5 mg/kg, IP). Conversely, nor-BNI (10 mg/kg, SC) failed to reverse the PPI disruption mediated by both apomorphine (.25 mg/kg, SC) and dizocilpine (.1 mg/kg, SC).*

Conclusions: *Our results support a pivotal role of KOR in the regulation of preattentional functions and sensorimotor gating, pointing to these receptors as a possible neurobiological substrate especially relevant to the clusters of psychosis unresponsive to typical antipsychotics.*

Key Words: Prepulse inhibition, kappa opioid receptors, U50488, clozapine

Converging lines of evidence support the concept that kappa opioid receptors (KOR) are involved not only in the regulation of analgesia, diuresis, and sedation (Dykstra et al 1987), but also in the modulation of cognitive functions (Hiramatsu and Kameyama 1998). However, while KOR involvement in mnemonic and learning processes has been documented, the role of these receptors in attentional and perceptual mechanisms is still elusive and controversial. The notion that KOR agonists produce perceptual distortions and hallucinatory effects (Pfeiffer et al 1986) was particularly challenge by evidence that the potent KOR agonist spiradoline possesses anti-psychotic-like properties in animal behavioral tests (Wadenberg 2003). Nevertheless, clinical investigations of novel, highly selective KOR agonists, such as enadoline, have revealed the ability of these compounds to induce depersonalization and sensory misrepresentations (Walsh et al 2001). In keeping with this evidence, the intriguing discovery that salvinorin A, the active ingredient of the hallucinogenic plant *Salvia divinorum,* is a highly selective KOR agonist (Roth et al 2002) supports the pathophysiological involvement of KOR in sensory impairment and hallucinatory phenomena, thus fostering new studies on the mechanism of action for such effects.

Scores of experiments have shown that one of the foremost neurophysiological endophenotypes for perceptual disorders is the impairment of sensorimotor gating. This preattentional function, aimed at ignoring irrelevant or background inputs and selecting salient stimuli for further processing, is critical for the organism to properly interact with a stimulus-laden environment (Braff and Light 2004). Deficits in informational filtering are conjectured to alter sensitivity to sensory stimuli, leading to perceptual overload and cognitive fragmentation (Braff 1999). The best validated paradigm to evaluate and measure sensorimotor gating is the prepulse inhibition (PPI) of the acoustic startle reflex (ASR), consisting in the reduction of the ASR that occurs when the eliciting stimulus is immediately preceded by a nonstartling prestimulus (Graham 1975). Prepulse inhibition is typically impaired in several neuropsychiatric disorders characterized by attentional or somatosensory deficits (for a review, see Braff et al 2001), such as schizophrenia spectrum disorders (Braff et al 1978; Geyer et al 1990), bipolar disorder (Saccuzzo and Braff 1986; Perry et al 2001), Huntington's disease (Swerdlow et al 1995), attention-deficit/hyperactivity disorder (Hawk et al 2003), obsessive-compulsive disorder (Swerdlow et al 1993), and Tourette syndrome (Castellanos et al 1996). Prepulse inhibition can be disrupted by many psychotomimetic drugs, such as N-methyl-D-asparate (NMDA) receptor antagonists and dopaminergic agonists (Geyer et al 2001). Besides, all antipsychotics prevent PPI disruption produced by dopaminergic agonists in an affinity- and potency-related fashion (Swerdlow et al 1994), while some atypical antipsychotics, unlike most classical antipsychotics, reduce PPI deficits mediated by NMDA receptor antagonists (Bakshi et al 1994; Bakshi and Geyer 1997). Taken together, these premises are in support of the heuristic validity of PPI in establishing preclinical distinctions between atypical and typical antipsychotics.

To the best of our knowledge, although interactions between opioid receptors and sensorimotor gating have been investigated (Swerdlow et al 1991), the role of KOR in PPI has not been studied to the present date. Thus, this study was undertaken to evaluate the ability of the potent KOR agonist U50488 (Vonvoigtlander et al 1983) to disrupt PPI, as well as the capacity of haloperidol (a typical antipsychotic) and clozapine (the prototype atypical antipsychotic) to reverse it. Besides, we tested the ability of the selective KOR antagonist norbinaltorphimine (nor-BNI) in preventing PPI disruption mediated by apomorphine (a

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dopaminergic agonist) and dizocilpine (an NMDA receptor antagonist). Some of the results displayed in this article have been already presented by our group during the 34th Annual Meeting of the Society for Neuroscience (Bortolato et al 2004).

Material and Methods

Animals

Two hundred seventy-eight male Sprague-Dawley albino rats (Harlan, Italy) weighing between 200 g and 300 g served as subjects in the present study. Rats were housed four per cage in the animal care quarters, maintained at a temperature of 22 ± 2°C on a reversed 12-hour light-dark cycle (lights went off at 7 AM and on at 7 PM). Food and water were available ad libitum, and each rat was handled for 5 minutes on each of the 5 days prior to experiment to minimize stress effects. All experimental procedures were approved by the local ethical committee and carried out in strict accordance with the Economic Community (EC) guidelines for care and use of experimental animals (86/609/European EC).

Drugs and Chemicals

The following drugs were used: U50488 (trans-(-)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]-benzeneacetamide hydrochloride), nor-BNI dihydrochloride, apomorphine hydrocloride, dizocilpine maleate, haloperidol, and clozapine. U50488 and nor-BNI was purchased from Tocris Cookson, United Kingdom. All other drugs were purchased from Sigma Aldrich, Italy. The U50488 and nor-BNI were dissolved in distilled water. Apomorphine was dissolved in .9% saline with .1 mg/mL ascorbic acid. Dizocilpine was dissolved in .9% saline. Haloperidol was dissolved in 10% acetic acid buffered with sodium hydroxide (NaOH) and diluted with saline, while clozapine was dissolved in a single drop of 1 N hydrogen chloride (HCl) and diluted with saline. The pH was adjusted to 7 using sodium bicarbonate ($NaHCO_3$). All drugs were weighed out as salts and administered in an injection volume of 1 mL/kg.

Apparatus

The apparatus for the detection of the startle reflexes (Med Associates, St Albans, Vermont) consisted of four standard cages placed in sound-attenuated chambers with fan ventilation. The cage was a Plexiglass cylinder of 9 cm diameter, mounted on a piezoelectric accelerometric platform connected to an analogue-digital converter. Background noise and acoustic bursts were conveyed by two separate speakers, placed at 7 cm beside the startle cage so as to produce a variation of sound within 1 dB across it. Both speakers and startle cages were connected to a main personal computer (PC), which detected and analyzed all chamber variables by means of custom software. Acoustic stimuli were monitored and balanced before each testing session through a digital sound level meter (Extech Instruments, Waltham, Massachusetts), while the mechanical response of each cage was set and equalized in all chambers via a 10-Hz spinner calibrator provided by Med Associates.

Procedure

Three days before the experiment, all rats went through a brief baseline startle session. Rats were exposed to a background noise of 70 dB, and after an acclimatization period of 5 minutes, they were presented with a randomized sequence of 12 40-millisecond bursts of 115 dB, interposed with three trials in which a 82-dB prestimulus preceded the same pulse by 100 milliseconds. Rats exhibiting baseline very high or very low startle values (more than two standard deviations above or below group mean values) were excluded from the study. Subsequently, treatment groups were established so that the average startle response and percent PPI of each group were equivalent in all groups. On the testing day, each rat was placed in a cage for a 5-minute acclimatization period with a 70-dB white noise background, which continued for the remainder of the session. Each session consisted of three consecutive sequences of trials (periods). Unlike the first and the third periods, during which rats were presented with only five pulse-alone trials of 115 dB, the second period consisted of a pseudorandom sequence of 50 trials, including 12 pulse-alone trials; 30 trials of pulse preceded by 73-dB, 76-dB, or 82-dB prepulses (10 for each level of prepulse loudness); and 8 no-stimulus trials, where only the background noise was delivered. Intertrial intervals (ITI) were selected randomly between 10 and 15 seconds. The startle session lasted about 30 minutes.

Experiments Description

This study consisted of seven experiments. In the first experiment (n = 32; 4 groups of animals), we evaluated the intrinsic effect of KOR activation on PPI by treating animals with either .9% saline or U50488 (1.25, 2.5, and 5 mg/kg, subcutaneous [SC]). Following the discovery that the KOR agonist dose-dependently reduces PPI, the second experiment (n = 64; 8 groups of animals) was carried out to verify whether nor-BNI (10 mg/kg, SC) prevents the PPI deficit induced by U50488 (5 mg/kg, SC). The third experiment (n = 48; 6 groups of rats) was designed to assess the ability of haloperidol (.1, .5 mg/kg intraperitoneal [IP]) to reverse U50488-induced PPI deficit (5 mg/kg SC). Since haloperidol exhibited no ability to reverse U50488-induced PPI disruption, the fourth experiment (n = 48; 6 groups) was conducted to verify that the same doses of the antipsychotic were effective against PPI deficit mediated by apomorphine (.25 mg/kg, SC). The fifth experiment (n = 48, 6 groups) aimed at evaluating the action of clozapine against U50488-mediated PPI deficit. Finally, the sixth and the seventh experiments (each one performed on 32 rats, divided into four treatment groups), tested the ability of nor-BNI (10 mg/kg, SC) to antagonize PPI deficits induced by apomorphine (.25 mg/kg SC) and dizocilpine (.1 mg/kg, SC), respectively. All experimental groups consisted of eight rats throughout the whole study. All substances were administered at a convenient time interval before experimental testing, compatible with their pharmacokinetic characteristics, so as to elicit their effects during the startle session. The time intervals for nor-BNI (30 minutes and 120 minutes before behavioral testing) were selected according to the study by Endoh et al (1992), who proved that this drug starts presenting its antagonistic effects on KOR at 30 minutes and becomes selective for KOR at 2 hours after its administration. Table 1 presents a synopsis of the whole study, detailing the time intervals for each treatment in all the experiments.

Data Analysis

For each animal, the mean startle amplitudes for the first and the second halves of the second period of the session (blocks, six pulse-alone trials each) were analyzed with a two-way or three-way analysis of variance (ANOVA), with pretreatment (where present) and treatment as between-subjects factors and blocks as repeated measures. The percent PPI was calculated with the following formula: 100 − [(mean startle amplitude for prepulse + pulse trials / mean startle amplitude for pulse-alone trials) × 100] and analyzed in multifactor ANOVAs (with specific