Tolerance to the Convulsions Induced by Daily Nicotine Treatment in Rats

Masato Okamoto, Taizo Kita, Hirotsugu Okuda and Toshikatsu Nakashima

Department of Pharmacology, Nara Medical University, Kashihara 634, Japan

Received March 31, 1992 Accepted May 19, 1992

ABSTRACT—Development of tolerance to the nicotine-induced convulsions in rats was examined. Acute intraperitoneal (i.p.) administration of nicotine (2.5, 3.75 and 5 mg/kg) produced convulsions in a dose-dependent manner. Mecamylamine (1 mg/kg, i.p.) antagonized the convulsions, but hexamethonium (5 mg/kg, i.p.) did not modify them. Daily nicotine administration (2.5, 3.75 and 5 mg/kg, i.p.) once a day for 6 days developed tolerance to the convulsions induced by nicotine. After the daily administrations of nicotine for 6 days, the effects of a challenge administration of nicotine (2 mg/kg) on the nicotine-induced convulsions were tested on the 7th-day. Further tolerances were also developed by the 7th-day challenge administration. After the 7th-day test, nicotine levels of the brain and blood 15 min after the challenge injection were measured. With nicotine (5 mg/kg once a day)-treatment, nicotine levels of all the brain regions were increased. In contrast, a similar challenge injection had no effect on blood nicotine level. These results indicate that the development of tolerance to the nicotine-induced convulsions is produced relatively earlier and day by day by daily administrations to rats, which is closely related with the increase in brain nicotine level.

Keywords: Nicotine, Tremor, Convulsion, Tolerance, Brain nicotine level

A variety of studies have already demonstrated the nicotine-induced behaviors in rodents (1–3). Acute systemic injection of nicotine at high concentrations depresses locomotor activity (3, 4) and produces tremors (1), prostration (5) and convulsions (1, 2) in rats and mice. In pharmacological studies, it has been shown that the nicotine-induced convulsions are blocked by small doses of ganglion blocking agents such as mecamylamine and chlorisondamine (6, 7). With intracerebroventricular (i.c.v.) injections of nicotine, Caulfield and Higgins (8) reported that the nicotine-induced convulsions in mice possessed a similar pharmacological profile to those by an activation of ganglionic (C6) receptors, rather than neuromuscular (C10) receptors. Beleslin and Krestic (9) also demonstrated that the i.c.v.-injection of mecamylamine and C6 antagonized the convulsions evoked by nicotine in cats. These results suggest that the nicotine-induced convulsions may be mediated by nicotinic acetylcholine receptors (nAChRs) in the brain.

There is a relationship between the nicotine-induced convulsive movements and nicotine levels of the blood and brain. Mice exhibiting a higher nicotine concentration in the brain suffered severe convulsions with a shorter latency than mice exhibiting a lower nicotine level after an acute i.p.-injection of nicotine, although the nicotine levels of the blood were unaffected (10).

On the other hand, the tolerance to the nicotine-induced behavior, especially the depressant effect on the locomotor activity, in rats and mice has also been studied (3, 11, 12). In a study of tolerance to nicotine-induced convulsions, Miner and Collins (13) reported that mice pretreated with a single dose of nicotine showed an increase in the ED50 for nicotine-induced seizures. However, there is little information on the time- and dose-dependent development of tolerance to the convulsions induced by daily administrations of nicotine. Therefore, we would like to determine if and how the tolerance to nicotine-induced convulsions is developed. Also, we tried to characterize the relationships between nicotine levels of the brain and blood and development of tolerance to the nicotine-induced convulsions.
MATERIALS AND METHODS

Animals
Male Wistar rats (Kiwa Experimental Laboratories, Wakayama), weighing 180–200 g, were used. Rats were acclimated to an environmentally controlled vivarium at 25 ± 2°C, and they were provided food (MF: Oriental Yeast Co., Tokyo) and water ad libitum for one week. Cycles of light and dark were controlled by a fluorescent lamp, and the light time was from 6:00 A.M. to 6:00 P.M.

Drugs
Nicotine (Maruwaka Kagaku, Osaka), Mecamylamine (Sigma, Chemical Co., St. Louis, MO, U.S.A.) and hexamethonium (Sigma) were dissolved in physiological saline (Otsuka, Naruto). All rats received intraperitoneal (i.p.) injections of the drugs in a volume of 1 ml/kg. The drugs solutions were freshly prepared each time.

Behavioral procedure
Rats were injected with nicotine (2.5, 3.75 and 5 mg/kg, i.p.) or saline once a day for 6 successive days. The drugs were constantly administered between 9:30 A.M. and 1:00 P.M. After placing each chronically drug administered rat in a test-cage (37 × 21 × 15 cm) for 30 min, they were observed for 30 min after an injection of nicotine. The onset time and duration of tremor and clonic convulsions were recorded, and the incidence of tonic convulsions were also observed. In addition, the recovery time from the paralysis of rat hind legs after the injection of nicotine was measured. All experiments were done between 10:00 A.M. and 2:00 P.M. After rats were daily injected with nicotine (2.5, 3.75 and 5 mg/kg), their nicotine-induced convulsive movements for 6 successive days were recorded. On the 7th-day, both nicotine- and saline-treated rats were challenged with nicotine (2 mg/kg) and immediately monitored for their nicotine-induced convulsions for 15 min.

Antagonists test
After placing each naive rat in a test-cage for 30 min, mecamylamine (1 mg/kg, i.p.) and hexamethonium (5 mg/kg, i.p.) were given to naive rats 15 min before a single injection of nicotine (3.75 mg/kg). Their convulsive movements were observed for 30 min after a single injection of nicotine. Control rats were injected with saline 15 min before the injection of nicotine.

Measurement of blood and brain levels of nicotine
On the 7th-day, a single challenge dose of nicotine (2 mg/kg, i.p.) was injected to both nicotine- and single-treated rats. Rats were sacrificed by decapitation 15 min after an injection of a single challenge dose of nicotine. The blood was immediately collected and centrifuged at 1,000 × g for 20 min to obtain the serum. The brain was removed rapidly, dissected on ice into the cortex, midbrain, cerebellum, medulla, hypothalamus, hippocampus and striatum, according to the method of Glowinski and Iversen (14), and then each section was weighed. Serum and brain tissues were stored at −100°C until the assay.

Tissues and serum preparation
The brain tissues were homogenized in 10 volumes of 0.05 N trichloroacetic acid for 2 min at 4°C, and the solution was centrifuged at 10,000 × g at 2°C for 30 min to obtain the resultant supernatant. Nicotine was extracted with diethyl ether from the supernatants and the serum, and then was measured by a gas chromatographic method, a modification of the procedures described by Jacob et al. (15). A 1-ml aliquot of the serum or supernatant was added to centrifuge tubes containing 0.5 ml of 2 N sodium hydroxide and 1 μg of quinoline as an internal standard. Two milliliters of diethyl ether was then added to each tube, which was mechanically shaken for 20 min and centrifuged at 3,000 × g for 10 min. The extraction with ether was performed three times. After the combined ether layers were transferred to tubes containing 1 ml of 1 N HCl, the tubes were mechanically shaken and centrifuged, and then the ether layers were removed and discarded. One milliliter of 2 N sodium hydroxide and 1 ml of ether were added to the aqueous layer; the tubes were again shaken. The ether layers were separated and concentrated by a centrifugal concentrator (Taiyo Chemical Industrial Co., Ltd., model VC-360) to a final volume of 50 μl. A 1-μl aliquot of the ether layer was injected onto the chromatographic column. The ratio of the peak height for quinoline to that of nicotine were determined.

A Hitachi model 163 gas chromatograph equipped with a flame thermionic detector was used. The column was a 2 m × 3 mm glass tubing packed with 10% Apiezon L and 10% KOH on WAX 80–100 mesh. The operating conditions were as follows: injection block temperature, 300°C; column temperature, 190°C–240°C (5°C/min); detector temperature, 300°C; carrier gas (helium) flow rate, 50 ml/min; air flow rate, 85 ml/min; and hydrogen flow rate, 1.8 ml/min.

The calibration curves, which were constructed by adding nicotine and quinoline as internal standards to blank solutions of sample type, was linear over the working range. The recovery of nicotine was 93%. The retention time for quinoline and nicotine were 2.5 and 3.0 min, respectively.
Statistical analysis

Values are the means ± S.E.M. Tolerance to the nicotine-induced convulsive movements was assessed by $\chi^2$ analysis or two-way analysis of variance (ANOVA). Effects of daily nicotine treatment on nicotine concentrations in the brain or serum at different concentrations were analyzed by one-way ANOVA. All other comparisons were made by Student’s $t$-test.

RESULTS

Development of tolerance to nicotine-induced convulsions

The development of tolerance to the nicotine-induced convulsions in rats was examined (Fig. 1). On the first day, after i.p.-injections of nicotine (2.5, 3.75 and 5 mg/kg), typical convulsions were dose-dependently produced in all the rats. Prostration, tremor and clonic convulsion followed by tonic convulsion were observed in the rats. Effects of antagonists on the nicotine (3.75 mg/kg)-induced convulsions were studied (Table 1). Pretreatment of mecamylamine (1 mg/kg, i.p.) antagonized all the nicotine-induced convulsive effects (tremor, prostration, and clonic and tonic convulsions), but hexamethonium (5 mg/kg, i.p.) did not affect them. These data are summarized in Table 1.

In ANOVA analysis for daily nicotine administration, the onset time of tremor was significantly slowed time-dependently [$F(5,126) = 13.262, P < 0.01$] and dose-dependently [$F(2,126) = 20.663, P < 0.01$] (Fig. 1A).

![Graphs showing development of tolerance to nicotine-induced convulsions](image-url)
The onset time of clonic convulsion was also slowed time-dependently \[F(5,126) = 22.182, \ P < 0.01\] and dose-dependently \[F(2,126) = 46.082, \ P < 0.01\] (Fig. 1B). In the duration of clonic convulsion, time- and dose-dependences were observed \[F(5,126) = 81.395, \ P < 0.01,\] and \[F(2,126) = 116.443, \ P < 0.01, \text{respectively}\] (Fig. 1C). The recovery time from the paralysis of the hind legs was shortened time- and dose-dependently \[F(5,126) = 86.993, \ P < 0.01,\] and \[F(2,126) = 74.197, \ P < 0.01\] (Fig. 1E). In addition, the incidence of tonic convulsion was decreased significantly (Fig. 1D).

Effects of the challenge injection on the 7th-day on the development of tolerance were examined. The incidence of clonic and tonic convulsions significantly decreased at only the concentration of 5 mg/kg, as shown in Table 2. Both the durations of clonic convulsion and the recovery time from the paralysis of the hind legs were decreased in a dose-dependent manner.

### Table 1. Effects of antagonists on nicotine-induced convulsant effects in rats

|          | Tremor /n | Prostration /n | Convulsion Clonic /n | Convulsion Tonic /n |
|----------|-----------|----------------|----------------------|--------------------|
| Saline   | 8/8       | 8/8            | 8/8                  | 7/8                |
| Mecamylamine (1 mg/kg) | 0/8**     | 0/8**          | 0/8**                | 0/8**              |
| Hexamethonium (5 mg/kg) | 8/8       | 8/8            | 8/8                  | 3/8                |

Antagonists or saline were intraperitoneally (i.p.) administered 15 min before an injection of nicotine (2 mg/kg, i.p.) in naive rats. **P < 0.01; Significantly different from the saline groups. Data were analyzed by the \(\chi^2\)-test. n: number of rats.

### Table 2. Effects of challenge injections on convulsant effects induced by chronic nicotine treatments in rats

|                | Duration of clonic convulsion (sec) | % of convulsion Clonic (\%/n) | Tonic (\%/n) | Recovery time (sec) |
|----------------|-------------------------------------|-----------------------------|--------------|---------------------|
| Saline         | 93.1 ± 9.4                          | 100 (8/8)                   | 62.5 (5/8)   | 845.4 ± 13.1        |
| Nicotine (2.5 mg/kg) | 46.3 ± 4.5**                        | 100 (8/8)                   | 25 (2/8)     | 727.8 ± 25.8**      |
| Nicotine (3.75 mg/kg) | 19.5 ± 4.4**                        | 87.5 (7/8)                  | 12.5 (1/8)   | 642.8 ± 19.3**      |
| Nicotine (5 mg/kg) | 5.5 ± 3.5**                         | 50 (4/8)**                  | 0 (0/8)**    | 482.1 ± 22.2**      |

Rats, intraperitoneally (i.p.) treated with 2.5, 3.75 or 5 mg/kg of nicotine or saline daily for 6 successive days, were given a challenge injection of nicotine (2 mg/kg, i.p.) on the 7th day. Data represent the mean ± S.E.M. (n = 8). *P < 0.05, **P < 0.01, with respect to the control. Data were analyzed by Student’s t-test or the \(\chi^2\)-test. n: number of rats.
DISCUSSION

It has been shown that an acute injection of nicotine elicits depressed locomotor activity in rats (3, 4, 16). In the present experiments, on the first day, the injections with nicotine (2.5, 3.75 and 5.0 mg/kg, i.p.) produced tremor with prostration rapidly followed by clonic and tonic convulsions. The convulsive effects were consistent with the results of several reports (1, 5–7, 17). Mecamylamine antagonized the nicotine-induced convulsive effects, but hexamethonium did not antagonize it. These results indicate that the nicotine-induced convulsive effects are due to the stimulation of nAChRs in the brain, but not due to neuromuscular activation.

In general, chronic drug treatment often results in alterations in the intensity of response to the drug. The development of tolerance to drugs might play a critical role in facilitating the continued use of the drugs. In this study, repeated injections of nicotine also produced the defined development of tolerance to the nicotine-induced convulsive effects. The tolerance was developed relatively rapidly during the first 2–3 days of nicotine treatment. This rapid tolerance was similar to development of tolerances to locomotor activity and hypothermia (16). Marks et al. (18) have reported that with chronic nicotine treatment, an increase in the receptor number is induced time- and dose-dependently, and the increase in receptors in DBA/2 mice roughly parallels the development of physiological and behavioral tolerances to nicotine. It was also reported that the increase in nAChR binding correlated with the development of tolerance to body temperature and locomotor activity (16). In addition, Collins et al. (19) suggested that chronic administrations of nicotine might cause prolonged desensitization and inactivation of the nAChRs, resulting from either an increase in the rate of receptor synthesis or a decrease in the rate of receptor catabolism. In the present experiments, therefore, it seems that the repeated nicotine treatment may produce an increase in the number of nAChRs in the brain. The great elevation in the brain nicotine concentration suggests that the desensitization of nAChRs may probably be elicited. Furthermore, up-regulation of nAChRs by chronic nicotine treatment has been widely supported.
by several investigators (18, 20, 21).

After a challenge injection, repeated injections with nicotine produced opposite effects on brain nicotine levels and those of the blood. Daily injections of nicotine increased the nicotine levels of all brain regions. However, no change in serum nicotine level occurred. It appears that the increase in brain nicotine levels in the nicotine-treated rats may be due to the increase in the absolute number of nAChRs, which were desensitized. The elevation of the nicotine level in the brain, but not in the blood, is evidence that the nicotine-induced convulsions were caused by the activation of nAChRs in the brain, especially in the cortex, hippocampus and striatum. Several investigators reported that nicotine-induced convulsive movements were concerned with upper brain regions (cortex, hippocampus and diencephalon) (22–24). Furthermore, it has been shown that nicotine may increase the release of adrenal steroids (19). Certain steroids may alter GABA receptor binding and functions. Thus, the tolerance to chronic nicotine treatment might be influenced by the effects of adrenal steroids on response to nicotine and nicotine receptor binding. In addition, Yamamoto et al. (25) reported that the dopamine level in whole rat brain was significantly increased when nicotine induced convulsions in rats. Nicotine-induced convulsions also might be associated with the dopaminergic system in rat brain. Further experiments are required to elucidate the complex mechanisms.

In conclusion, these results have demonstrated that the repeated injections with nicotine cause a dose-dependent development of tolerance to nicotine-induced convulsive effects. The tolerance appeared relatively earlier after the nicotine injections. The development of tolerance to the nicotine-induced convulsions are closely related to the increase in brain level of nicotine, presumably resulting from the increase in the number of nAChRs and from the desensitization of nAChRs in the brain.

Acknowledgments

We are very grateful to Dr. Hiroyasu Satoh for his criticism and help in the preparation of the manuscript. We also thank Ms. Kazuko Sakata for technical assistance. This work was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science and Culture, Japan, and in part, by a grant from The Smoking Research Foundation in Japan.

REFERENCES

1 Silvette, H., Hoff, E.C., Larson, P.S. and Haag, H.B.: The actions of nicotine on central nervous system functions. Pharmacol. Rev. 14, 137–173 (1962)
2 Sershen, H.: Nicotine. In Handbook of Neurochemistry, Second Edition, Edited by Lajitha, A., Vol. 10, p. 263–278, Plenum press, New York and London (1985)
3 Clarke, P.B.S.: Nicotine and smoking: a perspective from animal studies. Psychopharmacology (Berlin) 92, 135–143 (1987)
4 Kita, T., Nakashima, T., Shirase, M., Asahina, M. and Kurogochi, Y.: Effects of nicotine on ambulatory activity in mice. Japan. J. Pharmacol. 46, 141–146 (1988)
5 Abood, L.G., Reynolds, D.T., Booth, H. and Bidlack, J.M.: Sites and mechanisms for nicotine's action in the brain. Neurosci. Biobehav. Rev. 5, 479–486 (1981)
6 Stone, C.A., Meckelnburg, K.L. and Torchiana, M.L.: Antagonism of nicotine-induced convulsions by ganglionic blocking agents. Arch. Int. Pharmacodyn. Ther. 117, 419–434 (1958)
7 Aceto, M.D., Bentley, H.C. and Dembinski, J.R.: Effects of ganglion blocking agents on nicotine extensor convulsions and lethality in mice. Br. J. Pharmacol. 37, 104–111 (1969)
8 Caufield, M.P. and Higgins, G.A.: Mediation on nicotine-induced convulsions by central nicotinic receptors of the 'C6' type. Neuropharmacology 22, 347–351 (1983)
9 Beleslin, D.B. and Krestic, S.K.: Nicotine-induced convulsion in cats and central nicotinic receptors. Pharmacol. Biochem. Behav. 24, 1509–1511 (1986)
10 Tepper, J.M., Wilson, J.R. and Schlesinger, K.: Relations between nicotine-induced convulsive behavior and blood and brain levels of nicotine as a function of sex and age in two inbred strains of mice. Pharmacol. Biochem. Behav. 10, 349–355 (1978)
11 Stolerman, I.P., Fink, R. and Jarvik, M.E.: Acute and chronic tolerance to nicotine measured by activity in rats. Psychopharmacologia (Berlin) 30, 329–342 (1973)
12 Keenan, A. and Johnson, F.N.: Development of behavioral tolerance to nicotine in the rats. Experientia 28, 428–429 (1972)
13 Miner, L.L. and Collins, A.C.: Effect of nicotine pretreatment on nicotine-induced seizures. Pharmacol. Biochem. Behav. 29, 375–380 (1988)
14 Glowinski, J. and Iversen, L.L.: Regional studies of catecholamines in the rat brain. J. Neurochem. 13, 655–669 (1966)
15 Jacob, P., Wilson, M. and Benowitiz, N.L.: Improved gas chromatographic method for the determination of nicotine and cotinine in biologic fluids. J. Chromatogr. 222, 61–70 (1981)
16 Collins, A.C., Romm, E. and Wehner, J.M.: Nicotine tolerance: an analysis of the time course of its development and loss in the rat. Psychopharmacology (Berlin) 96, 7–14 (1988)
17 Orcutt, J.A., Michaelson, S.M. and Prytherch, J.P.: The inhibition of nicotine-induced convulsions in the rats. Arch. Int. Pharmacodyn. Ther. 146, 238–245 (1963)
18 Marks, M.J., Romm, E., Gaffney, D.K. and Collins, A.C.: Nicotine-induced tolerance and receptor changes in four mouse strains. J. Pharmacol. Exp. Ther. 237, 809–819 (1986)
19 Collins, A.C., Bhata, R.V., Pauly, J.R. and Marks, M.J.: Modulation of nicotine receptors by chronic exposure to nicotine agonists and antagonists. In The Biology of Nicotine Dependence, Edited by Bock, G. and Marsh, J., Vol. 152, p. 68–82, John Wiley & Sons, New York (1990)
20 Wonnacott, S.: The paradox of nicotinic acetylcholine receptor upregulation by nicotine. Trends Pharmacol. Sci. 11, 216–219 (1990)
21 Pauly, J.R., Marks, M.J., Gross, S.D. and Collins, A.C.: An autoradiographic analysis of cholinergic receptors in mouse brain after chronic nicotine treatment. J. Pharmacol. Exp. Ther. 258, 1127–1136 (1991)

22 Longo, V.G., Von Berger, G.P. and Bovet, D.: Action of nicotine and of the “gangloplegiques centraux” on the electrical activity of the brain. J. Pharmacol. Exp. Ther. 111, 349–359 (1954)

23 Sacra, P. and McColl, J.D.: Effect of ataractics on some convulsant and depressant agents in mice. Arch. Int. Pharmacodyn. Ther. 117, 1–8 (1958)

24 Dunlop, C.W., Stumpf, C., Maxwell, D.S. and Schilder, W.: Modification of cortical, reticular and hippocampal unit activity by nicotine in the rabbit. Am. J. Physiol. 198, 515–518 (1960)

25 Yamamoto, I., Nagai, K. and Inoki, R.: The contents of dopa and catecholamines in several rat tissues and nicotine-induced convulsions. Japan. J. Pharmacol. 16, 295–305 (1966)