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Iptakalim Preferentially Decreases Nicotine-induced Hyperlocomotion in Phencyclidine-sensitized Rats: A Potential Dual Action against Nicotine Addiction and Psychosis

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Objective: Iptakalim is a putative ATP–sensitive potassium (K_{ATP}) channel opener. It is also a novel nicotinic acetylcholine receptor (nAChR) blocker and can antagonize nicotine–induced increase in dopamine release in the nucleus accumbens. Our recent work also shows that iptakalim exhibits a clozapine–like atypical antipsychotic profile, indicating that iptakalim may possess a dual action against nicotine addiction and schizophrenia.

Methods: The present study examined the potential therapeutic effects of iptakalim on nicotine use in schizophrenia. We created an animal model of comorbidity of nicotine addiction and schizophrenia by injecting male Sprague–Dawley rats with nicotine (0.40 mg/kg, subcutaneously [sc]) or saline, in combination with phencyclidine (PCP, 3.0 mg/kg, sc) or saline daily for 14 consecutive days.

Results: During the PCP/nicotine sensitization phase, PCP and nicotine independently increased motor activity over time. PCP also disrupted prepulse inhibition (PPI) of acoustic startle response. Acute nicotine treatment attenuated the PCP–induced hyperlocomotion and PCP–induced disruption of PPI, whereas repeated nicotine treatment potentiated these effects. Importantly, pretreatment with iptakalim (10–20 mg/kg, intraperitoneally) reduced nicotine–induced hyperlocomotion in a dose–dependent fashion. This reduction effect was highly selective: it was more effective in rats previously sensitized to the combination of PCP and nicotine, but less effective in rats sensitized to saline, nicotine alone or PCP alone.

Conclusion: To the extent that the combined nicotine and PCP sensitization mimics comorbid nicotine addiction in schizophrenia, the preferential inhibitory effect of iptakalim on nicotine–induced hyperlocomotion suggests that iptakalim may be a potential useful drug for the treatment nicotine abuse in schizophrenia.

KEY WORDS: Iptakalim; Phencyclidine; Nicotine; Motor activity; Prepulse inhibition; Rats.

INTRODUCTION

Clinical observations suggest that there is a serious comorbidity of nicotine use and schizophrenia.[1] Schizophrenic patients also tend to be heavy smokers, defined as those who smoke more than one and a half packs a day.[2] They are also less likely to attempt quitting[3] and have a higher risk of developing smoking-related illnesses, such as lung cancer and cardiovascular disease.[4] Thus, a drug that could reduce nicotine use while maintaining antipsychotic efficacy in the treatment of schizophrenia

would be highly desirable. Unfortunately, all currently used antipsychotics except clozapine,[5] are ineffective in this regard. Since clozapine use is limited because of its risk in causing life-threatening agranulocytosis, there is an urgent need to search and evaluate other effective pharmacological treatments with novel mechanisms of action.

Iptakalim is a novel adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channel activator that was originally developed for the treatment of hypertension.[6] It can pass easily through the blood-brain-barrier and shows a potential neuroprotective effect for neurons and astrocytes against ischaemia, trauma and neurotoxins.[7,8] Iptakalim is also a novel nicotinic acetylcholine receptor (nAChR) blocker and is shown to antagonize nicotine-induced increase in dopamine release in the nucleus accumbens.[8,9] Our recent work shows that iptakalim also possesses a clozapine-like antipsychotic activity.[10] We found that iptakalim is effective in reducing amphetamine- and phencycli-

dine-induced hyperlocomotion as well as selectively disrupting conditioned avoidance responding, three well-established behavioral indices of antipsychotic activity.[11,12] Similar to clozapine, iptakalim preferentially increases c-Fos expression in the medial prefrontal cortex, nucleus accumbens and lateral septum.[10]

Based on these findings, we hypothesized that iptakalim may be a drug with a dual action against nicotine use/abuse and psychosis. The objective of the present study was to provide a needed behavioral assessment of the potential therapeutic effects of iptakalim on nicotine use in schizophrenia using a preclinical animal approach. To establish an animal model of comorbidity of nicotine abuse in schizophrenia, we sensitized rats to phencyclidine (PCP, 3.0 mg/kg, subcutaneously [sc]), in combination with nicotine (0.4 mg/kg, sc) for 14 consecutive days. The PCP sensitization was chosen as a model of schizophrenia because it mimics many aspects of the illness, such as hypoglutamatergic activity,[13] prepulse inhibition (PPI) deficit,[14] persistent deficits in cognition,[15] social interaction impairment,[16] and increased depressive-like behavior during the forced swim test.[17] The effects of repeated PCP and/or nicotine treatment, and their interactions were assessed in a series of locomotor activity and PPI tests. The effects of iptakalim on nicotine-induced hyperlocomotion was taken as a measure of efficacy of iptakalim to ameliorate the psychomotor stimulant and/or rewarding effects of nicotine.[18,19]

METHODS

Animals

Male Sprague-Dawley rats (226-260 g upon arrival; Charles River, Portage, MI, USA) were housed two per cage, in 48.3×26.7×20.3 cm transparent polycarbonate cages under 12-hour light/dark conditions (light on between 6:00 am and 6:00 pm). Room temperature was maintained at 22±1°C with a relative humidity of 45-60%. Food and water were available *ad libitum*. Animals were allowed at least one week of habituation to the animal facility before they were used in experiments. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

Motor Activity Monitoring Apparatus

Each of the sixteen activity boxes (48.3×26.7×20.3 cm transparent polycarbonate cages) was equipped with a row of 6 photocell beams (7.8 cm between two adjacent photobeams) placed 3.2 cm above the floor of the cage.

Motor activity was recorded as the number of the photocell beam breaks by a custom-built program run on a computer.

PPI of acoustic startle response apparatus

The PPI test was performed using six Startle Monitor Systems (Kinder Scientific, Julian, CA, USA) controlled by a computer. Each system was housed in a compact sound attenuation cabinet (35.56 cm wide×27.62 cm deep×49.53 cm high). A speaker (diameter: 11 cm) mounted on the cabinet's ceiling was used to generate acoustic stimuli (70 dB-120 dB). The startle response was measured by a piezoelectric sensing platform on the floor, which was calibrated daily. During testing, rats were placed in a rectangular box made of transparent Plexiglas (19 cm wide×9.8 cm deep×14.6 cm high) with an adjustable ceiling positioned atop the box, providing only limited restraint while prohibiting ambulation.

Drugs

Doses of nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA) were expressed as free base dissolved in 0.9% saline. The nicotine solution was brought to a pH of 7.0±0.2 with a dilute NaOH solution. We chose the 0.4 mg/kg nicotine dose because it is a commonly used dose in a number of behavioral tasks, such as motor activity,[20] conditioned place preference,[21,22] and working memory.[23] The injection solutions of phencyclidine hydrochloride (a gift from NIDA Chemical Synthesis and Drug Supply Program) and iptakalim hydrochloride (IPT, 99.9%, a gift provided by the Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences of China to Dr. Hu) were obtained by mixing drugs with 0.9% saline. Nicotine and PCP were administered subcutaneously at a volume of 1.0 ml/kg. Iptakalim was administered intraperitoneally [ip], also at a volume of 1.0 ml/kg .[10]

Experimental Procedure

1. Habituation and baseline PPI test (Days 1-2)

Sixty-four male Sprague-Dawley rats (2 batches of 32) were used. On the first day, all rats were habituated to both the locomotor activity (LMA) chambers and PPI chambers for 30 minutes and 20 minutes respectively. On the second day, rats were habituated to the LMA chambers, followed by a baseline of PPI test. The PPI procedure was adapted from Culm and Hammer.[24] Each session lasted approximately 18 minutes and began with a 5 minutes period of 70 dB background noise (which continued throu-

ghout the duration of the session) followed by four different trial types: PULSE ALONE trials and three types of PREPULSE+PULSE trials, which consisted of a 20 mimiseconds (ms) 73, 76, or 82 dB prepulse (3, 6, and 12 dB above background) followed 100 ms later by a 120 dB pulse. Each session was divided into 4 blocks. Blocks 1 and 4 were identical, each consisting of 4 PULSE ALONE trials. Blocks 2 and 3 were also identical and each consisted of 8 PULSE ALONE trials and 5 of each PREPULSE+PULSE trial type. A total of 54 trials were presented during each test session. Trials within each block were presented in a pseudorandom order and were separated by a variable intertrial interval averaging 15 seconds (ranging from 9-21 seconds). Startle magnitude was defined as the maximum force (measured in Newtons) applied by the rat to the startle apparatus recorded over a period of 100 ms beginning at the onset of the pulse stimulus. Startle responses from testing Blocks 2 and 3 were used to calculate percent PPI (%PPI) for each acoustic prepulse trial type:

$$\%PPI = 100 - \left[\left(\frac{\text{Average startle response to PREPULSE+PULSE trials}}{\text{Average statle response to PULSE ALONE trials}} \right) \times 100 \right]$$

2. Sensitization induction by repeated PCP and/or nicotine treatment (Days 3−16)

After two days of habituation, the rats were randomly assigned to one of four groups (saline+saline [SAL+SAL], saline+nicotine [SAL+NIC], PCP+saline [PCP+SAL], and PCP+nicotine [PCP+NIC], n=16/group). Every day for 14 days, the rats were brought into the lab in their home cages, allowed to habituate for 30 minutes, and then injected with either PCP (3.0 mg/kg, sc) or saline. This dose of PCP was chosen because it has been shown to produce a robust behavioral sensitization and disruption of learning.[25-27] Five minutes later a second injection of either nicotine (0.4 mg/kg, sc) or saline was administered. This sensitization phase lasted for 14 consecutive days. On Days 1, 6, 11 and 13, the rats were also placed in the LMA chambers 4 minutes after the second injection. LMA was recorded for 90 minutes. On Days 3, 8, 12 and 14, the rats were placed in the PPI chambers 5 minutes after the second injection and their PPI performance was tested. On days when rats were not tested, rats were injected in their home cages only.

3. PCP challenge test (Day 23) and iptakalim test on nicotine−induced hyperlocomotion in the same LMA boxes (Day 25)

Seven days after the last PCP/nicotine injections, all rats received a challenge dose of PCP (1.5 mg/kg, sc) and were tested in the LMA chambers to assess the PCP-induced psychomotor sensitization for 90 minutes. Two days later, the effects of iptakalim treatment on nicotine-induced hyperlocomotion were assessed. Each of the original four groups was further divided into three subgroups: saline (n=8), iptakalim (10 mg/kg, ip, n=4), or iptakalim (20 mg/kg, ip, n=4). At the beginning of the test, rats were first injected with iptakalim or saline and 5 minutes later received a challenge dose of nicotine (0.2 mg/kg, sc). Four minutes after receiving nicotine, rats were placed in the LMA chambers and motor activity was recorded for 90 minutes.

4. Iptakalim test on nicotine−induced hyperlocomotion in a novel environment (Day 29)

Four days after the first iptakalim test in the familiar LMA boxes, the effects of iptakalim treatment on nicotine-induced increase in motor activity were assessed in a novel environment to assess the generality of the iptakalim effect on nicotine-induced hyperlocomotion. Rats were tested in 8 two-compartment chambers (64 cm wide×30 cm high×24 cm deep) housed in a ventilated, sound-insulated isolation cubicle (96.52 cm wide×35.56 cm deep×63.5 cm high; Med Associates, St. Albans, VT, USA). The motor activity was detected by a set of 16 photobeams (ENV-256-8P; Med Associates, St. Albans, VT, USA) affixed at the bottom of the box (3.5 cm above the grid floor) as number of beam breaks. The groups were the same as in the first iptakalim test. At the beginning of the test, rats received the first injection of either saline, iptakalim (10 mg/kg, ip), or iptakalim (20 mg/kg, ip), followed by a challenge dose of nicotine (0.2 mg/kg, sc) 5 minutes later. The motor activity test started immediately after the nicotine injection and lasted for 30 minutes.

Data Analysis

All data were expressed as mean+SEM. Motor activity from the PCP/nicotine sensitization induction phase were analyzed using repeated-measures analyses of variance (ANOVAs) with a within-subjects factor of *test day* and between-subjects factors of *PCP* and *nicotine* treatment. Percent PPI data at the three prepulse intensity levels (e.g., 73, 76 and 82 dB) on the 4 drug testing days were presented separately and analyzed similarly with the ex-

ception that the *prepulse level* was added as a within-subjects factor. One-way ANOVA followed by *post hoc* least significant difference (LSD) tests was used to determine group differences on specific test days. Motor activity data from the PCP challenge test were analyzed by a two-way ANOVA with *PCP* and *nicotine* as two between-subjects factors. Iptakalim data from the two nicotine challenge days were analyzed separately by a three-way ANOVA with *iptakalim*, *PCP* and *nicotine* as three between-subjects factors.

RESULTS

LMA during the Sensitization Induction Phase

Data for one rat in the SAL+SAL group on the last motor activity test was missing due to a technical error and were not entered in the analysis. As can be seen in Fig. 1, both PCP and nicotine treatment progressively increased motor activity throughout the four test days. Repeated measures ANOVA revealed a main effect of *PCP*, $F(1, 59)=332.68$, $p < 0.001$, and *nicotine*, $F(1, 59)=12.88$, $p=0.001$, but no *PCP×nicotine* interaction, $F(1, 59)=1.7879$, $p=0.187$. Both PCP and nicotine also induced behavioral sensitization as evidenced by the significant *PCP×test day*, $F(3, 177)=11.104$, $p < 0.001$, and *nicotine×test day* interaction, $F(3, 177)=16.980$, $p < 0.001$. One-way ANOVA followed by the *post hoc* LSD tests on each test day showed that both PCP groups showed greater numbers of activity
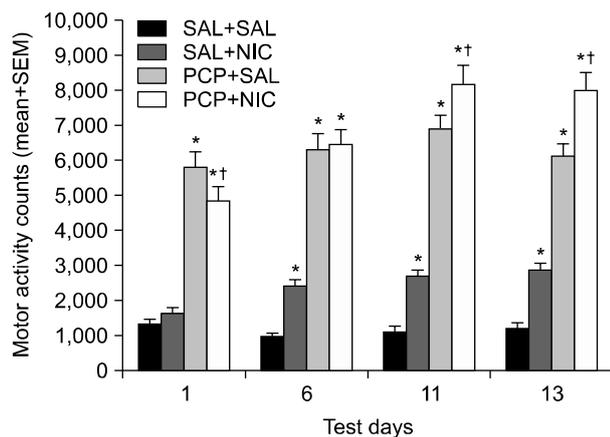

Fig. 1. Effects of repeated administration of SAL, PCP (3.0 mg/kg, sc), and NIC (0.4 mg/kg, sc) on locomotor activity (mean+SEM). A total of 64 rats were randomly assigned to one of four groups (n=16/group): SAL+SAL, SAL+NIC, PCP+SAL, and PCP+NIC and tested on day 1, 6, 11 and 13 of drug treatment. *p<0.05 significantly different from the SAL+SAL control group; †p<0.05 significantly different between two PCP groups (NIC vs. SAL). SAL, saline; PCP, phencyclidine hydrochloride; NIC, nicotine; SEM, standard error of the mean.

than the SAL+SAL group on all test days, $p < 0.001$. Nicotine treatment alone also significantly increased motor activity, as evidenced by the significant group differences between the SAL+NIC and SAL+SAL groups on the last three days of testing, $p < 0.011$. Interestingly, on the first day of testing, the PCP+NIC group had significantly *lower* motor activity than the PCP+SAL group, $p=0.034$, suggesting an acute inhibitory effect of nicotine on PCP-induced hyperlocomotion. This effect was in contrast to the potentiated effect seen in the latter part of testing. The PCP+NIC group showed significantly *higher* motor activity than the PCP+SAL group on the last two testing days, $p=0.009$ and $p < 0.001$, respectively.

PPI during the Sensitization Induction Phase

Data for two rats in the PCP+SAL group and two in the SAL+NIC groups were missing due to a technical error and were not analyzed. Fig. 2 shows PPI performance of the four groups of rats tested throughout the sensitization phase. PCP 3.0 mg/kg disrupted PPI at all three levels. Analysis of PPI data from the 4 drug test days revealed a main effect of *PCP*, $F(1, 56)=120.647$, $p < 0.001$, but no main effect of *nicotine*, $F(1, 56)=1.821$, $p=0.183$, nor *PCP×nicotine* interaction, $F(1, 56)=0.086$, $p=0.770$. The effect of *prepulse level* was significant, $F(2, 112)=512.147$, $p < 0.001$, but the effect of *test day* was not, $F(3, 168)=2.085$, $p=0.104$. There were also significant interactions between *PCP×test day*, $F(3, 168)=3.012$, $p=0.032$, *PCP×prepulse level*, $F(2, 112)=17.611$, $p < 0.001$, and *nicotine×test day*, $F(3, 168)=3.768$, $p=0.012$, suggesting that the effects of PCP and nicotine on PPI performance varied across the test days.

At the 73 dB prepulse level, one-way ANOVA followed by *post hoc* LSD tests revealed that the two PCP groups had significantly *lower* PPIs than the two SAL groups on all test days, $p < 0.031$ except on the second PPI drug test day, the two PCP groups did not differ from the SAL+SAL group, $p > 0.084$. At the 76 dB prepulse level, once again, the two PCP groups had significantly *lower* PPIs than the other two groups on all test days, $p < 0.012$. Interestingly, the PCP+NIC group had even lower PPI on the last test day than the PCP+SAL group, $p=0.041$, suggesting that repeated nicotine treatment worsened the PPI-disruptive effect of PCP. At the 82 dB prepulse level, the two PCP groups also exhibited significantly lower PPIs than the two SAL groups, $p < 0.002$. In addition, on the first PPI drug test day, the PCP+NIC group had significantly *higher* PPI on than the PCP+SAL group, $p=0.039$. However, this effect was reversed on the second drug test day,
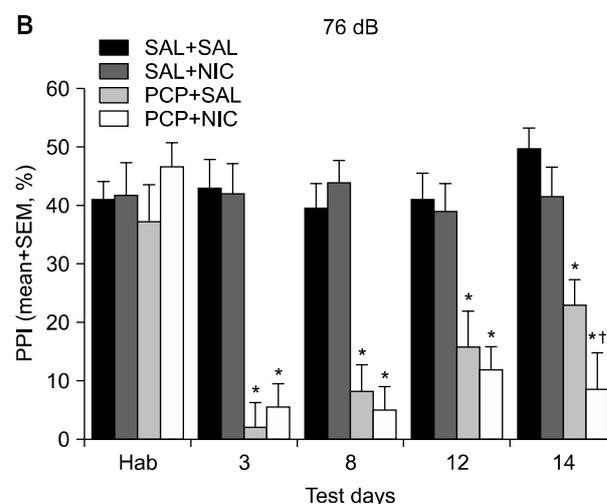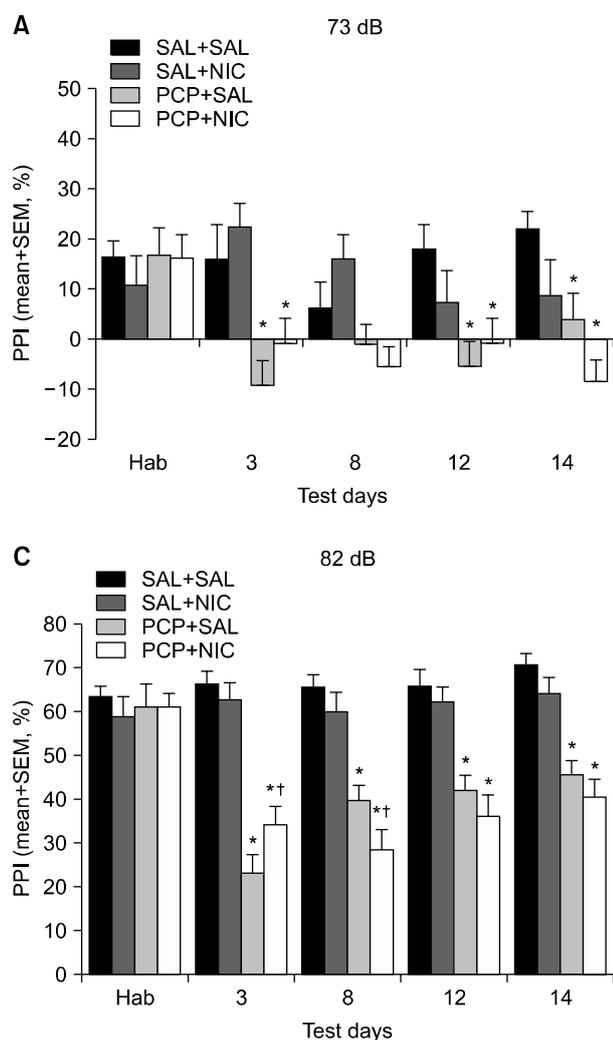
A

B

C

Fig. 2. Effects of repeated administration of SAL, PCP (3.0 mg/kg, sc), and NIC (0.4 mg/kg, sc) on PPI of acoustic startle response at the 73 dB prepulse level (A), 76 dB prepulse level (B) and 82 dB prepulse level (C) (mean+SEM). A total of 64 rats were randomly assigned to one of four groups (n=16/group): SAL+SAL, SAL+NIC, PCP+SAL, and PCP+NIC and tested on day 1, 6, 11 and 13 of drug treatment. The PPI tests were conducted on the second habituation day (Hab), and on day 3, 8, 12 and 14 of drug treatment. *$p<0.05$ significantly different from the SAL+SAL control group; $^{†}p<0.05$ significantly different between two PCP groups (NIC vs. SAL). SAL, saline; PCP, phencyclidine; NIC, nicotine; PPI, prepulse inhibition; SEM, standard error of the mean.

$p=0.037$. Inspection of the overall PPI patterns in the PCP+SAL and PCP+NIC groups across the four test days supports the observation that acute nicotine treatment tended to attenuate the PCP effect, while repeated treatment tended to worsen it. This pattern of nicotine effect on PCP in the PPI model is consistent with its effect on PCP-induced hyperlocomotion.

PCP Sensitization Assessment

Fig. 3 shows motor activity in the four groups of rats challenged with PCP 1.5 mg/kg. Two-way ANOVA revealed a main effect of *PCP*, $F(1, 60)=12.242$, $p=0.001$, confirming the PCP-induced behavioral sensitization. The nicotine effect was not significant, $F(1, 60)=2.572$, $p=0.114$, indicating that repeated nicotine treatment did not have a long-term impact on PCP sensitization. One-way ANOVA followed by *post hoc* LSD tested on the four groups showed that both PCP+SAL and PCP+NIC
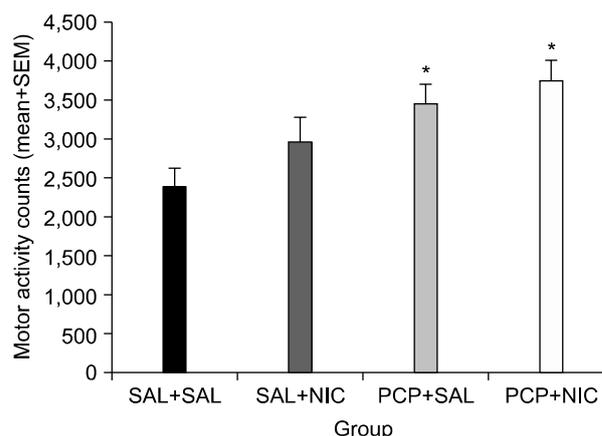
Fig. 3. Effects of repeated administration of SAL, PCP (3.0 mg/kg, sc), and NIC (0.4 mg/kg, sc) on the expression of PCP locomotor sensitization during the PCP challenge test in the four groups of rats (n=16/group) that were previously treated with SAL+SAL, SAL+NIC, PCP+SAL, and PCP+NIC. Each rat was injected with PCP 1.5 mg/kg, sc, and locomotor activity (mean+SEM) was recorded for 90 minutes. *$p<0.05$ significantly different from the SAL+SAL control group. SAL, saline; PCP, phencyclidine; NIC, nicotine; SEM, standard error of the mean; SEM, standard error of the mean.

groups showed significantly greater motor activity than the SAL+SAL group, $p < 0.007$.

Iptakalim test on nicotine-induced hyperlocomotion in the same LMA boxes

To examine the time course of the iptakalim effect on nicotine-induced increase in motor activity, we analyzed motor activity in each of 30 minutes blocks (a total of 3 blocks) separately (Fig. 4). During the first 30 minutes of testing, three-way ANOVA revealed a main effect of *nicotine*, $F(1, 52)=39.138$, $p < 0.001$, suggesting that rats previously treated with nicotine expressed a nicotine behavioral sensitization. There was also a significant *PCP×nicotine* interaction, $F(1, 52)=12.716$, $p=0.001$, indicating that the nicotine sensitization effect was modulated by PCP sensitization. One-way ANOVA followed by *post hoc* LSD tests revealed that rats previously sensitized with SAL+NIC had significantly higher motor activity in comparison to the SAL+SAL/SAL group, $p < 0.012$. Iptakalim at both doses (10 and 20 mg/kg) did not decrease the nicotine-induced hyperlocomotion or the expression of nicotine sensitization. Rats previously sensitized with PCP+NIC also showed significantly higher motor activity than the SAL+SAL/SAL rats when tested under saline and iptakalim 20 mg/kg conditions, $p < 0.006$, but not under iptakalim 10 mg/kg condition, $p=0.207$, suggesting that iptakalim 10 mg/kg reduced the nicotine-induced hyperlocomotion to some extent. This observation was also supported by the finding that the PCP+NIC/IPT 10 group exhibited significantly *lower* motor activity than the PCP+NIC/SAL group, $p=0.045$. In addition, rats previously sensitized with PCP+SAL showed significantly higher motor activity than the SAL+SAL/SAL rats, $p=0.009$ when they were tested under saline condition, indicating a possible cross-sensitization from PCP to nicotine. This significant effect disappeared under iptakalim 10 and 20 mg/kg conditions, $p > 0.236$. The overall pattern of the results indicates that iptakalim was more effective in decreasing nicotine-induced increase in motor activity in PCP sensitized rats than saline rats.

During the second 30 minutes of testing, there was still a main effect of *nicotine*, $F(1, 52)=15.821$, $p < 0.001$, a main effect of *PCP*, $F(1, 52)=6.957$, $p=0.011$, and a significant *PCP×nicotine* interaction, $F(1, 52)=8.568$, $p=0.005$. One-way ANOVA followed by *post hoc* LSD tests revealed that only rats previously sensitized with SAL+NIC showed significantly higher motor activity in comparison to the SAL+SAL/SAL group, $p < 0.011$. Comparing each of the three subgroups sensitized to

SAL+NIC to the corresponding subgroups sensitized to PCP+NIC revealed a significant between-group difference under the saline and iptakalim 10 test conditions, $p < 0.021$, suggesting that co-administration of PCP with nicotine attenuated the nicotine sensitization effect during this test period.

In the last 30 minutes, three-way ANOVA revealed a main effect of *nicotine*, $F(1, 52)=11.258$, $p=0.001$, a significant *PCP×nicotine* interaction, $F(1, 52)= 6.645$, $p=0.013$, and *iptakalim×nicotine* interaction, $F(2, 52)=5.634$, $p=0.006$. Group comparisons revealed that in comparison to the SAL+SAL/SAL group, rats previously sensitized with SAL+NIC showed significantly higher motor activity when tested under saline and iptakalim 20 mg/kg conditions, $p < 0.041$. Interestingly, iptakalim 20 mg/kg potentiated nicotine-induced hyperlocomotion in rats previously sensitized to PCP+NIC. Rats in the PCP+NIC/IPT20 group showed significantly higher motor activity than the rats in the SAL+SAL/SAL group, $p=0.030$. They also had significantly higher activity than those sensitized to PCP+NIC but tested under saline or iptakalim 10 mg/kg, $p < 0.018$.

Iptakalim Test on Nicotine-induced Hyperlocomotion in a Novel Environment

In this 30 minutes test, once again, iptakalim showed a selective inhibitory effect on nicotine-induced hyperlocomotion in rats that were previously sensitized to PCP+NIC. It had little effect in rats previously sensitized to other combinations of drugs. Three-way ANOVA revealed a main effect of *nicotine*, $F(1, 52)=7.613$, $p=0.008$, a significant *PCP×nicotine* interaction, $F(1, 52)=8.020$, $p=0.007$. The main effect of *iptakalim* was also significant, $F(2, 52)=5.041$, $p=0.010$. *Post hoc* LSD tests indicated that *iptakalim* 10 mg/kg differed significantly from saline, $p=0.004$; whereas iptakalim 20 mg/kg differed marginally from the saline, $p=0.051$. Group comparisons using one-way ANOVA followed by *post hoc* LSD tests revealed that in comparison to the SAL+SAL/SAL group, rats previously sensitized with SAL+NIC showed significantly higher motor activity when tested under saline and iptakalim 20 mg/kg conditions, $p < 0.024$. This significant group difference was abolished by iptakalim 10 mg/kg, $p=0.802$. Rats previously sensitized with PCP+NIC also showed significantly higher motor activity than the SAL+SAL/SAL rats when tested under saline condition, $p=0.029$, but not under iptakalim 10 or 20 mg/kg condition, $p > 0.598$, suggesting that iptakalim decreased the nicotine-induced increase in motor activity.
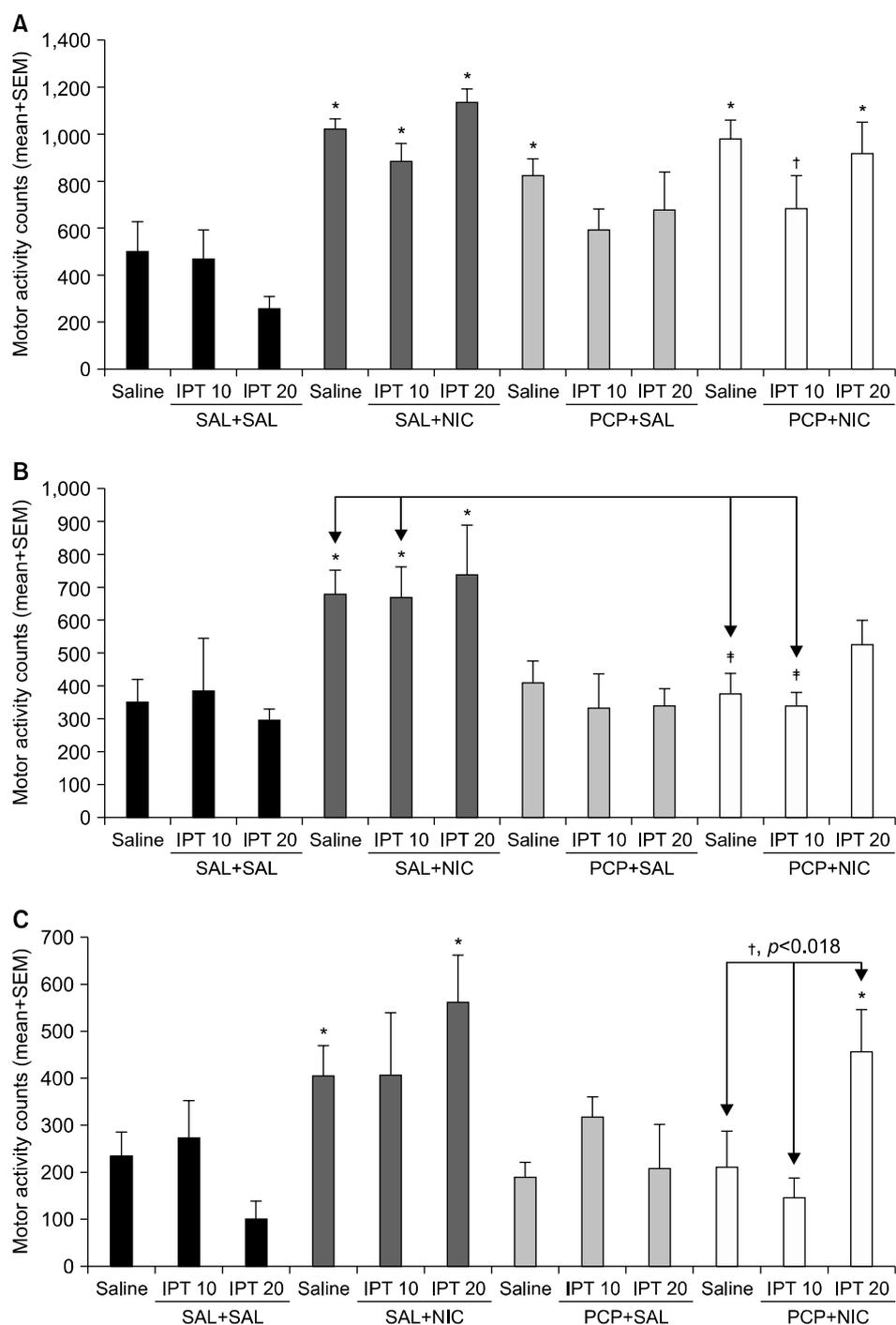
Fig. 4. Effects of iptakalim treatment (IPT; 0, 10 and 20 mg/kg, ip) on the expression of nicotine locomotor sensitization in the familiar environment during the nicotine challenge test in the four groups of rats (n=16/group) that were previously treated with SAL+SAL, SAL+NIC, PCP+SAL, and PCP+NIC. Each rat was injected with nicotine 0.2 mg/kg, sc and locomotor activity was recorded for 90 minutes. Locomotor activity (mean+SEM) was separated into (A) first 30 minutes, (B) second 30 minutes, and (C) last 30 minutes. *$p<0.05$ significantly different from the SAL+SAL/SAL control group; [†]$p<0.05$ significantly different between an iptakalim group and its SAL control; [†]$p<0.05$ significantly different between one of the SAL+NIC groups and one of the PCP+NIC groups. SAL, saline; PCP, phencyclidine; NIC, nicotine; SEM, standard error of the mean.

The inhibitory effect of iptakalim was confirmed by the finding that the PCP+NIC/IPT 10 and PCP+NIC/IPT 20 groups showed significantly lower motor activity than the PCP+NIC/SAL group, $p < 0.029$.

DISCUSSION

This present study investigated the potential therapeutic effect of iptakalim on nicotine use in schizophrenia. We created a rat model of comorbid nicotine addiction in schizophrenia using a sensitization regimen in which rats were being repeatedly injected with a combination of nicotine (0.40 mg/kg, sc) and PCP (3.0 mg/kg, sc) for 14 consecutive days. We confirmed the nicotine and PCP sensitization during the induction phase (i.e., 14 days of repeated drug injection) as well as in the expression phase (i.e., in the challenge tests). Because behavioral sensitization induced by a drug of abuse is often thought to reflect the addictive potential of the drug,[28] the confirmation of nicotine sensitization indicates that certain features of nicotine addiction had been successfully modeled. PCP sensitization is often regarded as a faithful model of schizophrenia.[11,14,29,30] The demonstration of persistent PPI deficits induced by PCP sensitization suggests that PCP-sensitized rats exhibited a schizophrenia-like cognitive deficit, as PPI deficit is one of hallmark features associated with schizophrenia and is thought to contribute to its various symptoms (e.g., sensory flooding and cognitive fragmentation).[31,32] Overall, the 14-day sensitization regimen used in this study seems valid in capturing certain aspects of behavioral symptoms associated with nicotine use in schizophrenia. This allowed us to examine the effects of iptakalim on nicotine-induced hyperlocomotion as a way to assess its therapeutic potential against nicotine addiction in schizophrenia. We found that pretreatment of iptakalim significantly and dose-dependently reduced nicotine-induced hyperlocomotion. This effect lasted for approximately 30 minutes and was found in two distinct testing environments. The iptakalim's attenuation effect on nicotine-induced hyperlocomotion was also selective as it was more effective in rats previously sensitized to the combination of PCP and nicotine, but less effective in non-sensitized rats or those sensitized to nicotine alone or PCP alone.

In the present study, we found two interesting interactions between nicotine and PCP. *Acute* treatment of nicotine reduced PCP-induced hyperlocomotion and PCP-nduced disruption of PPI, whereas *repeated* treatment of nicotine potentiated both effects of PCP (Figs. 1, 2).

Previous work on mice also found that acute nicotine ameliorates the PCP-induced deficit of PPI,[33,34] and decreases PCP-induced hyperactivity[35] in some strains of mice. This acute reversal effect of nicotine may be related to the well-documented acute motor suppressive effect of nicotine[20,36,37] and is likely mediated by the reversal of the blocking effect of PCP on nAchRs.[35] We are not aware of any work that has reported the potentiated effect of repeated nicotine treatment on repeated PCP-induced hyperlocomotion and PPI impairment. Result on the motor activity indicates a clear additive effect between these two drugs as they share a common psychomotor stimulation effect. The PPI result is not so easy to understand because nicotine alone did not significantly disrupt PPI (Fig. 2), and chronic nicotine treatment actually *increases* baseline PPI in Sprague-Dawley rats.[38,39] Mechanistically, both nicotine and PCP have a common action in increasing extracellular dopamine release in the nucleus accumbens.[40-43] Nicotine does so by stimulating nicotinic cholinergic receptors in the ventral tegmental area and the nucleus accumbens,[44] and PCP does so by primarily blocking glutamatergic N-methyl-D-aspartate (NMDA) receptors.[45-47] Thus repeated nicotine treatment may potentiate PCP-induced hyperlocomotion by enhancing PCP-induced increase in dopamine release in the nucleus accumbens.[48] Alternatively, because both VTA and the nucleus accumbens receive direct glutamatergic input from the prefrontal cortex and action of PCP in this area has been implicated in the regulation of its psychomotor effect,[49] it is also possible that repeated nicotine potentiates PCP-induced hyperlocomotion by enhancing PCP's activity in the prefrontal cortex, e.g., potentiating PCP-induced increase in glutamate release in the prefrontal cortex.[13,50,51] In addition to increasing dopamine release in the prefrontal cortex,[49,52] both nicotine and PCP also cause an excessive glutamate release in the prefrontal cortex[50,51] and PCP's noncompetitive antagonist actions on NMDA receptors and non-NMDA receptors are thought to mediate its PPI-disruptive effect.[14] Therefore, it is possible that the potentiated disruption of PCP-induced PPI deficit by repeated nicotine treatment is due to the additive effect of both drugs' actions on the glutamate system in the prefrontal cortex.

The main finding of the present study was that iptakalim dose-dependently reduced nicotine-induced hyperlocomotion. This finding is to some extent expected as evidence from electrophysiological and microdialysis studies shows that iptakalim selectively inhibits $\alpha 4 \beta 2$-nAChRs,[53] and antagonizes nicotine-induced increase in
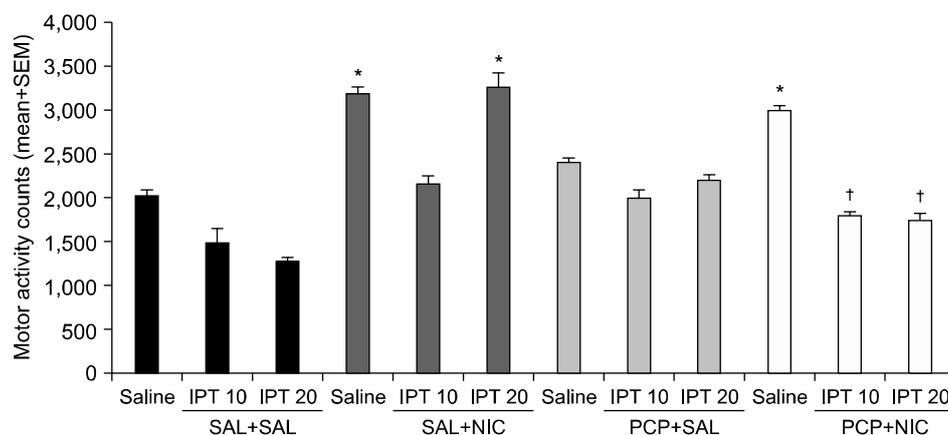
Fig. 5. Effects of iptakalim treatment (IPT; 0, 10 and 20 mg/kg, ip) on the expression of nicotine locomotor sensitization in the novel environment during the nicotine challenge test in the four groups of rats (n=16/group) that were previously treated with SAL+SAL, SAL+NIC, PCP+SAL, and PCP+NIC. Each rat was injected with nicotine 0.2 mg/kg, sc and locomotor activity (mean+SEM) was recorded for 30 minutes in a new testing apparatus different from the one used in the inducton of NIC/PCP sensitization. *$p<0.05$ significantly different from the SAL+SAL/SAL control group; †$p<0.05$ significantly different between an iptakalim group and its saline control. SAL, saline; PCP, phencyclidine; NIC, nicotine.

dopamine release in the accumbens.[9] Our finding provides additional *behavioral* support for iptakalim's inhibitory action on nicotinic receptors. The novel finding is its preferential effect in PCP+NIC sensitized rats: iptakalim was more efficacious in decreasing nicotine-induced increase in motor activity in PCP sensitized rats than in non-sensitized rats or rats sensitized to nicotine alone or PCP alone. During the first 30 minutes of testing in a familiar environment, iptakalim 10 mg/kg only significantly decreased nicotine-induced hyperlocomotion in rats previously sensitized to the combination of PCP and nicotine, but not in rats previously sensitized to saline, nicotine alone or PCP alone. The same pattern was found during the second test in a novel environment. Because nicotine challenge induced as much higher motor activity in rats sensitized to nicotine alone as in those sensitized to both nicotine and PCP (Figs. 4A, 5), the preferential effect of iptakalim could not be attributed to different levels of motor response to nicotine challenge.

Because iptakalim is also effective in reducing PCP-induced LMA,[10] and attenuates excessive dopamine and glutamate release in the nucleus accumbens induced by nicotine and cocaine,[9,54,55] this preferential action of iptakalim may be mediated by its preferential action against excessive dopamine and glutamate release induced by nicotine. As discussed in our previous study,[10] iptakalim might do so by opening the K_{ATP} channels (the putative target sites of iptakalim). Opening of K_{ATP} channels is known to result in hyperpolarization of the cell membrane and limitation of Ca^{2+} influx, blocking subsequent neurotoxic biochemical cascades[56] and reducing neurotransmitter

release.[7] Alternatively, recent receptor binding tests indicate that iptakalim may have an inhibitory action against sigma 2 and 1 receptors (with 97% and 81% inhibition rates as measured in the radioligand binding assays, thanks to National Institute of Mental Health Psychoactive Drug Screening Program). Because both nicotine and PCP effects have been suggested to be mediated at least partially by sigma receptors,[57-59] iptakalim may thus decrease nicotine-induced hyperlcomotion by inhibiting sigma receptors, as well as antagonizing nicotinic receptors. In addition, iptakalim may also antagonize β_1 adrenergic receptor (86% inhibition rate), μ opioid receptor (75% inhibition rate) as well as $\varDelta$ opioid receptor (67% inhibition rate). This multi-receptor binding profile of iptakalim may explain the unexpected result that iptakalim 20 mg/kg seems less effective than iptakalim 10 mg/kg in decreasing nicotine-induced hyperlocmotion. We speculate that with the increase of dose, iptakalim may act on more types of receptors, raising the possibility of diluting its main action against nicotine. Obviously, this possibility can be addressed in future studies using selective agonists again these potential targets of iptakalim.

We should point out several limitations with the present study. First, we only used the LMA model to identify the anti-nicotine property of iptakalim. Other models such as nicotine-induced conditioned place preference or intravenous nicotine self-administration should also be used. Second, we did not examine the molecular mechanisms responsible for iptakalim effects in this model. As mentioned above, iptakalim may target multiple receptors or molecules to achieve its inhibitory effect on nicotine.

Third, we have not compared iptakalim with other nicotinic receptor antagonists such as mecamylamine in this study, thus how it may differ from other drugs as pharmacotherapy for nicotine addiction remain to be determined. Finally, our chosen animal model could not capture many aspects of schizophrenia; therefore the clinical implication of our findings awaits further investigation. Nevertheless, the present study provides important preliminary evidence that iptakalim may possess a dual action against nicotine and PCP. This property is extremely important given that managing substance abuse including nicotine addiction is suggested to be a key target of schizophrenia treatment.[60] If iptakalim's therapeutic potentials are confirmed, it would contribute to broader understanding of the neuropathophysiology of co-morbidity of nicotine addiction in schizophrenia.

■ **Acknowledgments**

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