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Abstract
Sensorimortor gating and locomotion are behaviors that reflect pre-attentive sensory filtering and higher order, top-down, sensory processing, respectively. These processes are thought to affect either the perception of novelty in an environment (filtering) or cognition (higher order processing), salient features of models of altered states of consciousness (ASC). Drugs with highly selective receptor affinities that produce ASC can help to establish neural correlates, pathways, and mechanisms underlying ASC. Furthermore, screening for substances that selectively reverse drug-induced sensory processing departures is valuable for development of experimental antipsychotics. This study investigated the anomalous opioid sub-type, the kappa opioid (KA) system, within the two ASC models. Significant interaction and reversal effects between KA and the serotonin/2A (5-HT2A) system – the serotonin sub-type associated with classical psychedelics – were observed in three BPM measures. These measures showed that KA activation-induced effects could be reversed by 5-HT2A deactivation. These results suggest that KA could function as an atypical antipsychotic medications and/or as a screening tool for new antipsychotic medicines. The experimental work for this study comprised dose-response and reversal experiments with drugs that activate and deactivate kappa opioid and serotonin systems in the two behavioral models for the first time in mice.

Introduction

Behavioral models of psychosis are without parallel in their ability to elucidate higher order neural processing. Cell and receptor assays are necessarily restricted from taking systems approaches. The utilities of models like prepulse inhibition (PPI), a measure of preattentional sensorimotor gating, and exploratory behavior have been demonstrated in both basic and clinical pursuits. PPI is more accurately defined as the natural suppression of an extreme stimulus when a similar but milder stimulus precedes the extreme. Commonly, a strong acoustic noise is made in an enclosed area with the subject. The startle response, seen as a full body flinch in mice, is compared with successive startle responses from less intense auditory stimuli. PPI measures the ability of the subject to adapt and diminish their startle response. Within the PPI paradigm adjustments of PPI function are postulated to arise from striato-thalamo-cortical (CSTC) feedback loops by modulation within and between several neurotransmitter systems (Vollenweider and Geyer 2001.) PPI can be extrapolated as the ability to perform pre-attentive filtering of information within the CSTC feedback loop. PPI is thought to affect perception of the novelty of an environment or cognition. These features have applications in the characterization of psychoactive drug mechanisms and screening for atypical antipsychotics. Pharmacologic manipulation makes selective isolation of neural systems within and without the CSTC loops possible. Our understanding of schizophrenia has particularly benefited. Deficits for PPI are present in schizophrenic patients and may be restored by neruoleptic treatment (Braff et al. 1978). Experimental drugs that reverse PPI deficits are therefore potential candidates for antipsychotic medications. Drugs that disrupt PPI have been traditionally restricted to those that replicate the prototypical neurological states associated with schizophrenia. The most pervasive theories are associated with NMDA hypo activity, excess dopamine (DA), or excess serotonin (5-HT.) When induced pharmacologically (e.g. ketamine, amphetamine, and DOI, respectively) all drug types dose-dependently disrupt PPI and alter locomotor and exploratory behaviors in rodents (Peng et al. 1990; Geyer 1998; Bakshi et al. 1999) These effects may all be reversed by pretreatment with neuroleptics specific to those systems (Hamm A.; Weike A.; Bauer; Vaitl D.; Gallhofer B. 1995.) While the DA system is associated with typical neuroleptic action, only excess serotonergic activity in the 5-HT2A system and hypo-NMDA states are associated with atypical action. Atypical neuroleptic action is particularly sought after due to the absence of side effects from typical neuroleptics, such as tardive dyskinesia. Therefore, as the etiology of schizophrenia is multifaceted and incomplete, it is advantageous, both medically and theoretically, to seek out new routes to atypical antipsychotic action.

The kappa opioid (KA) system may represent such a novel system. The kappa receptor stands in stark contrast to its mu and delta counterparts in the opioid triad as it does not, by itself, produce euphoria or addiction. In animals KA stimulation produces generally adverse reactions, congruous with administration of the endogenous KA ligand dynorphin. Laboratory animals have been shown to not self administer the selective KA agonist U50488 (Tang and Collins 1985.) Until 1962 its activity in man had been elusive to the scientific community. After the western introduction of the plant Salvia Divinorum, which contains endogenously the selective KA agonist Salvinorin A, by Gordon Wasson, a body of reports on indigenous use and anecdotal bioassays has since emerged suggesting a diverse psychoactive profile. Effects that have been reported include dissociation, derealization, radical shifts in perception, (Walsh et al. 2001) as well as experiences contacting and communicating with non-human entities (Siebert.) A survey of Salvinorin A users reported 17.7% of respondents describing Salvinorin A experiences as being most similar to serotenergic hallucinogens (Baggott et al. 2004.) These effects suggest that KA agonists may represent a novel class of psychotomimetic drugs among other applications. If valid, it follows that their antagonists may have atypical neuroleptic profiles.

Dose dependent disruptions in acoustic PPI in rats have been reported through subcutaneous injection of the selective KA agonist Trans-U50488 (Bortolato et al. 2005.) This effect was reversed by pretreatment with the KA antagonist nor-binaltorphimine (Nor-BNI.) Nor-BNI failed to reverse PPI disruptions induced by the DA agonist apomorphine or the NMDA antagonist dizclopine. Similarly, the typical antipsychotic Haloperidol failed to reverse U50488 induced PPI disruption. Interestingly, though Bortolato and colleagues found that the atypical antipsychotic Clozapine was able to reverse U50488-induced PPI disruption. The finding that Clozapine, but not Haloperidol, prevented U50488-mediated PPI disruption suggests a potential role for the KA system in atypical neuroleptic action. If such a role exists, it is unclear at what level and through what intermediaries, if any, it functions. It is also unclear whether the role could represent a novel atypical antipsychotic mechanism. A serotonergic mechanism might account for the observed difference between haloperidol and clozapine in reversing U50488-induced PPI disruption, in view of the fact that the latter antipsychotic antagonizes 5-HT2 and that this specific characteristic has been suggested to explain some of its atypical properties and to distinguish it from the former (Meltzer and Nash 1991; Ichikawa and Meltzer 1999). Furthermore, contributions from the DA and NMDA systems were explicitly shown to not attenuate KA activity, as Nor-BNI did not reverse PPI disruption from either apomorphine or dizclopine, both of which are reversed by Haloperidol. In addition, it has also been shown in vivo that KA activation increases extracellular levels of serotonin (Gavend et al. 1987; Ho and Takemori 1989), suggesting an overall activation of 5-HT systems. It is also well established that 5HT releasers, as well as 5HT2 receptor agonists, disrupt PPI (Mansbach et al. 1989; Rigdon and Weatherspoon 1992; Sipes and Geyer 1994) There is also some human evidence, albeit mixed, that the nonselective opioid Naltrexone may provide relief from negative symptoms of schizophrenia (Marchesi G.F.; Franco G.; Santone G.; Cotani P.; Giordano A.; Chelli F. 1995.) Evidence that the behavioral syndrome induced by the KA agonist Cyclazocine is prevented by antagonists with affinity for both 5HT1 and 5HT2 receptors, such as Metergoline and Amitryptiline (Gavend et al 1986), indicates that KA activity may modulate other behaviors, such as locomotion and exploratory behavior.

Future genetic and behavioral studies involving the KA system – like those involving genetic knockouts or transfectants will invariably require dose- response data from a more genetically malleable species, like the mouse. As of the time of this writing no such information is available. Additionally, it has not been determined whether KA drugs can reverse 5-HT2A induced PPI disruptions. Such information may aid in expanding the repertoire for screening novel atypical antipsychotics. As most studies of 5-HT2A modulation of PPI and exploratory behavior have been examined in rats, to complement KA work in mice, it is necessary to replicate the findings in mice. Therefore, this study seeks to accomplish the following objectives: (1) to characterize dose-dependent effects of the highly selective KA agonist U50488 in both acoustic PPI and exploratory behavior paradigms for the first time in mice, as well as KA antagonist-induced reversal of such effects if they were to occur, and (2) to investigate whether secondary 5-HT2A modulation of the KA system occurs through reversal of U50488 effects on PPI or exploratory behavior through AC90179 pre-treatment.

Materials and Methods

Apparatuses

BPM: Measurement of exploratory and locomotor behavior took place in nine mouse behavior pattern monitors (BPM; San Diego Instruments, San Diego, CA). Each BPM consisted of a clear Plexiglas chamber with an opaque holeboard floor with the dimensions 30 x 60 cm. The test chamber had a total of 11 holes, with three along a mid and central axis of the floor, and eight distributed equally along the walls. Holes were 1.25 cm in diameter and 1.9 cm from the floor. The entirety of each chamber were separated and contained in a series of black tracked drawers, providing adequate shielding from external light and ambient noise. A two-dimensional array of 24 x 12 X-Y infrared photobeams (1cm from the floor and 2.5 cm apart from each other) were used in each chamber to track and record the subjects. Consecutively occurring coordinates were used to construct and visualize subject position, transitions, nosepokes, spatial d, time spent in center or corners or along walls, locomotor paths, and overall distance traveled. A third dimension above and along the Y-axis consisted of 16 photobeams, 6.9 cm from the floor and 1.9 cm apart served to record subject rearing. Recordings were made every 0.1 s. Subject position was defined across nine unequal regions (four corners, four walls and center; Geyer et al, 1986). For construction and detailed dimensionality please refer to the analogous rat BPM, as described previously (Geyer et al, 1986).

Startle and PPI: Startle magnitude and PPI testing were conducted in 8 commercially-produced startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA). Each chamber contained a clear Plexiglas cylinder with a 3.7 cm diameter atop an opaque platform. Chambers were ventilated and housed a loudspeaker to provide a background white noise (65 dB) continuously during the experiment and acoustic pre pulse and pulse stimuli. The entirety of each chamber was insulated from acoustic stimuli from neighboring chambers. Vibrations of whole-body startle responses in subjects were transduced to analog signals by a piezoelectric unit attached underneath the platform. The startle signals were then digitized and recorded by a standard computer. All startle chambers were calibrated monthly to ensure consistency between units and time.

Drugs, subjects, and treatment

trans-(-)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide-HCl (U50488) was procured from Sigma-Aldrich and certified in authenticity and purity. 2-(4-Methoxyphenyl)-N-(4-methyl-benzyl)-N-(1-methyl-piperidin-4-yl)-acetamide HCl (AC90179) was obtained from Acadia Pharmaceuticals. BPM and PPI dose-response studies examined the following doses: (U50488; 1.25, 2.5, 5.0, 10.0 mg/kg). The BPM U50488/AC90179 reversal study utilized the 1.0 mg/kg dose of AC90179 and 7.5, 10.0 mg/kg dosages of U50488. Both U50488 and AC90179 were dissolved in water. All drugs were administered intraperitoneally (i.p.). All injection volumes were prepared for 5 ml of solution/kg of body weight. Each solvent served as a control for the vehicle of its respective drug group. The male C57BL/6 mice used in this study were obtained from Harlan. Subjects were housed in an AAALAC-approved animal facility that meets Federal and State requirements for care and treatment of laboratory animals. Subjects were kept under a 12-hour reverse light/dark cycle with lights off at 8AM. The Institutional Animal Care and Use Committees approved all experimental protocols.

Experimental Overview

This paper reports on the findings from three experiments. One experiment (n=47; 5 groups of subjects) accomplishes objective (1) by examining U50488 effects on PPI. A second experiment (n=36; 6 groups of subjects) addressed objectives (1) and (2) by characterizing both U50488 BPM effects and their reversal using two U50488 dosages with AC90179 pretreatment.

Procedures

PPI: On the day of the experiment mice were brought into the lab and allowed to habituate for at least one hour in their home cage. Mice were then removed from their home cage, weighed, and separated into compartmentalized cages (four to a cage.) Mice were then injected i.p. and placed in the startle chamber. In all dose-response studies behavioral testing for both paradigms began 15 minutes after i.p. injection. Subjects were then transferred to the startle chambers.

The experimental session consisted of a 5 min acclimatization period to a 65 dB background noise (continuous throughout the session), followed by a variable prepulse intensity test. During the session, 5 trial types were presented: a 40 ms, 120 dB startle pulse (P120); a no stimulus trial (NOSTIM); and three 20 ms prepulse + pulse

combinations [68, 71 or 77 dB prepulses followed 100 ms later by a P120 stimulus (prepulse intensity test), or 77 dB prepulses followed 50, 100 or 200 ms later by a P120 stimulus (ISI test)]. Trial types were presented in a pseudorandom order (12 presentations of P120 trial, 10 presentations of each prepulse + pulse combination, 8 presentations of NOSTIM trial) with an average inter-trial interval (ITI) of 15 s. In addition, five P120 trials were presented at both the beginning (Block 1: to assess startle reactivity before appreciable habituation) and the end of the acoustic test session (Block 6). The 24 P120 trials in each test session were divided into four blocks of 6 trials (Blocks 2-5) to assess habituation across the session (Blocks 1-6). Mean startle magnitude for each trial type presentation, the dependent measure, was determined by averaging 65 one-ms readings taken from the onset of the startle P120 stimulus. All data were recorded and stored by a standard computer.

BPM: Mice were brought into the lab and allowed to habituate for at least one hour before each session. Only red light was used whenever mice were present. A white noise generator produced a background noise level for the entire preparation and execution of the experiment. Mice were weighed and separated as described in the PPI procedure section. For the reversal study, pretreatment (i.p) was thirty minutes prior to the session and treatment (i.p) occurred fifteen minutes before the session. Subjects were then transferred to the BPMs for a 60-min session. All data were recorded and stored by a standard computer.

Statistical analyses

All measurements were analyzed using analyses of variance (ANOVA) and covariance with repeated measures. Drug treatment was a between-subject variable and time or prepulse intensity were within-subject variables, where appropriate. Alpha level was set to 0.05. The only exception was in an external study of genetic interactions (relevant data from this study is presented in the discussion.) The Biomedical Data Programs (BMDP) statistical software (Statistical Solutions Inc., Saugus, MA) was used for all analyses. Post-hoc analysis was performed using Fischer's least significant difference test.

Results

Effects of U50488 on PPI

In a genetic study, external to the present, 10.0 mg/kg U50448 significantly disrupted PPI as a main effect [$F(1,13)=7.41$, $p<0.01$] and as an interaction with varying prepulse intensities [$F(2,26)=3.91, p<0.05$]. The disruption was most significant at a prepulse intensity of 4dB (figure 1, $p<0.01$, Fisher's LSD). Unexpectedly, these main drug effects were not seen, even at the 10.0-mg/kg dose, in the non-crossover U50488 dose-response experiment. Additionally, no effects of interactions with prepulse intensities or ISI blocks were observed.

Based on prior findings, 10.0 mg/kg and 1.0 mg/kg doses of U50488 and AC90179, respectively, were thought to be optimal for behavioral effects and subsequently used in the BPM U50488/AC90179 reversal experiment.

Effects of U50488 on center entries, transitions, and counts

U50488 time-dependently reduced measures of center entries, transitions and counts: [Center Entries: U50488 X Time, $F(10,150)=3.79$, $p<0.001$], [Transitions: U50488 X Time, $F(10,150)=3.5$, $p<0.001$], [Counts: U50488 X Time, $F(10,150)=3.82$, $p<0.001$]. Post hoc analysis revealed that all three measures maintained a $p<0.01$ significance level between drug and control during each time block (figure 4a,b,c.)

Effects of AC90179 pretreatment and U50488 treatment on center entries, transitions, and counts

AC90179 was able to reverse U50488 induced effects on center entries.[Center Entries: Reversal X Time: $F(10,150)=2.32$, $p<0.05$)]. Reversal of U50488 was observed in all time blocks but was found to only be significant during the third time block (figure 5b, $p<0.05$, Fisher's LSD). Likewise, reversal effects on counts were also seen [Counts: Reversal X Time, $F(10,150)=2.02$, $p<0.05$]. Count reversal effects were significant at a liberally adjusted alpha level ($a=0.1$) during the second and third time block (figure 5c, $p<0.1$, $p=0.1095$, respectively). There was a distinct, though non-significant, reversal of U50488-induced transition effects throughout the entire testing period. The pretreatment utilized the 1.0 mg/kg dose of AC90179 as this dose was not shown to statistically differ from control.

Discussion

The objectives addressed in this study are as follows: (1) to characterize effects of U50488, in both acoustic PPI and exploratory behavior paradigms and (2) examine whether the effects of U50488 may be reversed through pretreatment with AC90179.

There is an apparent contradiction stemming from the opposing results of the two U50488 PPI experiments. Although strain differences have been show to significantly alter results, the C57 strain of mice was used in both the initial pilot and as the backcrossed strain for the external genetic experiments. A remaining confounding variable may be the age of the subjects; the subject's in the pilot were considerably older than the genetic control subjects. It has been reported that with age the C57 strain are prone to hearing loss (Ouagazzal et al. 2006) As the PPI stimuli were acoustic, hearing loss would clearly diminish the accuracy of the PPI measure. Age was only a concern in the pilot U50488 animals. All other subjects were of an unquestionably suitable age.

The findings from the U50488 crossover experiment provide further evidence, complementing findings from Bortolato et al. (2005) in rats, for the existence of kappa opioid modulation of sensorimortor gating in mice. A robust disruption of PPI can be seen at all pre-pulse intensities. As the initial pilot study did not find any effects at all, it is especially advisable to replicate the findings from both this study and those of Bortolato et al. (2005).

Unfortunately, as the crossover experiment was not designed to address whether the disruption could be restored by AC90179, the original question of secondary 5-HT2A modulations of kappa opioid effects on PPI is still unknown. The replication study should be sure to include such tests. If the attenuated effects of DOI and AC90179 in mice relative indicate a cross species trend for PPI and BPM models, it would be comparatively worthwhile to reproduce Bortalatos findings in rats.

AC90179's ability to reverse U50488 effects on center entries, transitions, and counts provide positive findings for some reversal modulation in the BPM experiments (see results above). In all of the measures for which reversal was found, there was a trend for reversal to be greater as the session proceeded. This phenomenon may not be due to the onset period of the drugs as U50488 effects did not consistently increase in any of the measures with time. Secondary neural pathways may instead be responsible for the delay in modulation. Future studies should be careful to examine this potential relationship.

Future studies may benefit from utilizing Salvinorin A as a preferred KA as it is higher in potency and more selective than U50488. Salvinorin A is also preferred as the psychotropic effects in humans have been documented, while U50488 remains un-assayed in humans. U50488's kappa opioid agonist activity does not ensure psychoactivity in humans, as exemplified by the case of lisuride, which is a 5-HT2A agonist that is not psychoactive due to secondary signaling (Gonzalez-Maeso et al. 2007) The compound TRK-820 is a highly selective kappa opioid agonist without psychotropic effect in humans. Agonists likely preferentially stabilize one of three known isoforms of the kappa opioid receptor (Bi et al. 2003)**.** These subtypes are likely responsible for differences in psychoactivity. While U50488 does not substitute for TRK-820 in discrimination studies (Mori et al**.** 2004), there is currently insufficient research to confirm that U50488 is comparable in its isoform affinity or psychotropic effects to Salvinorin A. Furthermore, as the population using Salvinorin A outside of formal research grows, it becomes increasingly necessary to understand its health implications. Ultimately**,** human studies will be needed to verify these phenomena as some cross**-**species differences have been shown to exist (Vollenweider et al. 1999.)

In conclusion, the finding that U50488 is capable of PPI disruption in mice may imply a potential for a kappa opioid model of psychosis; as the disruption is likely reversed by a kappa opioid antagonist (i.e. Nor-BNI) this also suggests a role for Kappa opioid antagonists as a novel class of antipsychotics. Additionally, AC90179's ability to reverse U50488 BPM measures indicate further evidence for the hypothesis that Clozapine's, but not haloperidol's, ability to reverse U50488 PPI effects is indirectly due to Clozapine's 5-HT2A profile.

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