Chronic Treatment with Amitriptyline Produces Supersensitivity to Nicotine

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The authors used a thermoregulation paradigm to evaluate effects of amitriptyline (AMI) on the sensitivity of a nicotinic mechanism involved in the regulation of core temperature in rats. Treatment with this tricyclic was associated with a significant increase in the hypothermic response to nicotine. Supersensitivity persisted for a minimum of 7.5 days following the last dose of AMI, and a significant proportion of animals displayed increased sensitivity after 14.5 days of abstinence. Implications for the mechanism of action of AMI are highlighted.

Introduction

Tricyclic antidepressants (TCAs) directly bind to muscarinic acetylcholine receptors (mAchRs) and produce blockade of muscarinic mechanisms (Dilsaver 1986a). Supersensitization of these mechanisms is an effect of agents that directly block access of acetylcholine (Ach) to the postsynaptic mAchR or inhibit its release (Dilsaver et al. 1987). We reported that amitriptyline (AMI), 10 mg/kg twice daily for 12 days, increased sensitivity to the hypothermic effects of oxotremorine (OXO) in rats (Dilsaver et al. 1987). Although their muscarinic effects have been thoroughly investigated, influences of these agents on parameters influenced by nicotinic mechanisms have received little attention.

Nicotinic and muscarinic agonists may act at receptor sites that have mixed muscarinic–nicotinic responsiveness (Morley and Kemp 1981). Westfall (1973) observed that a muscarinic agonist blocked the release of norepinephrine in the rat hypothalamus, whereas a nicotinic agent increased its release. Thoenen et al. (1973) reported that increased preganglionic (i.e., nicotinic) neurotransmission enhanced the activity of tyrosine hydroxylase in noradrenergic neurons. Increased nicotinic neurotransmission lasting 60 min increased the level of tyrosine hydroxylase activity 48 hr later. The findings that nicotine releases catecholamines and that nicotinic neurotransmission increases the activity of the enzyme governing the rate-limiting step in catecholamine synthesis are consistent with the hypothesis that decreased noradrenergic and dopaminergic function are involved in the pathophysiology of depressive disorders.

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Methods

This report includes three experiments. Experiments 1 and 2 involved the measurement of change in core temperature in response to challenge with nicotine (base), 1 mg/kg, ip, before and after treatment with AMI, 15 mg/kg, ip, twice daily for 7 days. The second experiment replicated a portion of the first. A third experiment involved measurement of the hypothermic response to four doses of nicotine (base), 1 mg/kg, which were injected at 7-day intervals. This experiment was based on the possibility that multiple injections of nicotine could produce carryover effects that confound our data. All three experiments involved adult male Sprague-Dawley rats. Experiments 1 and 2 required single samples that weighed 294 ± 1.1 g (mean ± SEM, n = 10) and 289 ± 6.7 g (mean ± SEM, n = 7), respectively. The third experiment involved 8 animals weighing 303 ± 9.4 g.

Measurement of Core Body Temperature

Model VM Mini-Mitters (Mini-Mitter Co., Sun River, OR) were surgically implanted into the peritoneal cavity. These instruments emit radio waves at a rate 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 display stopwatch. This measurement was converted to temperature using a linear regression equation that was derived by measuring the emission rate of each instrument at three different temperatures in a temperature-controlled water bath. Tocco-Bradley et al. (1985) established the validity of this method.

Nicotine Challenge

Nicotine challenges were conducted at the same time of day. The first challenge preceded the initiation of treatment with AMI by 24 hr. The second challenge occurred 12 hr after the last dose of AMI. Temperature was measured immediately prior to and every 10 min after the injection of nicotine (1 mg/kg). Baseline temperature for a given challenge is defined as the temperature immediately prior to the injection of nicotine. Temperature was recorded every 10 min for 120 min.

Pharmaceuticals

AMI and nicotine were purchased from Sigma Chemical Company (St. Louis, MO). Nicotine doses refer to the base form. Dose of AMI refers to the salt form. Both agents were administered intraperitoneally on a milligram per kilogram basis.

Experimental Design: Study 1

This study was divided into three phases. In Phase I, the thermosensors were implanted into 10 animals, and the animals were then allowed 5 days to recover. During Phase II, the baseline hypothermic response to nicotine (1 mg/kg, ip) was measured. The animals then received twice daily injections (at 12-hr intervals) of AMI, 15 mg/kg. Twelve hours after the 14th dose of AMI, the animals were rechallenged with nicotine. Phase III, a drug withdrawal phase, extended over 14 days. During this period, nicotine challenges 3 and 4 were conducted. This allowed assessment of the duration of any effect of AMI treatment on the hypothermic response to nicotine after the TCA was discontinued.

Experimental Design: Study 2

This experiment included Phases I and II without modification, but Phase III was omitted.

Experimental Design: Study 3

Thermosensors were implanted into 8 rats, and the animals were allowed 5 days to recover. The sample then received nicotine (1 mg/kg, ip) every 7 days for 21 days. The thermic response was measured every 10 min for 120 min following the first and fourth injections of nicotine.

Statistical Analysis

Data from experiments 1 and 2 entering into statistical analyses were derived by calculating the difference between the thermic response between corresponding time points across weeks of the study. Change in temperature at each of the 12 points, difference in mean temperature change between challenges for each animal and each sample, and the mean difference in the maximum hypothermic response between challenges were assessed for significance. Mean change in core temperature was the dependent variable in experiment 3.

The Student's paired *t*-test was used to assess level of significance in mean response at each of the 12 time points in experiments 1 and 2. Availability of data on the thermic response of each animal at 12 time points before and after treatment with AMI allowed us to determine whether or not the responsiveness of individual animals to nicotine was altered using Student's paired *t*-test. Significance of the difference in mean response was also determined using Student's paired *t*-test in experiment 3.

The binomial test was applied to calculate the probability of a given number of animals exhibiting statistically significant responses at any given level of α. Siegel (1956) outlined the principles governing the use of this test. The Sign test was used to determine the probability of a sample exhibiting an increase to the hypothermic response to nicotine at a given proportion of time points (Siegel 1956).

Results

Experiment 1

Mean maximum hypothermic response. One week of treatment with AMI was associated with a significant increase in the mean maximum hypothermic response of 1.21 ± 0.12°C (mean ± SEM; $p < 0.001$). The mean maximum response remained elevated 1 (1.31 ± 0.23°C; $p < 0.001$) and 2 (1.00 ± 0.35°C; $p < 0.02$) weeks after the discontinuation of AMI.

Change in the hypothermic response at individual time points. One week of treatment with AMI was associated with a significant enhancement of the hypothermic response 10 ($p < 0.02$), 30 ($p < 0.05$), 40 ($p < 0.01$), 50 ($p < 0.001$), 60 ($p < 0.05$), and 120 ($p < 0.01$) minutes after the injection of nicotine. There was an increase in the hypothermic response at all 12 time points ($p = 0.002$, Sign test).

There was significant enhancement of the hypothermic response at three points after 1 week of abstinence ($p = 0.0029$, binomial test) and an increase in the response at all 12 points ($p = 0.0002$, Sign test). No point exhibited a significantly enhanced hypo-

thermic response after 14.5 days of abstinence, but the hypothermic response was increased at 9 points ($p = 0.045$, Sign test).

Change in the hypothermic response across weeks. Change in the thermic response to nicotine over the 4 weeks of this experiment was evaluated by assessing the significance of the mean reduction in core temperature relative to baseline. Mean reduction in core temperature (°C) after 7 days of treatment with AMI relative to the pre-AMI baseline was 0.46 ± 0.15°C ($p < 0.02$); after 7.5 days of abstinence, it was 0.54 ± 0.20°C ($p < 0.05$); and after 14.5 days of abstinence 0.19 ± 0.19°C (NS). Tables 1–3 summarize data of the mean hypothermic response for each week of experiment 1 for each animal and the sample as a whole.

Experiment 2

Maximum hypothermic response. Treatment with AMI was associated with an increase in the mean maximum hypothermic response to nicotine of 0.69 ± 0.16°C (mean ± SEM; $p < 0.001$). The maximum response to nicotine was greater after treatment in all animals ($p = 0.0039$, Sign test).

Change in the hypothermic response across time. The sample demonstrated an increase in the hypothermic response across time of 0.52 ± 0.10°C (mean ± SEM; $p < 0.01$).

Experiment 3

The mean hypothermic response to nicotine was 1.37 ± 0.23°C (n = 8) when the sample was first challenged with nicotine (1 mg/kg, ip) and 1.32 ± 0.20°C (n = 8) after the fourth challenge (NS, $t = 0.67$, df = 7).

Table 1. Mean Reduction in Core Body Temperature (°C) (Average for Each 10-min Point from 10 to 120 min Postinjection of Nicotine) ± SEM in Response to Nicotine (1 mg/kg, ip) 12 hr after the Discontinuation of a Course of Amitriptyline Hydrochloride (15 mg/kg, ip, Twice Daily for 7 Days) Relative to the Preamitriptyline Baseline

Animal no.	Mean	SEM	df	*t*	*p*
1	−0.44	0.04	11	−2.93	<0.02
2	−1.06	0.05	11	−8.83	<0.001
3	+0.21	0.18	11	+0.95	NS
4	−0.75	0.08	11	−4.69	<0.01
5	−0.36	0.16	11	−2.4	<0.05
6	−0.25	0.14	11	−1.79	NS
7	−0.57	0.09	11	−2.48	<0.05
8	+0.34	0.18	11	+1.54	NS
9	−0.49	0.15	11	−3.30	<0.02
10	−1.20	0.15	11	−13.30	<0.001
Mean ± SEM = −0.46 ± 0.15			9	−3.07	<0.02

The 98% confidence interval for this mean is 0.46 ± 0.42.

The probability of 7 or more animals out of 10 exhibiting enhanced responses to nicotine is 6.6×10^{-10} when the critical value of *t* is set to $\alpha < 0.05$ (Siegel 1956).

Table 2. Mean Reduction in Core Body Temperature (°C) (Average for Each 12 Time Points from 10 to 120 min ± SEM) in Response to Nicotine (1 mg/kg, ip) 7.5 Days after the Last Dose of Amitriptyline Hydrochloride

Animal no.	Mean	SEM	df	*t*	*p*
1	+0.27	0.18	11	+1.5	NS
2	−0.63	0.15	11	−4.20	<0.01
3	−0.61	0.11	11	−5.08	<0.001
4	−0.47	0.16	11	−2.76	<0.001
5	+0.04	0.21	11	+0.19	NS
6	−0.08	0.07	11	−1.00	NS
7	−1.68	0.20	11	−8.4	<0.001
8	+0.44	0.21	11	+2.55	(<0.05)
9	−1.02	0.20	11	−5.1	<0.001
10	−0.81	0.19	11	−4.26	<0.01
Mean ± SEM = 0.54 ± 0.20			9	−2.70	<0.05

The 95% confidence interval for this mean is 0.54 ± 0.45.

The probability of 6 or more animals out of 10 exhibiting significant enhancement of hypothermic responses (at $\alpha < 0.05$) to nicotine is 4.6×10^{-8}.

The probability of 1 or more animals exhibiting a significant rise in the thermic response to nicotine is 0.25.

Discussion

These data indicate that AMI produces supersensitivity to nicotine. This effect occurs within 7 days of starting treatment with AMI and slowly decays after it is discontinued. Although the mean hypothermic response relative to the pre-AMI baseline was not significant after 14.5 days of abstinence, 5 of 10 animals still exhibited a significant increase in the hypothermic response ($p = 2.2 \times 10^{-6}$, binomial test) (Siegel 1956). Furthermore,

Table 3. Mean Reduction in Core Body Temperature (°C) (Average for Each 12 Time Points from 10 to 120 min ± SEM) in Response to Nicotine (1 mg/kg, ip) 14.5 Days after the Last Dose of Amitriptyline Hydrochloride

Animal no.	Mean	SEM	df	*t*	*p*
1	+0.44	0.19	11	+2.44	(<0.05)
2	−0.11	0.27	11	−0.38	NS
3	−0.91	0.17	11	−4.3	<0.01
4	−0.54	0.16	11	−2.8	<0.02
5	+0.95	0.12	11	+6.39	(<0.001)
6	−1.32	0.35	11	−8.25	<0.001
7	−0.20	0.09	11	−2.22	<0.05
8	+0.86	0.20	11	+3.44	(<0.01)
9	−0.01	0.16	11	−1.0	NS
10	−1.06	0.22	11	−5.36	<0.001
Mean ± SEM = 0.19 ± 0.19			9	−0.79	NS

The probability of 5 or more animals out of 10 exhibiting significant enhancement of the hypothermic responses to nicotine is 2.17×10^{-6}.

The probability of 3 or more animals exhibiting a significant rise in the thermic response to baseline is 0.003.

the maximum hypothermic response remained elevated. This suggests that treatment with AMI produced prolonged supersensitivity to the hypothermic effects of nicotine. Observation that the injection of nicotine at 7-day intervals did not produce a carryover effect suggests that the decrease in thermic responsiveness following the discontinuation of AMI is due to the decay of a pharmacological effect of the TCA and not an artifact of design.

Schofield et al. (1981) found that AMI and nortriptyline produced a voltage- and time-dependent decrease in the peak amplitude of the end-plate current in frog skeletal muscle. They proposed that the primary site of action is the ion channel. Similarly, Slaker et al. (1981) presented evidence that imipramine binds to sites on the ionic channel of the nicotine receptor of the Torpedo electric organ. However, the results reported here are, to our knowledge, the first indicating that a TCA alters a nicotinic mechanism in a mammalian species.

Nicotine promotes the release of dopamine in the nigrostriatal and mesolimbic tracts (Andersonn et al. 1981) and triggers the release of norepinephrine in the hypothalamus (Westfall 1973). Activation of mAchRs in the peripheral ganglia increases the activity of tyrosine hydroxylase (Thoenen et al. 1973). Thus, the findings reported here are consistent with the hypothesis that TCAs potentiate monoaminergic mechanisms via effects on nicotinic mechanisms.

It would be advantageous to study the hypothermic effects of nicotine in animals in which a highly effective, yet nontoxic, nicotinic antagonist of nicotinic receptors in the periphery is employed. An agent with this selectivity may not exist. Ganglionic blockade produces vasodilitation, which can produce heat loss. Secondly, it is important to determine the degree to which our findings can be applied to other somatic treatments and physiological paradigms before their importance can be determined.

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