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Amitriptyline-Induced Supersensitivity of a Central Muscarinic Mechanism: Lithium Blocks Amitriptyline-Induced Supersensitivity

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Abstract. Chronic treatment with amitriptyline produces dose-dependent supersensitivity of a central muscarinic cholinergic mechanism involved in the regulation of core body temperature. The authors demonstrated that chronic treatment with lithium prevents the induction of this response. The potential clinical and theoretical significance of this finding is set forth.

Key Words. Affective disorders, amitriptyline, cholinergic, depression, lithium, mania, muscarinic.

Amitriptyline (AMI) produces dose-dependent supersensitivity of a central muscarinic mechanism (Dilsaver et al., 1987; Dilsaver and Snider, 1988). Paradoxically, supersensitivity of muscarinic systems is implicated in the pathophysiology of depression (Janowsky et al., 1972; Dilsaver, 1986*a-d*). Lithium (Li^+) potentiates (de Montigny et al., 1983; Heninger et al., 1983) the therapeutic effects of tricyclic antidepressants (TCAs). Dilsaver (1984) proposed that Li+ might prevent the supersensitization of muscarinic networks. This hypothesis was subjected to a test by measuring the influence of Li^+ on the capacity of AMI to affect supersensitivity to the hypothermic effects of oxotremorine.

Methods

The dependent variable in the experiments below is change in core temperature in response to oxotremorine (OXO). Core temperature was measured using a telemetric thermosensor, the model VM Mini-Mitter (Mini-Miter Co., Sun River, OR). These instruments, which were implanted into the peritoneal cavity of adult male Sprague-Dawley rats, emit radio waves at a rate proportional to temperature. A transistor radio set to an AM frequency served as receiver. Time to emit 10 sounds was measured using a digital display stopwatch. This measure was converted to temperature using a linear regression equation derived by measuring the emission profile of each individual thermosensor in a temperature-controlled water bath. This procedure is sensitive to a 0.1 °C change in temperature (Tocco-Bradley et al., 1985). Dilsaver and Majchrzak (in press) established the reliability and validity of this methodology, in psychopharmacological research paradigms.

OXO challenges were started between 9 and 10 a.m., 17 hours after the evening dose of AMI

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and/or LiCl. Methylscopolamine nitrate, 1 mg/kg i.p., was given 30 min before the injection of OXO to block its peripheral effects. Baseline temperature was measured 30 min after injecting methylscopolamine. Baseline core temperature was defined as the postmethylscopolamine measurement. This was the measure of core temperature (before OXO challenge) used in the data analysis. OXO was given after the measurement of baseline temperature. Temperature was recorded every 10 min for 120 min after the injection of OXO. Core temperature was measured at baseline (before the injection of OXO) and every 10 min thereafter for 120 min. The mean thermic response is the average of the 12 measurements 10, 20, 30, ..., 120 min following the injection of OXO relative to baseline. Two doses of OXO, 1 mg/kg and 0.25 mg/kg i.p., were used. The use of two doses of a drug allows one to illustrate that an effect is not peculiar to a given dose.

OXO (base), AMI HCl, and scopolamine methylnitrate were purchased from Sigma Chemical Company (St. Louis, MO). Lithium chloride (LiCl) was obtained from Fisher Scientific. Doses of OXO refer to the base form. Doses of all other drugs are expressed as the salt form. All drugs were given intraperitoneally (i.p.) on a milligram per kilogram (mg/kg) basis. The doses of LiCl and AMI were always 160 mg/kg (3.77 mEq/kg) and 10 mg/kg i.p. These drugs were always given at 9 a.m. and 5 p.m.

Data were assessed for significance using Student's paired t test. The mean hypothermic response of each animal entered into the calculation of t statistics. Measures of variance refer to the standard deviation.

Peak and trough plasma levels of Li^+ were measured in separate groups of Sprague-Dawley rats given LiCl, 160 mg/kg i.p., for 7 and 14 days. Blood was collected by cardiac puncture. Levels were determined by atomic absorption spectrophotometry in the Clinical Laboratory of the Department of Psychiatry at the University of Michigan. This assay has intra-assay and interassay coefficients of variation of 4.2% and 5.0%, respectively.

Results

Experiment 1 (Determination of plasma trough and peak Li^+ levels after treatment with LiCl for 7 or 14 days): (1) Eleven ($n = 11$) rats weighing 232.7 ± 15.6 g were treated with LiCl for 7 days. Blood was drawn 16 hours after the 14th dose on day 8 by cardiac puncture for the measurement of the trough Li^+ level. (2) Nine ($n = 9$) rats weighing 228.3 ± 15.9 g were similarly treated. These animals were individually given LiCl, 160 mg/kg i.p., at 2-min intervals between 8:50 a.m. and 9:10 a.m. on day 8. Blood was drawn 60 min after injection for determination of peak plasma levels. (3) Trough levels were measured in 16 rats weighing 267.5 ± 15.2 g treated with LiCl for 14 days. Blood was drawn 16 hours after the 28th dose of Li^+. (4) Eleven ($n = 11$) rats weighing 254.6 ± 24.5 g were given LiCl for 14 days. Blood was collected in the manner described above for determination of peak Li^+ levels.

The mean peak and 16 hour trough Li^+ levels following 7 days of treatment with LiCl were 3.10 ± 0.39 ($n = 9$) and 0.25 ± 0.10 ($n = 11$) mEq/l, respectively. The corresponding levels after 14 days of treatment were 3.31 ± 0.76 mEq/l ($n = 11$) and 0.72 ± 0.20 mEq/l ($n = 16$), respectively. These results are also presented in Table 1.

Experiment 2 (Illustration that Li+ blocks the capacity of AMI to produce supersensitivity to OXO): LiCl, 160 mg/kg i.p., was given to six rats weighing 346.7 ± 9.1 for 7 days. On the morning of the 8th day (18 hours after the last dose of LiCl), the animals were challenged with OXO, 1 mg/kg i.p. The rats were then treated with a combination of LiCl and AMI, 10 mg/kg i.p., for 7 days. The hypothermic response to OXO was measured on the morning of the 15th day. This experiment assessed whether treatment with Li^+ followed by concurrent treatment with Li^+ and AMI is associated with a lack of supersensitization to the thermic effects of OXO.

Table 1. Peak and trough lithium levels (Experiment 1)

Treatment with LiCl[2]	Type of level	*n*	Level[1] (mEq/l)
7 days	Trough	11	0.25 ± 0.10
7 days	Peak	9	3.1 ± 0.39
14 days	Trough	11	3.31 ± 0.76
14 days	Peak	16	0.72 ± 0.20

1. Mean ± SD.
2. 160 mg/kg at 9 a.m. and 5 p.m.

Mean core temperature of the sample at baseline was 37.1 ± 0.5 °C. The mean thermic response to OXO, 1 mg/kg i.p., at baseline was -1.4 ± 1.0 °C. The thermic response to OXO, 1 mg/kg i.p., after 7 days of treatment with Li^+ alone was -1.3 ± 0.7 °C (net change in core = 0.1 °C, $t = 0.25$, $df = 5$, $p > 0.8$). Thus, treatment with LiCl did not alter the thermic response to OXO.

This sample used in this experiment began concurrent treatment with LiCl and AMI following 7 days of treatment with LiCl. The mean thermic response to OXO after 1 week of concurrent treatment was -0.90 ± 0.8 °C. This did not differ from the response exhibited during treatment with LiCl alone ($t = 0.85$, $df = 5$, $p > 0.40$). This is remarkable given that the regularly recurring effect of treatment with AMI (10 mg/kg i.p. twice daily) is the development of supersensitivity to OXO (Dilsaver et al., 1987; Dilsaver and Snider, 1988).

Experiment 3 (Illustration that the concurrent administration of Li^+ blocks the capacity of AMI to produce supersensitivity to OXO): This experiment involved the co-administration of LiCl and AMI to seven rats weighing 341.1 ± 29.1 g. The animals were challenged with OXO, 1 mg/kg, before and after 1 week of concurrent treatment with Li^+ and AMI.

Mean core temperature of the sample at baseline was 37.8 ± 0.5 °C. The baseline thermic response to OXO, 1 mg/kg i.p., was -1.4 ± 0.9 °C ($n = 7$). The sample exhibited a mean thermic response of -1.0 ± 2.38 °C after 1 week of concurrent treatment with LiCl and AMI ($t = 0.47$, $df = 6$, $p > 0.65$). Thus, the simultaneous treatment of rats with LiCl and AMI was not associated with the development of supersensitivity to OXO.

Experiment 4 (Illustration that chronic treatment with AMI produces supersensitivity to OXO): This involved the administration of AMI to 10 rats weighing 272.5 ± 18.3 g. These rats were challenged with OXO, 0.25 mg/kg, before treatment with AMI and on the morning of the 8th day of the experiment (i.e., 18 hours after the last dose of AMI). The objective was to demonstrate that treatment with AMI produces supersensitivity to OXO.

Mean core temperature at baseline was 37.0 ± 0.3 °C. Before treatment, the sample exhibited a mean thermic response to OXO of -0.3 ± 0.79 °C. One week of treatment with AMI increased the thermic response to -1.1 ± 0.6 °C ($t = 3.65$, $df = 9$, $p < 0.006$). Thus, treatment with AMI produced supersensitivity to OXO.

Experiment 5 (Illustration that chronic treatment with Li^+ blocks the capacity of AMI to produce supersensitivity to OXO using a second dose of OXO): LiCl and AMI were simultaneously given to 13 rats weighing 243.5 ± 31.4 g. The objective was to demonstrate that concurrent treatment with Li^+ blocks the capacity of AMI to produce supersensitivity to OXO, 0.25 mg/kg.

Mean core temperature of the sample at baseline was 37.7 ± 0.7 °C. The mean thermic response to OXO, 0.25 mg/kg i.p., before treatment was -0.9 ± 0.54 °C. The sample exhibited a mean thermic response of -0.8 ± 0.7 °C 1 week after concurrent treatment with AMI and Li^+. This did not differ from the response to OXO before treatment ($t = 0.22$, $df = 11$, $p > 0.80$).

Experiment 6 (Illustration that Li^+ does not alter the thermic response to OXO): The objective of this experiment was to demonstrate that treatment with Li^+ does not alter the thermic response to OXO. LiCl was given to rats weighing 250.0 ± 19.0 ($n = 10$). These animals were challenged with OXO (base), 0.25 mg/kg i.p., before and after 7 days of treatment.

Mean core temperature at baseline was 38.2 ± 0.41 °C. The mean thermic response to OXO, 0.25 mg/kg, was -0.70 ± 0.79 °C. The mean response to OXO following 1 week of treatment with Li^+ was -0.56 ± 1.0 °C ($t = 0.75$, $df = 9$, $p > 0.47$). Thus, treatment with Li^+ alone did not alter the thermic response to OXO.

Table 2 outlines the effects of the various treatments on the thermic response to OXO.

Table 2. Effects of various treatments on thermic response to oxotremorine (OXO)

Experiment	Mean core temperature at baseline (°C)	Pretreatment	Treatment	OXO dose	Mean hypothermic response to OXO at baseline (°C)	% of hypothermic response to OXO relative to baseline	*p*
2	37.1 ± 0.5	Li	Li+AMI	1.0	1.4 ± 1.0	64.3%	>0.40
3	37.8 ± 0.5	None	Li+AMI	1.0	1.4 ± 0.9	71.4%	>0.65
4	37.0 ± 0.3	None	AMI	0.25	0.3 ± 0.79	366.7%	<0.006
5	37.7 ± 0.7	None	Li+AMI	0.25	0.9 ± 0.54	88.9%	>0.80
6	38.2 ± 0.41	None	Li	0.25	0.7 ± 0.79	80.0%	>0.47

Note. Table 2 presents the results of treating rats with lithium (Li^+) for 1 week followed by the simultaneous administration of Li^+ and amitriptyline (AMI) for 1 week (OXO dose 1.0 mg/kg i.p.) (Experiment 2), lithium and AMI simultaneously (Experiment 3), AMI alone (Experiment 4), Li^+ and AMI simultaneously (OXO dose 0.25 mg/kg i.p.) (Experiment 5), or Li^+ alone (Experiment 6). Only the sample (Experiment 4) treated with AMI alone developed supersensitivity to OXO. Note that a hypothermic response of 1.4 ± 1.0°C (Experiment 2) is a thermic response of -1.4 ± 1.0°C.

Discussion

These data indicate that treatment with Li^+, 3.77 mEq/kg i.p. at 9 a.m. and 5 p.m., has no effect on the thermic response to OXO, 1.0 mg/kg i.p. (Experiment 2) or 0.25 mg/kg i.p. (Experiment 6). They also illustrate that treatment with AMI, 10 mg/kg i.p. at 9 a.m. and 5 p.m., produces supersensitivity to the hypothermic effects of OXO, 0.25 mg/kg i.p. (Experiment 4). We established that treatment with AMI produces dose-dependent supersensitivity of a central muscarinic mechanism to OXO (Dilsaver et al., 1987; Dilsaver and Snider, 1988). Thus, the results reported in this article indicate that Li^+ blocks the capacity of AMI to supersensitize this mechanism.

Lerer (1985) reported that Li^+ enhanced the hypothermic response to pilocarpine in rats treated with scopolamine. The study reported here is *not* a failure to replicate his

findings. Both AMI and scopolamine are muscarinic antagonists, but they do have unshared properties. For example, AMI and its principal metabolite inhibit the reuptake of amines. It is conceivable that there is an interaction between Li^+ and AMI which does not exist between scopolamine and Li^+.

Lerer (1985) gave scopolamine, 15 mg/kg/day, by a osmotic mini-pump to rats. This is equivalent to 39 m*M*/kg of scopolamine daily. In contrast, we injected AMI, 66 m*M*/kg/day. On a molar basis, our animals received 1.7 times more muscarinic receptor antagonist than did Lerer's animals. However, Snyder and Yamamura (1977) estimated that the affinity of scopolamine for the muscarinic receptor (mAchR) in rat brain is 33.3 times greater than that of AMI. Thus, Lerer's animals received a much higher dose of mAchR antagonist. This raises the possibility that Li^+ might block the capacity of a lower dose of scopolamine to supersensitize the mAchR. It is important to note that the administration of Li^+ by osmotic mini-pump results in a constant blood level of the mAchR antagonist. The mean Li^+ blood level in Lerer's sample was 0.86 mEq/l (range 0.48-1.36 Eq/l). The administration of LiCl by a bolus injection twice daily results in a high peak level (> 3.1 mEq/l). This could affect the capacity of Li^+ to stabilize the receptor.

The findings reported here have potential clinical relevance. Reports indicate that Li^+ potentiates the efficacy of tricyclic antidepressants (TCAs) (de Montigny et al., 1983; Heninger et al., 1983; Charney et al., 1984, Price et al, 1984). Investigators have proposed that this is due to activation of presynaptic serotonergic mechanisms (Heninger et al., 1983; Charney et al., 1984). This may be a factor, but other effects of Li^+ could be contributory.

Chronic treatment with TCAs produces supersensitivity of a central muscarinic mechanism (Dilsaver et al., 1987; Dilsaver and Davidson, 1987; Dilsaver and Snider, 1988). Hyperfunction of central muscarinic mechanisms may be involved in the biology of depression (Janowsky et al., 1972; Dilsaver, 1986*a-d*). Cohen and Baldessarini (1985) reported that some patients developed tolerance to the antidepressant effects of TCAs. The capacity of TCAs to supersensitize muscarinic mechanisms may exacerbate a pathophysiological defect underlying melancholia. The capacity of Li^+ to block supersensitization of muscarinic mechanisms by TCAs could contribute to its capacity to potentiate their action.

Levy et al. (1982) reported that Li^+ prevents atropine-induced up-regulation of mAchRs in vivo. Pestronk and Drachman (1980) found that Li^+ reduced the proliferation of extrajunctional nicotinic receptors in denervated skeletal muscle in vivo. However, Tollefson and Senogles (1983) concluded that Li^+, 1 mEq/l in vitro, caused a 50% ($p < 0.05$) reduction in affinity and a 23% decrease ($p < 0.10$) in β_{max} of ^{3}H-quinuclidinyl benzilate binding sites in homogenates of the human caudate nucleus. Dilsaver (1984) thoroughly reviewed the often incongruous literature describing the effects of Li^+ on cholinergic mechanisms in vivo and in vitro. He proposed that in vivo studies simulating the clinical situation are of primary importance and suggested that if an agent which supersensitizes muscarinic mechanisms were coadministered with Li^+ that the latter would be revealed to stabilize the mAchR. The results obtained in the in vivo experiments reported here support this idea.

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