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# Nicotine-Induced Convulsions in Cats and Central Nicotinic Receptors

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BELESLIN, D. B. AND S. K. KRSTIĆ. *Nicotine-induced convulsions in cats and central nicotinic receptors.* PHARMACOL BIOCHEM BEHAV 24(6) 1509–1511, 1986.—The effects were investigated of intracerebroventricular (ICV) injections in the cat of ganglionic blocking agents, antimuscarinic drugs, alpha and beta adrenergic blocking substances, dopamine antagonists, an antihistamine, reserpine and a 5-hydroxytryptamine antagonist as well as the inhibitors of catecholamines, 5-hydroxytryptamine and acetylcholine synthesis upon convulsions produced by nicotine, which was similarly injected. Mecamylamine and hexamethonium but not atropine, scopolamine, yohimbine, phenoxybenzamine, tolazoline, propranolol, practolol, chlorpromazine, haloperidol, antazoline and methysergide abolished the convulsions evoked by nicotine. Furthermore, reserpine, but not 6-hydroxydopamine, as well as 5,6-dihydroxytryptamine and hemicholinium blocked the convulsions caused by nicotine. It appears, therefore, that the convulsions produced by nicotine are mediated through central nicotinic receptors. However, the depressed catecholaminergic, 5-hydroxytryptaminergic and histaminergic mechanisms induced by reserpine can also suppress the convulsions evoked by nicotine.

Convulsions Nicotine Nicotinic receptors Central aminergic mechanisms

PREVIOUS pharmacological analyses of central effects of intracerebroventricular (ICV) nicotine in unanaesthetized cats have shown that vomiting and ear twitching are mediated through central nicotinic receptors [2,12]. Salivation and panting are mediated by central receptors having mixed nicotinic and muscarinic properties [2,4]. On the other hand, although nicotine-induced convulsions are well known [17], there is little evidence on receptors or on the mechanism/s underlying these convulsions in the cat. For instance, it is reported that the nicotinic ganglionic agonist dimethylphenylpiperazinium produces convulsions by an action on central nicotinic receptors in the cat [13]. Therefore, in the present experiments the pharmacological properties as well as the biochemical mechanisms underlying the convulsions caused by nicotine injected into the cerebral ventricles (ICV) of unanaesthetized cats were studied.

## METHOD

In these experiments 194 cats of both sexes, weighing 2–4 kg were anesthetized with sodium pentobarbital (35–40 mg/kg IP). Following aseptic precautions, a hole was drilled 7–8 mm from the zero line and 4–5 mm from the midline. A Collison cannula was then screwed into the calvarium, the tip of the cannula resting in the left lateral ventricle [9]. The lower end of the cannula shaft was made of polythene tubing with a side opening 1.0 mm from its closed tip, and positioned with the lumen facing the foramen of Monro. Post mortem dye studies indicated that the injected material passed from the lateral ventricle into the third and fourth ventricle. Postoperatively, penicillin was administered intramuscularly. An interval of five days was allowed to elapse before an experiment began.

The substances injected into the cerebral ventricles were dissolved in sterile, pyrogen-free 0.9% sodium chloride. These solutions were then injected manually from a 1.0 ml syringe in a volume of 0.1–0.2 ml over a period of 15–20 sec and washed in with 0.1 ml of saline under the same conditions. The injected animals were observed for a period of 4 hours and intermittently for 24 hours.

On the test day, cats were habituated for one hour in a wire mesh cage (110×130×150 cm) before any activity was monitored. The behavior of the animals was monitored continuously by two experienced observers who were blind to the drug condition of the animals. The correlation coefficient for these checks ranged consistently between 0.92–0.98.

The compounds used were: nicotine hydrogen tartrate, atropine sulfate, scopolamine bromide, hexamethonium bromide, mecamylamine hydrochloride, yohimbine chloride, phenoxybenzamine chloride, tolazoline hydrochloride, propranolol chloride, practolol chloride, chlorpromazine chloride, haloperidol, methysergide bimaleate, antozoline chlorhydrate, reserpine (Serpasil, Ciba-Geigy), 6-hydroxydopamine bromide, 5,6-dihydroxytryptamine creatinine sulfate and hemicholinium-3. Haloperidol was dissolved in warm lactic acid (0.001 mg/ml), whereas 6-hydroxydopamine and 5,6-dihydroxytryptamine were always freshly dissolved in 0.9% (w/v) sodium chloride containing 0.2% (w/v) ascorbic acid. The aqueous solutions of

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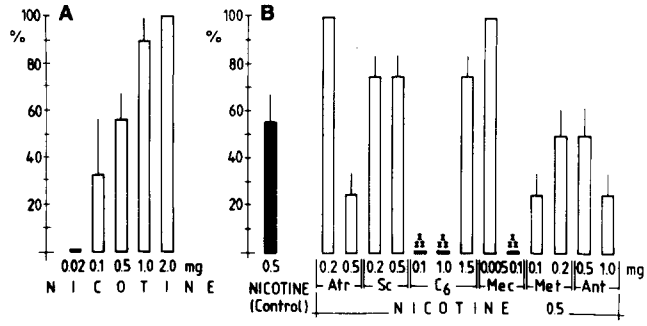


FIG. 1. A. Convulsions produced by nicotine injected into the cerebral ventricles of the cat. B. ICV effect of atropine (Atr), scopolamine (Sc), hexamethonium ($C_6$), mecamylamine (Mec), methysergide (Met) and antazoline (Ant) on convulsions after ICV nicotine. Ordinate denotes percent of cats showing convulsions. Abscissa in B, the first column (black) is control value for convulsions; values of atropine, scopolamine, hexamethonium, mecamylamine, methysergide or antazoline in mg doses are mean of at least 4 experiments ±S.E.M. ***Difference from control value is significant at $p<0.001$ (Student's $t$-test).

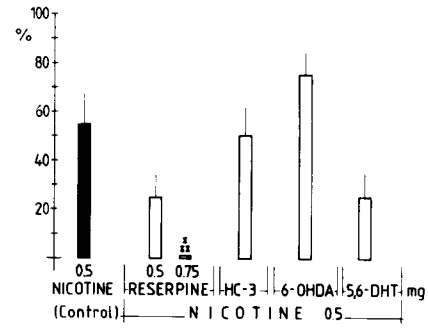


FIG. 2. Convulsions produced by ICV nicotine in reserpine-, hemicholinium (HC-3)-, 6-hydroxydopamine (6-OHDA)- and 5,6-dihydroxytryptamine (5,6-DHT) all given ICV. Ordinate: percent cats showing convulsions. Abscissa: first column (black) represents control, and other columns the mean of at least 4 experiments ±S.E.M. ***Difference from control value is significant at $p<0.001$ (Student's $t$-test).

ascorbic acid and lactic acid in a volume of 0.1–0.3 ml had no visible effects on the cat's behavior. All drug doses refer to the salts, except those of haloperidol, reserpine and hemicholinium-3 which refer to the base.

## RESULTS

### *Nicotine-Induced Convulsions*

Nicotine (0.1–2.0 mg) given ICV produced dose-related convulsions (Fig. 1A), which appeared a few seconds after the ICV injection and lasted about 20–40 seconds. They usually occurred once after the ICV injection; however, with the largest doses of nicotine (0.5–2.0 mg) they occurred sometimes several times within the first hour after the ICV administration.

Apart from convulsions, ICV nicotine also evoked licking, retching, vomiting, panting, respiratory irregularities, salivation, defecation, micturition, mydriasis, asynchronous twitching of the ears, ataxia, blind charging, ataxia and muscular weakness.

### *Effects of Ganglionic Blocking Substances, Antimuscarinic Drugs, Antihistamine and 5-Hydroxytryptamine Antagonist on Nicotine-Induced Convulsions*

Ganglionic blocking substances (hexamethonium and mecamylamine), antimuscarinic drugs (atropine and scopolamine), an antihistamine (antazoline) and a 5-hydroxytryptamine antagonist (methysergide) were injected into the cerebral ventricles 15–20 sec before the similar injection of nicotine. As shown in Fig. 1B hexamethonium (0.1–1.0 mg) and mecamylamine (0.1 mg) abolished the convulsions induced by nicotine (0.5 mg) while atropine (0.2–0.5 mg), scopolamine (0.2–0.5 mg), antazoline (0.5–1.0 mg) and methysergide (0.1–0.2 mg) had no significant effect.

### *Effects of Alpha and Beta Adrenergic Blocking Agents and Dopamine Antagonists on Nicotine-Induced Convulsions*

In this series of experiments, alpha-(yohimbine, phenoxybenzamine and tolazoline) and beta-(propranolol and practolol) adrenergic blocking agents and dopamine antagonists (chlorpromazine and haloperidol) were injected ICV 15–20 sec before nicotine was injected ICV. Yohimbine (0.5–1.0 mg; n=8), phenoxybenzamine (0.5–1.0 mg; n=8), tolazoline (0.5–1.0 mg; n=8), propranolol (0.5–1.0 mg; n=8), practolol (0.2–0.5 mg; n=8), chlorpromazine (0.5–1.0 mg; n=8) and haloperidol (0.5–1.0 mg; n=8) had virtually no effect on convulsions induced by nicotine (0.5 mg).

### *Effects of Reserpine, 6-Hydroxydopamine, 5,6-Dihydroxytryptamine and Hemicholinium on Nicotine-Induced Convulsions*

The effects of ICV 6-hydroxydopamine and 5,6-dihydroxytryptamine on convulsions evoked by ICV nicotine were evaluated over 10–14 days after 2 days' treatments, while the effect of ICV reserpine on the same response to nicotine was evaluated for 24 hours after its single injection. Hemicholinium was administered ICV twice a day for 5 days.

In cats treated with 6-hydroxydopamine (2.0 mg), 5,6-dihydroxytryptamine (0.4 mg) and hemicholinium (0.5 mg), convulsions caused by ICV nicotine (0.5 mg) were virtually unaltered (Fig. 2). On the other hand, in cats treated only with larger doses of reserpine (0.75 mg), convulsions evoked by nicotine did not occur, while smaller doses of reserpine (0.5 mg) had no significant effect on nicotine-induced convulsions (0.5 mg) (Fig. 2).

In control experiments 0.3 ml of 0.9% NaCl injected ICV into unanesthetized cats (n=4) did not evoke any visible behavioral, autonomic and motor effects or convulsions. However, miaowing was occasionally observed.

## DISCUSSION

Earlier experiments demonstrated that nicotine injected ICV in the cat produces convulsions [2–6, 11, 12]. The results of the present experiments show further that the convulsions evoked by ICV nicotine are dose-related and occur in all cats when the nicotine is injected in a dose of 2 mg.

These observations are in agreement with the findings that nicotine induces convulsions in mammals [17] and that nicotinic ganglionic agonists, i.e., dimethylphenylpiperazinium and tetramethylammonium, injected ICV in unanesthetized cats produce convulsions [6,13].

Classical pharmacological analyses of central effects of nicotine revealed that the vomiting and ear twitching are mediated through central nicotinic receptors [2,12], whereas salivation and panting are mediated through central receptors having mixed nicotinic and muscarinic properties [2,4]. As shown in this study, convulsions evoked by ICV nicotine are abolished by ICV ganglionic blocking substances, while they are not significantly changed by antimuscarinic drugs, a 5-hydroxytryptamine antagonist, alpha- and beta-adrenergic blocking agents, an antihistamine or dopamine antagonists given ICV. Thus, convulsions produced by ICV nicotine appear to be mediated through central nicotonic receptors which corresponds to the finding that ganglionic blocking agents injected parenterally abolish the convulsions evoked by parenteral administration of nicotine in rodents [8, 10, 17, 18]. Further, in the cat the ganglionic blocking agent hexamethonium, but not atropine, inhibits the convulsions induced by ICV nicotinic ganglionic agonist, dimethylphenylpiperazinium [13]. Finally, recent radioligand binding investigations have confirmed the existence of nicotinic receptors in the brain which are associated with the seizure sensitivity to nicotine [14].

The results obtained in cats pretreated with ICV reserpine, 6-hydroxydopamine, 5,6-dihydroxytryptamine and hemicholinium show that only in the animals pretreated with a large ICV dose of reserpine are convulsions produced by ICV nicotine abolished. Reserpine, also in large doses, is ineffective in antagonizing the convulsive action of nicotine in the mouse as well as in the rabbit [7,16]. These findings suggest a species difference with respect to the antagonism of nicotine convulsions evoked by carbachol and electroshock [1,15]. Since reserpine depletes catecholamines as well as 5-hydroxytryptamine and histamine, these results strongly suggest that depressed monoaminergic mechanisms in the brain inhibit the convulsions evoked by ICV nicotine in the cat.

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## REFERENCES

1. Arnold, P. S., R. J. Racine and R. A. Wise. Effects of atropine, reserpine, 6-hydroxydopamine and handling on seizure development in the rat. *Exp Neurol* **40:** 457–470, 1973.
2. Beleslin, D. B., S. K. Krstić and S. Dožić. Central nicotinic receptors: Vomiting, ear twitching and panting. *Brain Res Bull* **11:** 299–302, 1983.
3. Beleslin, D. B., S. K. Krstić, K. Stefanović-Denić, M. Štrbac and D. Mićić. Inhibition by morphine and morphine-like drugs of nicotine-induced emesis in cats. *Brain Res Bull* **6:** 451–453, 1981.
4. Beleslin, D. B., S. K. Krstić and N. Tomić-Beleslin. Nicotine-induced salivation in cats: Effects of various drugs. *Brain Res Bull* **12:** 585–587, 1984.
5. Beleslin, D. B. and Z. S. Malobabić. Catalepsy produced by intraventricular injection of nicotine. *Experientia* **28:** 427, 1972.
6. Beleslin, D. B. and R. Samardžić. Comparative study of aggressive behaviour after injection of cholinomimetics, anticholinesterases, nicotinic and muscarinic ganglionic stimulants into the cerebral ventricles of conscious cats: failure of nicotinic drugs to evoke aggression. *Psychopharmacology (Berlin)* **60:** 147–153, 1979.
7. Chen, G. and B. Bohner. A study of certain CNS depressants. *Arch Int Pharmacodyn* **125:** 1–20, 1960.
8. Edge, N. D., S. J. Corne, G. E. Lee and W. R. Wragg. The ganglion-blocking activity of aminobicyclo-(2,2,1)heptanes (congeners of mecamylamine) and bicyclo(3,2)azaoctanes (bridged congeners of pempidine). *Br J Pharmacol* **15:** 207–214, 1960.
9. Feldberg, W. and S. L. Sherwood. A permanent cannula for intraventricular injection in cats. *J Physiol (Lond)* **120:** 3-5P, 1953.
10. Haas, H. and W. Klavehn. Uber 3,piperidino-1-phenyl-1-bicycloheptenyl-propanol-(1) (Akineton). *Naunyn Schmiedebergs Arch Pharmacol* **226:** 18–35, 1955.
11. Hall, G. H. and E. Reit. Analysis of some central actions of nicotine injected into the cerebral ventricles of cats. *J Physiol (Lond)* **185:** 400–417, 1966.
12. Krstić, S. K., R. Samardžić, B. Terzić, M. Štrbac and D. B. Beleslin. Central nicotinic receptors and the vomiting. *Period Biol* **85:** Suppl 2, 158–159, 1983.
13. Krstić, S. K., R. Samardžić, M. Štrbac and D. B. Beleslin. Nature of dimethylphenilpiperazinium-induced convulsions in cats. *Iugoslav Physiol Pharmacol Acta* **19:** 106–108, 1983.
14. Miner, L. L., M. J. Marks and A. C. Collins. Classical genetic analysis of nicotine-induced seizures and nicotinic receptors. *J Pharmacol Exp Ther* **231:** 545–554, 1984.
15. Radmanović, B., D. Beleslin and L. Grbović. The action of reserpine on the gross behavioural effects of choline esters injected into the cerebral ventricles of unanaesthetized cats. *Iugoslav Physiol Pharmacol Acta* **13:** 279–284, 1977.
16. Reuse, J. J. Modifications de certains effets de la nicotine chez le lapin par l'administration préalable de réserpine. *J Physiol (Paris)* **52:** 209–210, 1960.
17. Silvette, H., E. C. Hoff, P. S. Larson and H. B. Haag. The actions of nicotine on central nervous system functions. *Pharmacol Rev* **14:** 137–173, 1962.
18. Stone, C. A., K. L. Meckelnburg and M. L. Torchiana. Antagonism of nicotine-induced convulsions by ganglionic blocking agents. *Arch Int Pharmacodyn* **117:** 419–434, 1958.