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Effects of Nicotine on Spontaneous and Amphetamine-induced Motor Behaviors: The Differences between Nicotine Tolerant and Nontolerant Rats

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Abstract

This study was designed to investigate the effect of nicotine on spontaneous and amphetamine (AMP)-induced motor activity in rats with or without tolerance to nicotine. Tolerance were induced by treating the rats with nicotine (0.3 mg/kg, S.C.) 2hr before receiving challenge doses. Motor activity including locomotion and stereotypy was monitored automatically by videocamera every 15 min for 90 min. The results indicated that: (1) Nicotine increased spontaneous locomotion at 0.15 or 0.3 mg/kg (S.C.) in naive rats and at 0.6 mg/kg in tolerant rats. Nicotine also slightly affected AMP-induced locomotion at 0.15, 0.3 or 0.6 mg/kg in both naive and tolerant rats, and (2) Nicotine increased spontaneous stereotypy at 0.3 or 0.6 mg/kg in naive rats only and showed no effect on AMP-induced stereotypy in either naive or tolerant rats. Comparing the results of spontaneous motor activity between naive and tolerant rats, it revealed behavioral desensitization in locomotion at low doses (0.15 or 0.3 mg/kg) and hyperlocomotion at higher dose (0.6 mg/kg), and revealed desensitization in stereotypy at 0.3 or 0.6 mg/kg. Moreover, nicotine had temporary (at 0-15 min interval) attenuating effect on AMP-induced locomotion in naive rats but showed a potentiating effect on AMP-induced locomotion in tolerant rats. The present results indicated that acute tolerance modified the action of nicotine in both spontaneous and AMP-induced locomotion, while stereotypy was changed only in the spontaneous one but not in the AMP-induced one. In other words, acute tolerance modified the effect of nicotine on locomotion-related dopaminergic system, and it affected the stereotypy-related dopaminergic system only in the spontaneous one but not in the AMP-induced one.

Key Words: nicotine, amphetamine, acute tolerance, stereotypy, locomotion

Introduction

Effects of nicotine on spontaneous locomotor activity are complex. Nicotine can either stimulate or depress such activity, depending on the treated dosage, the duration of drug injection, previous exposure to nicotine or not (1, 6, 28), and the experience in the behavioral testing situation (11). Some of the stimulatory effects of nicotine on locomotor activity are mediated through the activation of nicotinic receptors located in the neuron of nigrostriatal dopaminergic system (NDS) or mesolimbic dopaminergic system (MDS). Acute administration of nicotine can stimulate the release of mesolimbic dopamine (DA) and a higher concentration is required to increase nigrostriatal DA release (2, 4, 23, 25).

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Reports regarding the effect of nicotine on stereotypic activity reveal an increase effect (18) or not (13).

Amphetamine (AMP) is an indirect dopaminergic agent. In rats, acute injection of a low dose of AMP increases the locomotion. This locomotor activation is mediated by an increase in synaptic dopamine levels in the nucleus accumbens (19, 20). At the higher dosage (> 3 mg/kg), AMP induces stereotyped behaviors. It is believed that AMP-induced hyperlocomotion is mediated through an action on MDS and that AMP-induced stereotypy is mediated through an influence on NDS (9, 15, 16, 24, 26). Because the motor effect of nicotine and AMP are both mediated through the dopaminergic system, it has been suggested that nicotine pretreatment may influence AMP-induced locomotion in nontolerant (6) or in chronic tolerant (7) rats. It is of special interest to know whether nicotine pretreatment may influence spontaneous or AMP-induced motor activity, in other words, whether nicotine pretreatment may modify dopaminergic system, in acute tolerant rats.

Acute tolerance to nicotine is easily induced by an I.P. administration of nicotine (27, 28). After administration, acute tolerance to the depressant action of a second dose becomes maximal after 2hr and wears off after about 8hr, in comparison with the behavioral response of naive rats to nicotine. In this study, we examined the effects of nicotine on spontaneous and AMP-induced motor activity in naive and in acute tolerant rats and demonstrated that acute tolerance could modify dopaminergic system and influence the action of nicotine.

Materials and Methods

Animals

Female Wistar rats weighing 200-280g were used. They were housed in a room with a 12-hr light-dark cycle (light on 06:00-18:00) at 24±2 °C and 6 0±10% relative humidity. Rat chow and water were available *ad lib*. For the consistency of hormonal effects on motor activity, only rats demonstrating two or more consecutive 4-day estrous cycle and being at the stage of diestrus were used. An i mals w ere adapted to the open field (50×50×35 cm) 60-90 min each day for at least one week prior to the formal experiment.

Apparatus

The activity monitor Video Path Analyzer (VPA, Model E61-21, Coulbourn Instruments) measured motor activity. The VPA followed the animal's path with the TV camera and analyzed a variety of behaviors including motor activity with the analyzer. The VPA system could separately record: total distance traveled, stereotypic movement, and other activities. The analyzer was fully operational alone. The analyzer took the camera picture, established an X-Y coordinate from 50×50 cm edge floor which was divided into 16×16 sets of coordinates, generated the cursor block to superimpose over the animal's image on the video monitor, and logged the coordinate data.

Treatment

Eighty female Wistar rats were divided equally into the naive and the tolerant batches. Each batch was divided into two equal groups: the N group and the NA group. In N group, rats were treated with nicotine and were subdivided into 4 subgroups (N_0, N_1, N_2, and N_3) which were given saline and 3 different doses of nicotine (0.15, 0.3 and 0.6 mg/kg), respectively. In NA group, rats were subdivided into 4 subgroups (NA_0, NA_1, NA_2, and NA_3) which were given saline and 3 different doses of nicotine (0.15, 0.3, and 0.6 mg/kg), respectively. They were given a dose of amphetamine (1 mg/kg) 5-min after the nicotine treatment. Nicotine was S.C. administered in the dorsal surface of the neck and amphetamine was I.P. administered. All drugs were administered in a volume of 1 ml per kg body weight. Both (-)-nicotine and d-amphetamine sulphate were purchased from Sigma Chemical Co. (St. Louis, MD USA).

Induction of Acute Tolerance to Nicotine

Rats were pretreated with nicotine (0.3 mg/kg free base, S.C.) or saline 2hr before receiving one of 3 different challenge doses of nicotine (0.15, 0.3, and 0.6 mg/kg, S.C.). The dose and the delay were optimal for demonstrating acute tolerance according to a previous study (27).

Motor Activity Recording

The experiment was carried out between 8:00 and 12:00 a.m. Each animal was allowed 30 minutes for adapting to the open field before drug administration. Motor activity was monitored for 90 min immediately after the animal had been placed into the open field. The Analyzer defined two measurements of motor activity: (a) total distance (of locomotion) - total distance traveled by the animal in cm, and (b) stereotypic movement - entering and reentering a given set of coordinates without going further than 1/16th of the distance of the 50×50 cm field before reentry, such as the movement of grooming or sniffing. The tallies in each category were collapsed into six 15-min intervals during 90-min period.

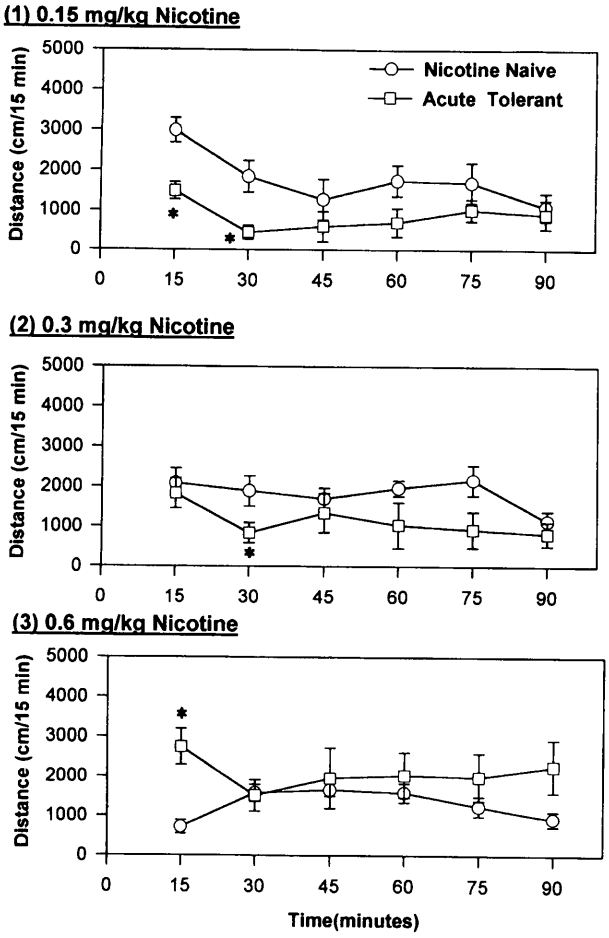

Fig. 1. Time-course for the development of acute tolerance. Tolerant rats were pretreated with 0.3 mg/kg nicotine and naive rats were pretreated with saline 2h before challenge with 0.15 mg/kg of nicotine at panel 1, with 0.3 mg/kg of nicotine at panel 2, and with 0.6 mg/kg of nicotine at panel 3. Each data point is represented as Mean±SEM. (*p < 0.05, paired t-test)

Statistical Analysis

Motor activity was analyzed by one-way analysis of variance (ANOVA) and further by Dunnetteës multiple comparisons against a single control group. Paired t-tests were used to compare the difference between two subgroups. A probability of 0.05 or less was accepted as significant statistically. All data were expressed as Mean±SEM of each group.

Results

Development of Acute Tolerance to Nicotine

Figure 1 presents the results of experiment regarding the time course for the development of acute tolerance. A challenge dose of nicotine at 0.15 or 0.3 mg/kg showed a decrease (reduced response) of locomotor activity at every 15-min intervals for 90 min in acute tolerant rats when comparing with that of naive rats. A challenge dose at 0.6 mg/kg elicited an increase of locomotion in acute tolerant rats.

Total Distance of Locomotion

Figure 2 depicts the dose-response curves for the effect of nicotine on spontaneous locomotion (N group) or AMP-induced locomotion (NA group) in naive and acute tolerant rats. The results indicate that tolerant rats show less sensitive to nicotine at lower dose and hyperlocomotion at higher dose in NA group. In naive rats, statistical significance is obtained in N group by one-way ANOVA ($F(3, 16)=3.97$, $p < 0.05$), but not in NA group. Take further comparison by Dunnette's method, it reveals that two subgroups (N_1 and N_2) shown significance when comparing to control subgroup (p , 0.05). In tolerant rats, significance is obtained in N group ($F(3, 16)=4.62$, $p<0.05$), but not in NA group. Dunnette's multiple comparisons indicate that N_3 subgroup shows a significance from the control subgroup ($p < 0.05$).

Figure 3 depicts the time course for the effect of nicotine on locomotion. The results indicate that tolerant rats show desensitization to nicotine. The desensitizing effects occurred at every intervals of low dose (especially at 0-15 min interval at the dose

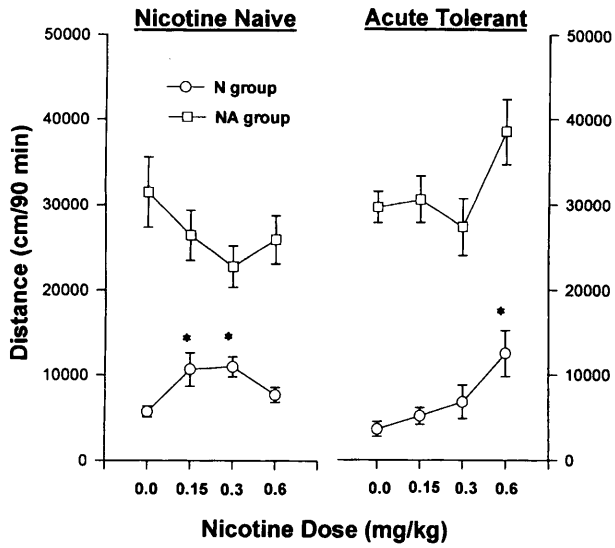

Fig. 2. Effect of nicotine on spontaneous locomotion (N group) or amphetamine-induced locomotion (NA group) in naive and acute tolerant rats. Nicotine was S.C. administered and amphetamine (1mg/kg) was I.P. administered. Each data point is represented as Mean±SEM. Comparisons were made using one-way ANOVA followed by Dunnette's test. *Represents a significant difference comparing with its control subgroup. N_0: control; N_1: nicotine 0.15 mg/kg; N_2: nicotine 0.3 mg/kg; N_3: nicotine 0.6 mg/kg; NA_0: AMP 1 mg/kg; NA_1: AMP 1 mg/kg + nicotine 0.15 mg/kg; NA_2: AMP 1 mg/kg + nicotine 0.3 mg/kg; NA_3: AMP 1 mg/kg + nicotine 0.6 mg/kg. (*p < 0.05)

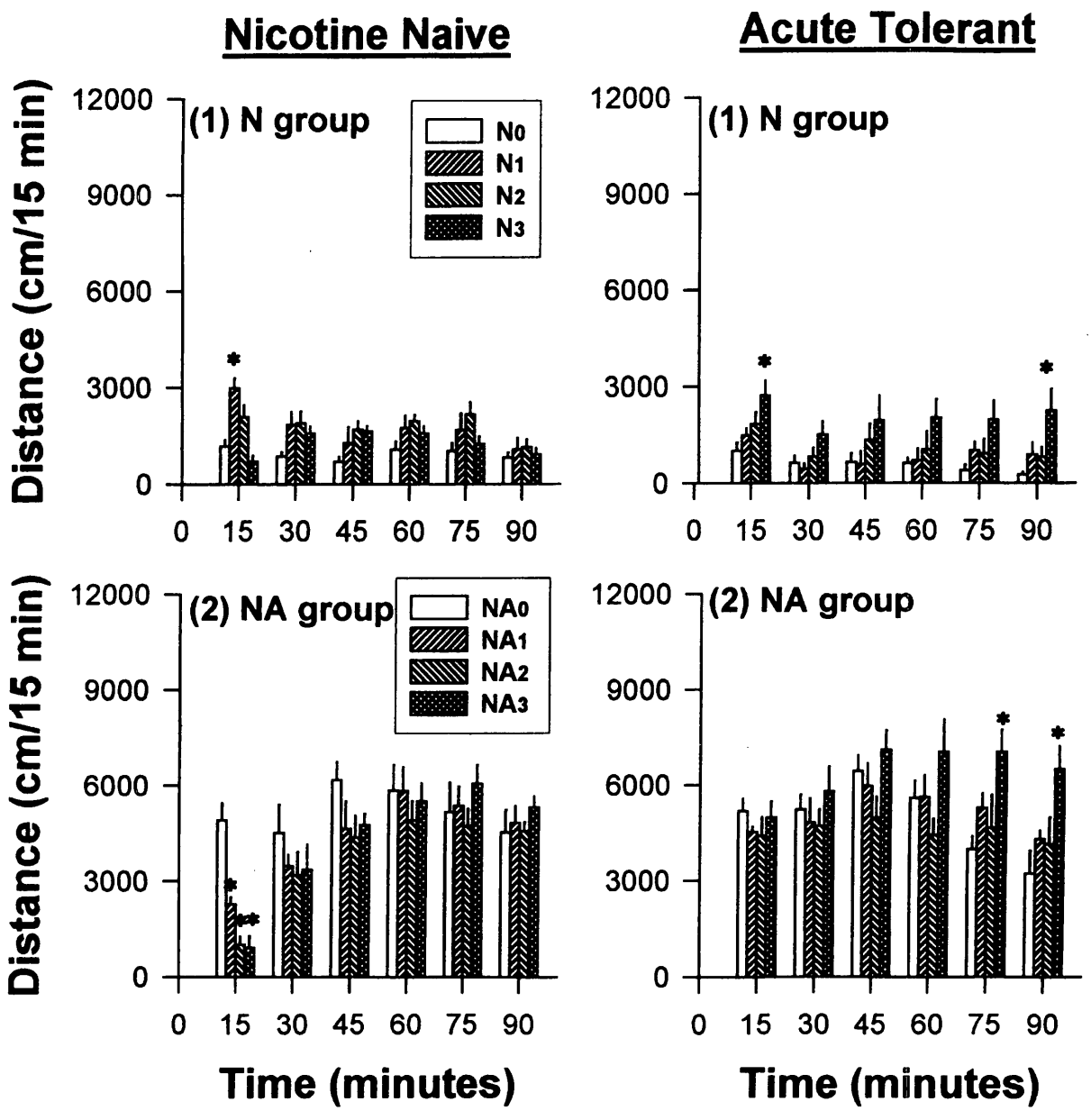

Fig. 3. Time-course for the effect of nicotine on locomotion. Nicotine was S.C. administered and amphetamine was I.P. administered. Locomotion was measured immediately after amphetamine administration and was measured at 15-min intervals for 90 min. Each data point is represented as Mean±SEM. Comparisons were made using one-way ANOVA followed by Dunnette's test. *Represents a significant difference comparing with its control subgroup. (*$p < 0.05$)

of 0.15 mg/kg) and the hyperlocomotor effect occurred at every intervals of high dose (especially at 0-15 and 75-90 min intervals at the dose of 0.6 mg/kg). In naive rats, significance is obtained at two 0-15 min intervals not only in N group ($F(3, 16)=13.7$, $p < 0.0001$) but also in NA group ($F(3, 16)=25.7$, $p < 0.0001$) by one-way ANOVA. Take further comparison by Dunnette's method, it reveals that not only N_1 subgroup but also three subgroups (NA_1, NA_2 and NA_3) show significance when comparing to control subgroup ($p < 0.05$). In tolerant rats, significance is obtained not only at 0-15 min interval ($F(3, 16)=4.69$, $p < 0.05$) and 75-90 min interval ($F(3, 16)=4.2$, $p < 0.05$) for N group but also at 60-75 min interval ($F(3, 16)=3.5$, $p < 0.05$) and 75-90 min interval ($F(3, 16)=4.44$, $p < 0.05$) for NA group. Take further comparison by Dunnette's method, it reveals that each N_3 subgroup of these four significant intervals also shows significance ($p < 0.05$) .

Stereotypy

Figure 4 depicts the dose-response curves for the effect of nicotine on stereotypy. Significant

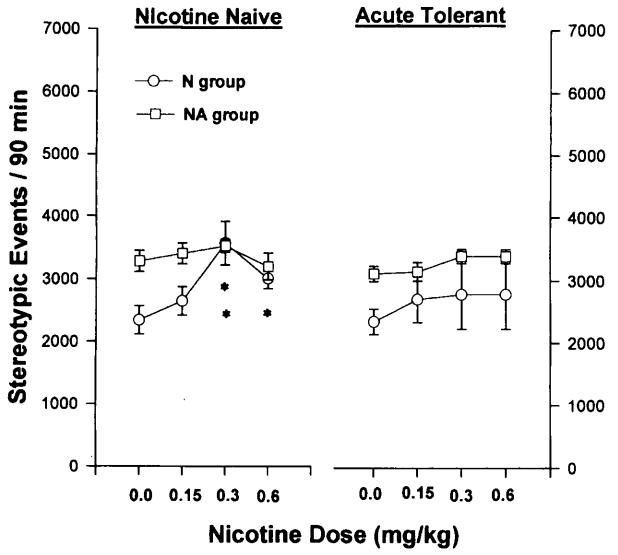

Fig. 4. Effect of nicotine on spontaneous stereotypy (N group) or amphetamine-induced stereotypy (NA group) in naive and acute tolerant rats. Nicotine was S.C. administered and amphetamine (1mg/kg) was I.P. administered. Each data point is represented as Mean±SEM. * Represents a significant difference comparing with its control subgroup. N_0: control; N_1 nicotine 0.15 mg/kg; N_2: nicotine 0.3 mg/kg; N_3: nicotine 0.6 mg/kg; NA_0: AMP 1 mg/kg; NA_1: AMP 1 mg/kg + nicotine 0.15 mg/kg; NA_2: AMP 1 mg/kg + nicotine 0.3 mg/kg; NA_3: AMP 1 mg/kg + nicotine 0.6 mg/kg. (*$p < 0.05$)

difference is obtained only in N group of naive rats ($F(3, 16)=6.32$, $p < 0.01$). Take further comparison by Dunnette's method, it reveals that two subgroups (N_1 and N_2) were significant from the control subgroup ($p < 0.05$).

Figure 5 depicts the time course for the effect of nicotine on stereotypy. Significance is obtained at 30-45 min interval ($F(3, 16)=4.41$, $p<0.05$) and at 45-60 min interval ($F(3, 16)=4.0$, $p < 0.05$) in N group of naive rats. Take further comparison by Dunnette's method, it reveals that not only N_2 subgroup at 30-45 min interval but also two subgroups (N_2 and N_3) at 45-60 min interval show significance ($p < 0.05$).

Discussion

With regard to the development of acute tolerance to nicotine, previous report (27) indicated that maximal tolerance requires 2hr to develop after an I.P. dose of nicotine and that tolerant rats showed a reduced locomotion in response to the same drug. As shown in Fig. 1, the reduced locomotion was observed in acute tolerant rats after challenge with 0.15 or 0.3 mg/kg of nicotine. In consistent with previous findings, our results demonstrated the existence of acute tolerance during 90-min session in this experiment. In addition, we found that the reduced response change into an increased one by challenge with a dose of 0.6 mg/kg, which is higher than pretreating dose (0.3 mg/kg), in acute tolerant rats. Such results indicated that acute tolerance modified the nicotine effect to result in a reducing locomotor response after a challenge dose which was lower than or equal to pretreating dose, and increased the locomotor response by challenge with a dose which was higher than the pretreating one.

In spontaneous locomotion, many studies (1, 6, 28) assessing nicotine effect on locomotion indicate that a low dose of nicotine increases locomotion but a high dose of nicotine decreases locomotion in nontolerant rats. Although it is not easy to determine the "typical" dose of nicotine for increasing or decreasing locomotion of rats, there is a general agreement (18) that nicotine-naive rats given doses greater than 0.4 mg/kg of nicotine show decrease in locomotion. In accordance with this view, our results (Fig. 2) indicated that nicotine increased spontaneous locomotion at the doses of 0.15 or 0.3 mg/kg in naive rats. Comparing the results between tolerant and naive rats (Fig. 2 and Fig. 3, upper two panel), it appeared that tolerant rats showed behavioral desensitization to nicotine at the doses of 0.15 or 0.3 mg/kg and hyperlocomotion at a higher dose (0.6 mg/kg). Such results indicated that acute tolerance modified the nicotinic influence by shifting the dose-response curve to the right. In other words, acute tolerance caused locomotion-related dopaminergic system less sensitive to nicotine.

In AMP-induced locomotion, it has been reported (2, 4) that nicotine administration to rodents produces behavioral effect mediated in part by stimulation of nicotine receptors located on dopaminergic nerve terminals. When continuous infusion with nicotine (1.5 mg/kg/day) by a subcutaneously implanted osmotic minipump for 1 day, the stimulating effect of AMP-induced locomotion is significantly attenuated at every 10-min intervals for 90 min (6). Our results (Fig. 3, lower two panel) revealed that AMP-stimulated locomotion was significantly attenuated only at the first (0-15 min) interval and the other intervals (15-90 min) showed a restoration or slight increase. Comparing with previous study (6), the doses of nicotine used in the present study were larger and the duration of nicotine exposure was temporary, so the attenuating effect occurred only temporarily. In accordance with previous reports (13, 17), our results suggest that dopaminergic neural activity may be affected by nicotine depending the dose and the duration of exposure in naive rats. In acute tolerant rats, the desensitizing effect occurred at the earlier interval at the doses of 0.15, 0.3, and 0.6 mg/kg, but the hyperlocomotor effect occurred at the later intervals (60-90 min) at high dose (0.6 mg/kg) when

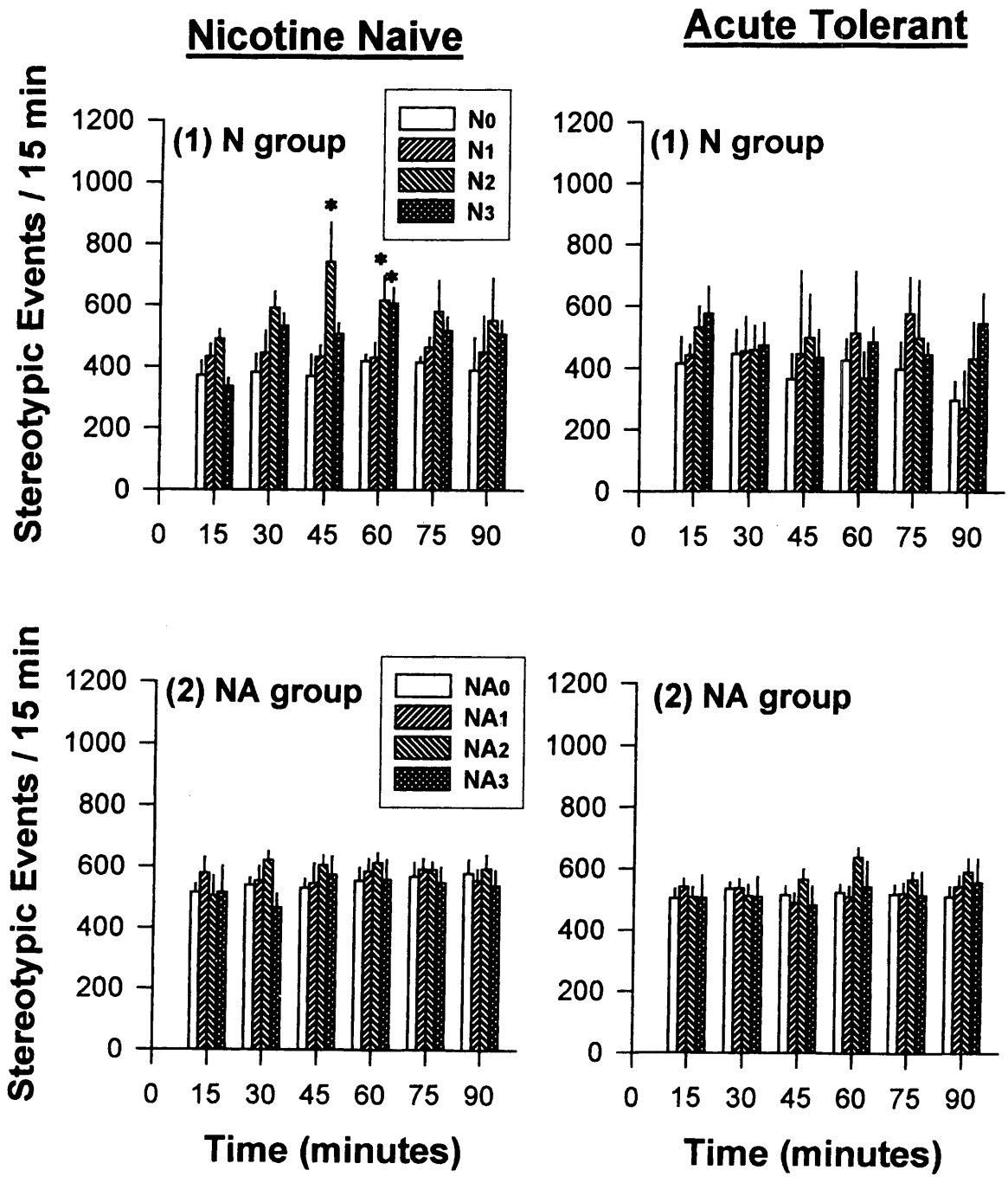

Fig. 5. Time-course for the effect of nicotine on stereotypy. Nicotine was S.C. administered and amphetamine was I.P. administered. Stereotypy was measured immediately after amphetamine administration and was measured at 15-min intervals for 90 min. Each data point is represented as Mean±SEM. *Represents a significant difference comparing with its control subgroup by post hoc of Dunnetteës test. (*$p < 0.05$)

comparing to naive rats. Such results indicate that acute tolerance also modifies the nicotinic influence on AMP-induced locomotion. In other words, such results also reveal that acute tolerance causes locomotion-related dopaminergic system less sensitive to nicotine.

Previous reports (5, 22, 26) have mentioned that nicotine or AMP alone can affect stereotypic activity. In this study, nicotine increased 52% and 30% of stereotypic activity at the doses of 0.3 and 0.6 mg/kg, respectively. AMP increased 40% of stereotypic activity at the dose of 1 mg/kg (Fig. 4). The promoting effects of both drugs were almost the same magnitude under the dosage used in this experiment. Behavioral desensitization to nicotine could be found in acute tolerant rats in comparison with naive rats. The desensitizing effect occurred mostly at the middle intervals (30-60 min) under the doses of 0.3 and 0.6

mg/kg. Such results indicated that acute tolerance modified the nicotinic influence on spontaneous stereotypy, in other words, acute tolerance caused stereotypy-related dopaminergic system less sensitive to nicotine. In the effect of nicotine on AMP-induced stereotypy, there was no significant difference between naive and tolerant rats. Such results revealed that nicotine could not influence the AMP-induced stereotypy and that acute tolerance failed to modify the nicotinic effect on AMP-induced stereotypy. The reason is unknown.

In conclusion, the present results indicated that acute tolerance modified the effect of nicotine on the locomotion-related dopaminergic system, and it affected the stereotypy-related dopaminergic system only in the spontaneous one but not in the AMP-induced one.

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