Short Report

Desipramine Subsensitizes Nicotinic Mechanism Involved in Regulating Core Temperature

Abstract. Desipramine HCl, 10 mg/kg i.p. twice daily, produced subsensitivity to the hypothermic effects of nicotine, 1 mg/kg, after 1 and 2 weeks of treatment in male Sprague-Dawley rats. Phenelzine sulfate, fluoxetine HCl, and bright artificial light produced the same effect. The capacity of three chemically distinct classes of antidepressants and bright artificial light (a treatment for seasonal depression) to produce this result suggests that effects on nicotinic mechanisms may be involved in the mechanism of action of these treatments.

Key words. Antidepressants, cholinergic, desipramine, nicotine, tricyclics.

The effects of antidepressants on nicotinic mechanisms have received minimal attention. Shaker et al. (1981) and Schofield et al. (1981) presented evidence that tricyclic antidepressants bind to sites on the ionic channel of the nicotinic receptor (nAchR). We studied the effects of fluoxetine, phenelzine, amitriptyline, lithium, and bright artificial light on a nicotinic mechanism involved in the regulation of core temperature in rats (Dilsaver and Hariharan, in press). We now report the effects of desipramine HCl (DMI) on this mechanism.

Methods

This report includes three experiments involving adult male Sprague-Dawley rats. Experiment 1 involves the measurement of change in core temperature in response to nicotine (base), 1 mg/kg i.p., before and after 1 and 2 weeks of treatment with DMI, 10 mg/kg i.p., at 9 a.m. and 5 p.m. in 12 rats with a mean weight of 319.2 (SD 46.1) g. Experiment 2 was a study of the effects of DMI on the disposition of nicotine in two

samples of 10 rats each. The first group was treated with DMI, 10 mg/kg i.p., at 9 a.m. and 5 p.m. for 14 days. The mean weight of this sample was 236.5 (SD 32.9) g. The second sample received saline injections, 1 mg/kg i.p., at 9 a.m. and 5 p.m. for 14 days. The mean weight of this sample was 288.0 (SD 13.3) g. Experiment 3 was a control experiment in which eight animals with a mean weight of 303 (SD 29.7) g were challenged with nicotine (base), 1 mg/kg i.p., every 7 days for 3 weeks. The mean hypothermic response to nicotine was measured after the first and fourth injections. The objective was to demonstrate that multiple injections of nicotine at the dose used in Experiment 1 do not produce subsensitivity to nicotine.

Measurement of Core Body Temperature.
Telemetric thermosensors (Mini-Mitter Corp., Sun River, OR) were implanted into the peritoneal cavity. Dilsaver et al. (in press) established the validity of this method.

Nicotine Challenge.
Nicotine challenges were conducted at 10 a.m. Challenge 1 occurred the day before treatment started. Challenges 2 and 3 occurred 20 hours after the 14th and 28th doses of DMI. Temperature was measured immediately before and every 10 min after the injection of nicotine (base), 1 mg/kg i.p., for 120 min. Baseline temperature for a given challenge is defined as the core temperature immediately before the injection of nicotine.

Assay of Plasma Levels of Nicotine and Metabolites.
Plasma levels of nicotine and cotinine were determined by a high performance liquid chromatography (HPLC) method using a UV detector (262 nm) and 2-phenylimidazole as an internal standard.

The mobile phase is a citrate-phosphate buffer mixture (0.03 M each) with a final pH of 5.0, and containing 7% acetonitrile and 2 mM solidum hepatanesulphonate. A C-18 Shandon column (Keystone Co., State College, PA) with a length of 150 mm and a diameter of 2 mm is used. The analytes are extracted from alkalinized plasma using methylene chloride. After separation, the organic solvent is evaporated using nitrogen gas. The residue is reconstituted with 50 μl of mobile phase, and 20 μl is injected into the column. The sensitivity of the assay is 1 ng/ml for nicotine and 2 ng/ml for cotinine. The method is linear from 0 to 600 ng/ml for both analytes. The precision of the assay is 5% (coefficient of variation) ($n = 30$) for both substances (Hariharan et al., 1987).

Pharmaceuticals. DMI HCl and nicotine (base) were purchased from Sigma Chemical Company (St. Louis, MO). Doses of DMI and nicotine refer to the salt and base forms, respectively. Both agents were administered i.p. on an mg/kg basis.

Experimental Design—Study 1. This study was divided into two phases. In Phase 1, the thermosensors were implanted into 12 animals, which were allowed a minimum of 5 days to recover. During Phase 2, the hypothermic response to normal saline, 1 mg/kg i.p., was measured. This provides a baseline taking into account the effects of handling and "placebo" injection. The thermic response to nicotine, 1 mg/kg i.p., was subsequently measured. The animals then received twice-daily injections (at 9 a.m. and 5 p.m.) of DMI, 10 mg/kg i.p. Twenty hours after the 14th and 28th doses of DMI, the animals were rechallenged with nicotine.

Experimental Design—Study 2. Ten animals were treated with DMI for 14 days. The animals were then injected with nicotine, 1 mg/kg i.p., between 0930h and 1230h. They were anesthetized with diethyl ether, and 30 min after the injection of nicotine, blood was drawn by cardiac puncture for the determination of plasma nicotine and cotinine levels. These levels were compared to those in the 10 animals treated with saline.

Experimental Design—Study 3. Thermosensors were implanted into eight rats, which were allowed 5 days to recover before experimentation began. The sample then received nicotine, 1 mg/kg i.p., every 7 days for 21 days. The thermic response to this drug was measured every 10 min for 120 min following the first and fourth injections of nicotine.

Statistical Analysis. Data from Experiments 1 and 3 were derived by calculating the mean thermic response across weeks for each rat. Mean temperature change between the challenges for each animal was then used to determine the significance of the mean change in temperature for the sample using Student's paired t test. The significance of the differences in the mean plasma nicotine and cotinine levels in Experiment 2 was determined using Student's two-sampled t test. Measures of variance refer to the SD.

Results

Experiment 1. Mean core temperature of this sample before the first experimental manipulation was 37.1 ± 0.45°C. Table 1 summarizes the results of this experiment. The thermic response to nicotine between 1 and 2 weeks of treatment did not differ ($t = 0.88$, $df = 11$; $p > 0.35$).

Table 1. Experiment 1

Mean thermic response to nicotine	Mean thermic response to nicotine after DMI treatment	
-1.43±0.62°C	**After 1 week:**	-0.58±0.76°C[1]
	After 2 weeks:	-0.80±0.73°C[2]

DMI = desipramine.
1. $t = 3.04$, $df = 11$, $p < 0.05$.
2. $t = 3.56$, $df = 11$, $p < 0.005$.

Experiment 2. The mean nicotine levels in the experimental and control groups were 318.2 (SD 32.9) and 220.3 (SD 48.5) ng/ml, respectively ($t = 5.57$, $df = 18$, $p < 0.00003$). The mean cotinine levels were 87.0 (SD 35.0) and 232.1 (SD 81.9) ng/ml, respectively ($t = 5.22$, $df = 18$, $p < 0.000006$). Thus,

treatment with DMI was associated with highly elevated levels of plasma nicotine. The elevated levels of cotinine in the control sample relative to the experimental group suggest that DMI retarded the metabolism of nicotine.

Experiment 3. The mean hypothermic response to nicotine, 1 mg/kg i.p., was -1.37 (SD 0.23)°C when the sample was first challenged, and -1.32 (SD 0.20)°C at the time of the fourth challenge ($t = 0.67$, $df = 7$, $p > 0.50$). Thus, multiple injections of nicotine did not produce subsensitivity to subsequent challenges.

Discussion

Treatment with DMI produced subsensitivity to nicotine. This effect was not due to a pharmacokinetic factor. Indeed, the DMI sample had a level of plasma nicotine that was 44% higher than that of the saline sample ($p < 0.00003$). Further, the saline sample had a much lower mean ratio of plasma nicotine to cotinine, 1.0 (SD 0.09) as opposed to 4.0 (SD 0.39) in the DMI group ($p < 0.000001$). We can conclude that the development of subsensitivity to nicotine is probably due to a pharmacodynamic effect of treatment with DMI.

Bright artificial light (Dilsaver and Majchrzak, 1988), fluoxetine (Dilsaver and Davidson, 1987), and phenelzine (Dilsaver and Hariharan, in press) also produce subsensitivity to the hypothermic effect of nicotine. Thus, drugs from three chemical classes and a nonpharmacological treatment for depression all blunt the hypothermic response to nicotine. There are two exceptions. Treatment for 1 or 2 weeks with amitriptyline (Dilsaver et al., 1988) and lithium (Dilsaver and Hariharan, in press) produces supersensitivity to nicotine. We suggest that the essential point is not that there are exceptions to the results presented here but that all treatments for depression assessed affect a nicotinic mechanism involved in the regulation of core temperature.

Nicotine promotes the release of norepinephrine in the hypothalamus (Westfall, 1973) and dopamine within the mesolimbic and nigrostriatal tracts (Andersonn et al., 1981). Should an antidepressant enhance aminergic activity, nicotinic mechanisms with which it interacts might become subsensitive. Alternatively, drugs supersensitizing nicotinic mechanisms, such as amitriptyline, might activate aminergic pathways. It is conceivable that both drugs which supersensitize and those which subsensitize nicotinic mechanisms have antidepressant properties. The significance of the phenomenon reported here is yet to be determined. However, the array of antidepressant treatments producing it suggests potential importance.

References

Andersonn, K., Fuxe, K., and Agnati, L.F. Effects of single injections on nicotine in the ascending dopamine pathways in the rat: Evidence for increases of dopamine turnover in neostriatal and mesolimbic dopamine neurons. *Acta Physiologica Scandinavica,* **112,** 345 (1981).

Dilsaver, S.C., and Davidson, R.R. Fluoxetine produces subsensitivity to nicotine. *Life Sciences,* **41,** 1165 (1987).

Dilsaver, S.C., and Hariharan, M. Nicotinic effects of antidepressants. In: Gershon, S., and Lerer, B., eds. *New Directions in Affective Disorders.* Springer-Verlag, New York (in press).

Dilsaver, S.C., and Majchrzak, M.J. Bright artificial light produces subsensitivity to nicotine. *Life Sciences,* **42,** 225 (1988).

Dilsaver, S.C., Majchrzak, M.J., and Alessi, N.E. Amitriptyline produces supersensitivity to nicotine. *Biological Psychiatry,* **23,** 169 (1988).

Dilsaver, S.C., Majchrzak, M.J., and Alessi, N.E. Telemetric measurement of core temperature in psychopharmacological research: Validity and reliability. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* (in press).

Hariharan, M., VanNoord, T., and Greden, J. A rapid and sensitive method for simultaneous quantitation of nicotine and cotinine in biological fluids. Presented at the Annual Meeting of the American Clinical Chemistry Association (1987).

Schofield, G.G., Witlop, B., Wernick, J.E., and Albuquerque, S.T. Differentiation of the open and closed states of the ionic channels of nicotinic acetylcholine receptors by tricyclic antidepressants. *Proceedings of the National Academy of Sciences of the United States of America,* **78,** 5240 (1981).

Shaker, N., Eldefrawi, A.T., Miller, E.R., and Eldetyawi, M.E. Interaction of tricyclic antidepressants with the ionic channel of the acetylcholine receptor of Torpedo electric organ. *Molecular Pharmacology,* **20,** 515 (1981).

Westfall, T.C. Effect of acetylcholine on the release of [³H]norepinephrine by nicotine and potassium chloride from rat brain slices. In: Usdin, E., and Snyder, S.H., eds. *Frontiers of Catecholamine Research.* Pergamon Press, New York, p. 617 (1973).

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