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FLUOXETINE SUBSENSITIZES A NICOTINIC MECHANISM INVOLVED IN THE REGULATION OF CORE TEMPERATURE

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Summary

Fluoxetine HCl, 10 mg/kg ip, twice daily produced subsensitivity to the hypothermic effects of nicotine (base), 1 mg/kg ip, after 1 ($p<0.02$) and 2 ($p<0.002$) weeks of treatment. Phenelzine sulfate, desipramine 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.

Fluoxetine HCl, a selective inhibitor of the uptake of serotonin (1-3), is an effective antidepressant (4-6). Wong, et.al. (7) reported that fluoxetine failed to change the concentration dependent binding of [^{3}H]-quinuclidinyl benzilate to muscarinic receptors, [^{3}H]-clonidine and [^{3}H]-dihydroalprenol to α_2-, and α_1-, and β-adrenergic receptors respectively, and [^{3}H]-pyrilamine to H_1-receptors or [^{3}H]-naloxone to opiate receptors.

The effects of fluoxetine, and other antidepressants on nicotinic mechanisms have received little attention. Schofield, et.al. (8) and Slaker, et.al. (9) presented evidence that amitriptyline, nortiptyline and imipramine bind to sites on the ionic channel of the nicotinic cholinergic receptor (nAchR). We have studied the effects of four different types of antidepressant treatment on a nicotinic mechanism involved in the regulation of core temperature. These treatments include bright artificial light (a treatment for Seasonal Affective Disorder) (10-14), desipramine HCl (a tricyclic antidepressant), phenelzine sulfate (a monoamine oxidase inhibitor), and fluoxetine HCl (a chemically distinct antidepressant which inhibits the uptake of serotonin). We report the effects of fluoxetine on this mechanism here.

Methods and Research Design

This report includes two experiments. Experiment 1 involves the measurement of the change in core temperature in response to challenge with nicotine (base), 1mg/kg ip, before and after 1 and 2 weeks of treatment with fluoxetine HCl, 10 mg/kg ip, at 0900 and 1700 hours. Experiment 2 was a control experiment in which 8 animals were challenged with nicotine (base), 1 mg/kg ip, every 7 days for 3 weeks. The mean hypothermic response to nicotine was measured after the first and fourth injections. The objective of this experiment was to demonstrate that multiple injections of nicotine, at the dose used in Experiment 1, do not produce subsensitivity to nicotine. Both experiments involved adult, male Sprague-Dawley rats. Experiment 1 involved 9 animals weighing 315±8.2 g (mean±SEM). The second experiment involved 8 animals weighing 303±9.4 g (mean±SEM).

Measurement of core body temperature. Model VM Mini-Mitters (Mini-Mitter Corp., Sun River, OR) were surgically implanted into the peritoneal cavity. These instruments emit radio waves at a rate directly proportional to temperature. A transistor radio set to an AM frequency served as a receiver. Time to emit 10 sounds was measured using a digital

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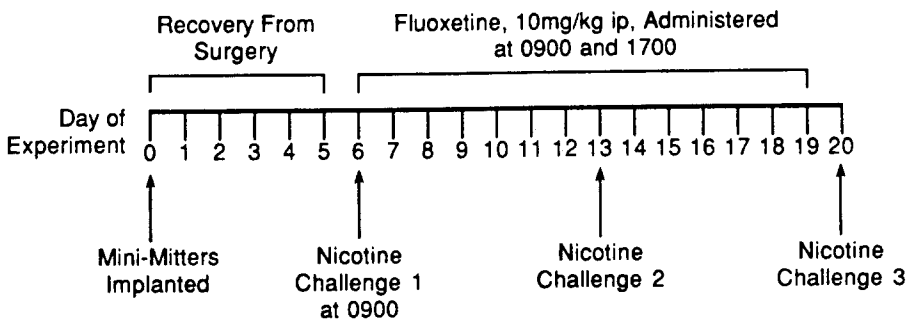
display stop watch. This measurement was converted to temperature using a linear regression equation which was derived by measuring the emission rate of each instrument at three different temperatures in a temperature controlled water bath. Tocco-Bradley, et.al. (14) established the validity of this method.

Nicotine Challenges. Nicotine challenges were conducted at the same time of day so as to control for circadian effects on temperature. The first challenge preceded the initiation of treatment with Fluoxetine by 24 hours. The second and third challenges occurred approximately 20 hours after the 14th and 28th doses of fluoxetine respectively. Temperature was measured immediately prior to and every 10 minutes for 120 minutes after the injection of nicotine, 1 mg/kg ip. Baseline temperature for a given challenge is defined as the core temperature immediately prior to the injection of nicotine.

Pharmaceuticals. Fluoxetine HCl was provided as a gift from Lilly Laboratories. Nicotine was purchased from Sigma Chemical Company, (St. Louis, MO). Doses of fluoxetine and nicotine refer to the salt and base forms respectively. Both agents were administered ip on a mg/kg basis.

Experimental Design: Study 1. This study was divided into three phases. In phase 1, the thermosensors were implanted into 9 animals and they were then allowed 5 days to recover before beginning experimentation. During phase 2, the hypothermic response to saline, 1 mg/kg ip, was measured. The baseline hypothermic response to nicotine, 1 mg/kg ip, was measured the next day. The animals then received twice daily injections (at 0900 and 1700) of fluoxetine, 10 mg/kg ip. Twenty hours after the 14th dose of fluoxetine, the animals were rechallenged with nicotine. They received a third challenge with nicotine 20 hours after the 28th dose (14 days of treatment). Phase 3, a drug withdrawal phase, extended over 7 days. At the end of this withdrawal phase, the animals were once again challenged with nicotine. This allowed assessment of the duration of the effect of fluoxetine on thermic responsiveness to nicotine after its withdrawal.



This figure illustrates the time course of Experiment 1. Mini-Mitters were implanted on Day 0. The animals were given 5 days to recover. The sample was challenged with nicotine on Day 5. This provided baseline data against which data from the other nicotine challenges were compared. Fluoxetine, 10mg/kg ip twice daily, was given on Days 6-19. The animals were rechallenged with nicotine on Days 13 and 20 at 0900.

Experimental Design: Study 2. Thermosensors were implanted into 8 rats and they were allowed 5 days to recover before beginning experimentation. The sample then received nicotine, 1 mg/kg ip, every 7 days for 21 days. The thermic response was measured every 10 minutes for 120 minutes following the first and fourth injections of nicotine.

Statistical Analysis. Data entering into statistical analyses were derived by calculating the mean difference in thermic responsiveness of each animal across weeks. Mean temperature change between challenges for each animal was then used to determine significance of the mean change in temperature for the sample using Student's paired t-test. Availability of data on the thermic response of each animal at 12 time points, before and after treatment with fluoxetine, allowed us to determine whether the responsiveness of individual animals to nicotine was altered using Student's paired t-test. These data are presented for the animals used in Experiment 1. Measures of variance in the text refer to the standard error of the mean (SEM).

Results

Experiment 1: Table I summarizes the data for the sample. Core temperature prior to challenge with saline and the first through fourth challenges with nicotine was 36.5±0.19°C, 37.1±0.18°C,37.1±0.38°C, 36.5±0.14°C and 36.5±0.14°C. The mean thermic response to saline was an increase in core temperature of 0.22±0.10°C. The sample exhibited a change in core temperature of -1.41±0.24°C in response to the first nicotine challenge. This was highly different from the response to saline ($p<0.0002$, $t=6.54$, $df=8$). The sample exhibited a thermic response to nicotine of -0.44±0.26°C following 1 week of treatment with fluoxetine. This differed from the baseline response to nicotine at the 0.002 level ($t = 4.86$, $df = 8$). The thermic response to saline 0.22±0.10°C, and to nicotine 0.16±0.14°C, did not differ after 2 weeks of treatment with fluoxetine ($p>0.70$, $t=0.33$, $df=8$). Finally, the thermic response to nicotine after 1 week of withdrawal was 0.91±0.25°C. This did not differ from baseline ($p>0.15$, $t=1.59$, $df=7$).

Table II summarizes the absolute difference in the mean thermic response after 2 weeks of treatment with fluoxetine, relative to baseline, for each animal. Eight (8) of the 9 animals exhibited blunting of the hypothermic response at $\alpha<0.0006$.

Experiment 2: The mean thermic response to nicotine, 1 mg/kg ip, was -1.37±0.23°C ($n=8$) when the sample was first challenged and -1.32±0.20°C ($n=8$) at the time of the fourth challenge ($p>0.50$, $t=0.67$, $df=7$). Thus, multiple injections did not produce subsensitivity to subsequent challenges.

Discussion

Antidepressants are not recognized as having physiologically or biochemically significant effects on nicotinic mechanisms. However, this report establishes that fluoxetine powerfully subsensitizes a nicotinic mechanism involved in the regulation of core temperature. This effect is not an artifact of multiple injections of nicotine. The relevance of this finding is highlighted by the recent finding that phenelzine sulfate (submitted for publication), desipramine HCl (submitted for publication), and bright artificial light (submitted for publication) produce the same effect. Thus, drugs from 3 distinct chemical classes and a non-pharmacologic treatment of depression all blunt the hypothermic response to nicotine. This suggests that rather than being adventitious, this capacity may be related to the mechanism of action of these drugs.

Systemically (15-17) and centrally (18-20) administered nicotine decreases core body temperature. Core temperature is partially regulated at the level of the hypothalamus. The microinjection of acetylcholine (21,22) or cholinomimetics (23,24) into hypothalamic nuclei dramatically changes core temperature. The authors (15) recently reviewed the literature describing the use of core temperature as a dependent variable in neuropharmacological research and reports of the effects of nicotine, muscarinic agonists and other agents on core temperature.

TABLE I

Thermic Response to Nicotine, 1 mg/kg ip

Animal #	A Saline	B Baseline	C 1 Week	D 2 Weeks
1	0.89±0.17	-0.77±0.28	-0.51±0.12	-0.28±0.10
2	0.12±0.05	-1.56±0.13	-0.85±0.10	0.25±0.08
3	0.03±0.07	-1.23±0.21	-0.74±0.10	-0.26±0.10
4	0.13±0.10	-1.72±0.22	1.30±0.09	-0.24±0.07
5	-0.16±0.05	-1.35±0.09	-0.81±0.12	-0.09±0.07
6	0.23±0.11	-2.32±0.19	-1.30±0.10	0.51±0.10
7	0.36±0.10	-2.50±0.17	-0.93±0.12	0.90±0.09
8	0.29±0.05	-0.85±0.18	0.29±0.12	0.14±0.04
9	0.06±0.16	-0.36±0.27	-0.45±0.30	0.50±0.14
Mean±SEM	0.22±0.10	-1.41±0.24	-0.44±0.26	0.16±0.14

Probability Statements

	Absolute Change		
A > B	1.63±0.25	p<0.0002	t=6.53
C < B	0.97±0.31	p<0.02	t=3.15
D < B	1.57±0.32	p<0.002	t=4.86
A = D	0.05±0.17	n.s.	t=0.33

TABLE II

Thermic Responsiveness of Individual Animals

(Thermic Response to Nicotine at Baseline) - (Thermic Response to Nicotine after 2 Weeks of Treatment with Fluoxetine)

Animal #	Absolute Mean Difference	SEM	p <	t =	df
1	0.11	0.16	n.s.	1.11	11
2	1.82	0.17	0.000001	10.86	11
3	0.99	0.21	0.0006	4.83	11
4	1.36	0.11	0.000001	11.54	11
5	1.28	0.15	0.000003	8.79	11
6	1.47	0.18	0.000008	7.89	11
7	2.86	0.21	0.000001	13.48	11
8	3.40	0.14	0.000001	24.14	11
9	1.06	0.19	0.0002	5.46	11

Nicotine promotes the release of norepinephrine in the hypothalamus (25) and of dopamine within the mesolimbic and nigrostriatal tracts (26.) It is conceivable that the development of subsensitivity to the hypothermic response to nicotine is an epiphenomenon. That is, should treatment with antidepressants enhance noradrenergic and dopaminergic neurotransmission, related nicotinic mechanisms which may serve a compensatory role when these monoaminergic networks are dampened or inefficient, may become subsensitive. Determining whether the effect is an epiphenomenon or of mechanistic importance will require further evaluation. The array of antidepressant treatments producing this effect highlights potential importance!

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