Effects of Acute Administration of Nicotine on Convulsive Movements and Blood Levels of Corticosterone in Old Rats

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ABSTRACT — The convulsive movements, blood levels of corticosterone and pharmacokinetics of nicotine after an acute intraperitoneal injection of nicotine (5 mg/kg) were examined in young (6-week-old) and old (2-year-old) rats. In pharmacokinetic study, blood nicotine levels during the elimination phase were significantly higher in old rats than in young rats. However, the duration of convulsions and the elevation of corticosterone levels after the nicotine injection showed significant decreases in old rats compared with those in young rats. These differences of nicotine-induced responses between young and old rats may be involved in the decrease in nicotine sensitivity.

Keywords: Nicotine-induced convulsion, Corticosterone, Aging

Epidemiological studies on the association of cigarette smoking with Parkinson’s disease or Alzheimer’s disease have suggested that smoking provides a protective effect against these disorders (1, 2). Moreover, a decrease in number of nicotinic acetylcholine receptors (nAChRs) in the brain with age has also been observed in experimental studies (3, 4). In behavioral studies, several investigators have demonstrated age-related changes in nicotine-induced convulsive movements (5) and spontaneous motor activity (6). However, there are few studies on the behavioral response and pharmacokinetics of nicotine in old rats. To demonstrate the age-related changes in sensitivity to nicotine, we investigated nicotine-induced convulsive movements and the pharmacokinetics of nicotine in old rats. Moreover, several studies on animals have demonstrated that the acute systemic injection of nicotine induces dose-dependent increases in blood corticosterone (CS) levels (7, 8). The nicotine-induced elevations of blood CS levels are regulated through nAChRs in the brain (8). The age-related difference in the nicotine-induced elevations of CS levels may reflect the brain sensitivity to nicotine. Thus, the effect of nicotine on blood CS levels was also assessed in young and old rats.

Male Wistar rats at the age of 6 weeks (young) and 2 years (old) were obtained from Kiwa Laboratory Animal Company (Wakayama); these rats weighed from 180 to 200 g and from 700 to 900 g, respectively. All rats were housed with a 12-hr light-dark cycle (light on from 6:00 to 18:00) and fed laboratory chow (MF: Oriental Yeast Co., Tokyo) and water ad libitum for one week before the experiment. Nicotine (free base; Maruwaka Kagaku, Osaka) was dissolved in physiological saline (Otsuka, Naruto). Rats received an acute intraperitoneal (i.p.) injection of nicotine (5 mg/kg) or saline at a volume of 1 ml/kg. All injections of the drugs were performed between 10:00 and 13:00.

We have already reported the methods for measuring nicotine-induced convulsive movements and blood nicotine levels (9). After placing the rats in a test-cage (37 × 21 × 15 cm) for 30 min, each rat was injected with nicotine or saline. The onset times and the durations of tremor and clonic and tonic convulsions were recorded by a stopwatch.

At 5, 10, 15, 20, 30, 45, 60, 90 and 120 min following nicotine or saline injection, blood samples (400–600 μl) were obtained from the retro-orbital sinus of each rat. After centrifugation of the blood samples, the serum was stored at −100°C until the measurements of nicotine and corticosterone levels. Nicotine was extracted from the serum into diethyl ether. Nicotine extracts containing quinoline as the internal standard were analyzed by a Hitachi model 163 gas chromatography equipped with a flame thermionic detector. The column was a 2 m × 3 mm glass tubing packed with 10% Apiezon L-10% KOH on WAW 80–100 mesh. The operating conditions were as described by our previous report (9). The blood concentration/time course data
for nicotine were evaluated by quantitation of semi-logarithmic plots. Pharmacokinetic parameters were derived as described by Kapetanovic et al. (10). The apparent blood elimination half-life ($T_{1/2}$) for nicotine was calculated by the elimination rate constant ($\beta$) obtained from the linear terminal segments of semi-logarithmic plots. The area under the concentration-time curve from time zero to infinity (AUC) was determined by the trapezoidal rule including extrapolation to infinity. The hybrid parameters, clearance per fraction of dose (CL/F) and apparent volume of distribution per fraction of dose (Vd/F), were calculated as dose/AUC and dose/(AUC $\times \beta$), respectively.

CS was extracted with methylene chloride by the method of Shimizu et al. (11). CS levels were determined by a high-performance liquid chromatograph (Model LC-6A, Shimadzu, Kyoto) equipped with a UV detector (Model SPD-6AV, Shimadzu) and an automatic sampler (Model SCL-6B, Shimadzu). A stainless-steel column (150 × 4.6 mm I.D.) was packed with Inertsil ODS-2 (5 $\mu$m; GL Science, Tokyo). The mobile phase was a solvent mixture composed of acetonitrile - methanol - water (3:1:6, v/v/v). The column temperature was 42°C, the flow-rate was 0.8 ml/min, and the detection wavelength was 254 nm. Ratios of the peak heights of CS and dexamethasone as an internal standard were evaluated by a standard curve established from CS standards ranging from 10 to 100 ng/ml. The recovery of CS after extraction was approximately 97%. The retention times of CS and dexamethasone were 9.8 min and 8.6 min, respectively.

Differences between young and old rats were analyzed by the unpaired Student’s t-test. The data are expressed as the mean ± S.E.M. A P value of less than 0.05 was considered to indicate a significant difference.

Table 1 shows the onset times of tremor and clonic and tonic convulsions and the durations of clonic and tonic convulsions induced by nicotine in young and old rats. There was no significant difference between young and old rats in the onset times of tremor and clonic and tonic convulsions. The durations of clonic and tonic convulsions in old rats, however, were decreased in comparison with those in young rats.

The concentration vs. time profiles of nicotine in the blood of young and old rats after nicotine injection are presented in Fig. 1, panel A. Although there was no significant difference in blood nicotine levels between young and old rats up to 15 min after nicotine injection, statistically higher blood nicotine levels in old rats were observed from 20 to 120 min after nicotine injection. Table 2 shows the pharmacokinetic parameters for nicotine in young and old rats. The values of $T_{1/2}$ and AUC in old rats were approximately 1.5- and 2.4-fold greater than those in young rats, respectively. The value of CL/F in old rats was approximately half of that in young rats.

Figure 1, panel B shows the effect of acute nicotine administration on blood CS levels in young and old rats. There was no difference between young and old rats in CS levels at any time after saline injection. Acute nicotine injection caused significant elevations of blood CS levels in young rats from 5 to 45 min after nicotine injection, while those in old rats were observed at 20 and 30 min after nicotine injection, compared to each saline-treated group. From 5 to 30 min after the injection, the nicotine-induced elevation of CS levels in young rats was significantly greater than that in old rats.

Our previous experiment indicated that nicotine (2.5 to 5 mg/kg, i.p.)-induced convulsive movements were dose-dependent in young rats (9). In the present results, nicotine (5 mg/kg, i.p.) produced tremor with prostration, rapidly followed by clonic and tonic convulsions in young and old rats. The nicotine-induced convulsive movements observed in young and old rats disappeared within 5 min after the nicotine injection. The onset times of tremor and clonic convolution and the duration of clonic convolution in young rats agreed with our previous results (9).

There were no significant differences in blood nicotine levels between young and old rats at the early time points after the nicotine injection. However, the dura-
tions of nicotine-induced convulsions were deceased in old rats compared to those in young rats. Also, although the blood nicotine levels from 15 to 45 min were higher in old rats than in young rats, the nicotine-induced elevations of blood CS levels were less in old rats. The systemic pretreatment with mecamylamine, but not hexamethonium, has been shown to antagonize the nicotine-induced convulsive movements and the elevations of blood CS levels, suggesting that these nicotine-induced responses are mediated through nAChRs in the brain (8, 9). Furthermore, several studies suggest the age-related decrease in the number of nAChRs in rat brain (3, 4). Therefore, the decrease in the sensitivity of nAChRs in the brain may be one of the causes of both changes in the nicotine-induced convulsive movements and the elevations of CS levels between young and old rats.

Several studies have demonstrated that the adrenal stimulatory effects of nicotine on the release of catecholamines influence nicotine-induced convulsive movements (12, 13). Laurence and Stacey (12) observed that nicotine-induced convulsions were potentiated by the systemic pretreatment with adrenaline or noradrenaline, and they are suppressed by adrenalectomy. In addition, it has been shown that acute administration of nicotine stimulates the release of adrenocorticotropic hormone from the pituitary gland, resulting in the elevation of blood glucocorticoid level (7). Thus, the decreases in convulsive and CS responses induced by nicotine in old rats may also be related to the age-related decrease in sensitivity of the adrenal gland.

Dopamine levels significantly increase in young rat brain when nicotine induces convulsive movements (13, 14). In addition, it has been observed that the pretreatment with the specific dopamine D1 receptor antagonist SCH-23390 antagonizes nicotine-induced convulsive

Table 2. Pharmacokinetic parameters of nicotine in young and old rats

<table>
<thead>
<tr>
<th>Age</th>
<th>$T_{1/2}$ (hr)</th>
<th>AUC (ng/hr/ml)</th>
<th>CL/F (liters/hr/kg)</th>
<th>Vd/F (liters/kg)</th>
</tr>
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<tr>
<td>Young (6 weeks)</td>
<td>1.0 ± 0.1</td>
<td>1658 ± 44</td>
<td>3.0 ± 0.1</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Old (2 years)</td>
<td>1.5 ± 0.1**</td>
<td>3914 ± 545**</td>
<td>1.4 ± 0.2**</td>
<td>3.1 ± 0.6</td>
</tr>
</tbody>
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All rats were intraperitoneally injected with nicotine (5 mg/kg). The value represents the mean ± S.E.M. of 5–10 rats. **P < 0.01, significantly different from young rats.
movements (15). Ponzio et al. (16) suggested that dopamine levels in old rats were significantly reduced in the substantia nigra, limbic area and striata of rat brain. Furthermore, they indicated that dopaminergic neurons in the brain were affected by aging (16). Therefore, changes of the dopaminergic system in the brain with age may be also be involved in the difference of nicotine-induced convulsive movements between young and old rats.

The values of the elimination $T_{1/2}$, CL/F and $V_d/F$ for nicotine in young rats agreed with the previously data (17). However, in contrast to the data by Kyerematen et al. (17), the prolonged $T_{1/2}$ and the declined $CL/F$ were observed in old rats. The pharmacokinetic differences may be related to an age-dependent alteration in nicotine metabolism. We have already observed that the metabolism of nicotine decreases in old rats (14). The decrease in the nicotine metabolism of old rats might cause the decreased $CL/F$, resulting in the prolongation of $T_{1/2}$ for nicotine and higher nicotine levels in the blood. Age-related changes in body composition may also be responsible for the difference in the time-course of blood nicotine levels between young and old rats. The mean body weight of old rats in the present study was 3- to 4-fold greater than that of young rats. The greater body weight in old rats may be due to an age-related increase in the amount of adipose tissue. Since the widespread tissue distribution and high lipid-solubility of nicotine have been observed (18), the increased adipose tissues in old rats may result in an increase in nicotine retention, followed by higher blood nicotine levels in old rats at later time points.

From these results, the difference in both the nicotine-induced convulsive movements and the elevation of blood CS levels between young and old rats may be mainly due to the decrease in sensitivity to nicotine rather than to the change in pharmacokinetics of nicotine.

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