Stress Induces Supersensitivity of a Cholinergic System in Rats

Steven C. Dilsaver, R. Michael Snider, and Norman E. Alessi

Introduction

Cholinergic system supersensitivity may be involved in the pathophysiology of depressive disorders (Janowsky et al. 1972; Dilsaver and Gre- den 1984; Dilsaver 1986). Associations between stressful events and onset of depressive (Lloyd 1980) and manic (Kennedy et al. 1983) episodes are documented. Janowsky et al. (1983) proposed that stress increases the sensitivity of central cholinergic mechanisms and that the latter mediates some neurobiological effects of stress. Despite these points, animal models linking the physiologies of depression, mania, stress, and cholinergic systems are not available.

Thermoregulation is subject to muscarinic cholinergic control at the hypothalamic level (Lomax et al. 1964). Lomax and Jenden (1966) reported that oxotremorine produces dose-dependent hypothermia in rats. We utilized this fact to evaluate the effect of stress on a central muscarinic receptor mechanism.

Methods

Temperature Measurement

Thermosensors (Mini-Mitter Co., Sun River, OR) were implanted into the intraperitoneal cavity. The thermosensors emit hertzian waves at a rate proportional to temperature. A transistor radio set to an AM frequency served as a receiver. Time to emit 25 sounds or "clicks" was measured using a digital display stopwatch. This measure was converted to temperature using a linear regression equation, which was derived by measuring the emission rate of the thermosensors at three temperatures in a temperature-controlled water bath. This instrument allows the accurate detection of a change in temperature of 0.1°C. Details pertaining to the calibration and use of the thermosensor are available elsewhere (Tocco-Bradly et al. 1985).

Oxotremorine Challenge

All oxotremorine challenges were conducted between 11:00 AM and 2:00 PM and were preceded by the administration of methylscopolamine nitrate, 1 mg/kg ip, to block the peripheral effects of oxotremorine. Baseline temperature (i.e., time to the 25th "click") was measured 30 min later. Oxotremorine (base), 1 mg/kg ip, was then given and temperature recorded every 15 min for 120 min.
Swim Stress

Stress sessions started 5 days after thermosensor implantation. Swim stress, a widely accepted means of stressing rats (Weiss et al. 1981), was employed. Sessions were held morning and evening.

The duration of the sessions and water temperature were adjusted so as to produce exhaustion. Extreme fatigue caused the animals to sink. Observation that one or two did struggle to the surface terminated a session.

The first four sessions were at 22-24°C for 7, 10, and 15 min, respectively. Sessions 5 and 6 were both 8 min at 16-17°C. 7 and 8 lasted 7-9 min at 8-9°C, and sessions 9 and 10 for 6-7 min at 11 and 13°C, respectively.

Experimental Design

The study was divided into three phases, as depicted in Figure 1. Phase one (implantation): Five male Sprague-Dawley rats [301 ± 6.8 g (mean ± SEM)] participated in all phases of the experiment. The first (I) of five oxotremorine challenges marked the end of Phase I and provided a baseline against which subsequent data from challenges could be evaluated. Phase two (stress): This phase started with the first of 10 swim stress sessions and ended with the last session. During this phase, oxotremorine challenges (II, III) were administered. Challenge II followed the fourth stress session by 2 hr on day 8, and challenge III followed the 10th and final stress session by 4 hr on day 10. Phase III (recovery): During this 8-day period, the animals were not stressed. Challenge IV occurred on day 12, 48 hr after the last stress. The fifth challenge followed in 6 days on day 18.

Magnitude of the change in body temperature between oxotremorine challenges was the dependent variable. Data were analyzed using Analysis of Variance (ANOVA) with repeated measures.

Results

Swim stress produced increased sensitivity to the hypothermic effects of oxotremorine for at least 52 hr after the last swim session (Figure 2). Oxotremorine challenge II, which followed four swim sessions, did not disclose supersensitivity. However, there was a significant effect (increase in responsiveness to oxotremorine) following 6 more sessions, suggesting that chronic stress resulted in cholinergic supersensitivity (I versus III, \( p = 0.0049 \); II versus III, \( p = 0.0014 \); and I and II versus III, \( p = 0.0003 \)). Supersensitivity persisted for at least 52 hr (I versus IV, \( p = 0.019 \)), but a sufficient lapse of time resulted in a loss of supersensitivity (I versus V, NS). This indicates that a poststress recovery occurs within 8 days of the final stress session.

Table 1 highlights the strength of the effect of swim stress on oxotremorine-induced hypothermia. Every animal demonstrated significant increases in its hypothermic responses 4 hr after the last stress session, and four of five did after 52 hr. Thus, idiosyncratic responses by one or two animals did not determine the outcome of the study.

Figure 1. Presentation of experimental design. Phase one (P1) (implantation), days 0–6, oxotremorine challenge I; Phase two (P2) (stress), days 6–9, oxotremorine challenges II and III; Phase three (P3) (recovery), days 10–18, oxotremorine challenges IV and V.
Figure 2. Presentation of hypothermic response to oxotremorine (1 mg/kg) as a function of time (I) before stress, (II) 2 hr after the fourth stress session, (III) 4 hr after the 10th stress session, (IV) 52 hr after the 10th stress session, and (V) 8 days after the last stress session. Data were analyzed using ANOVA with repeated measures. I versus II (NS); I versus III (p = 0.0049); II versus III (p = 0.0014); I versus IV (p = 0.0019), and I versus V (NS). Stress produces significant increases in the sensitivity to oxotremorine, which decays over time.

Table 1. Baseline Hypothermic Response to Challenge with Oxotremorine (1 mg/kg ip) minus Response after the Last of 10 Stress Sessions

<table>
<thead>
<tr>
<th>Rat (mean ± SEM)</th>
<th>df</th>
<th>t</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>4 hr after Last Stress Session</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-1.02 ± 0.25</td>
<td>7</td>
<td>-3.995</td>
</tr>
<tr>
<td>2</td>
<td>-1.26 ± 0.25</td>
<td>7</td>
<td>-2.88</td>
</tr>
<tr>
<td>3</td>
<td>-0.46 ± 0.15</td>
<td>7</td>
<td>-3.10</td>
</tr>
<tr>
<td>4</td>
<td>-0.64 ± 0.20</td>
<td>7</td>
<td>-3.18</td>
</tr>
<tr>
<td>5</td>
<td>-1.3 ± 0.27</td>
<td>7</td>
<td>-4.78</td>
</tr>
<tr>
<td>52 hr after Last Stress Session</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-0.99 ± 0.16</td>
<td>7</td>
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<tr>
<td>2</td>
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<td>7</td>
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</tr>
<tr>
<td>3</td>
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<td>7</td>
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<td>-0.24 ± 0.08</td>
<td>7</td>
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<tr>
<td>5</td>
<td>-0.93 ± 0.11</td>
<td>7</td>
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</table>

Discussion
To our knowledge, these results are the first experimental evidence that stress sensitizes a central muscarinic mechanism. This could be of importance in the spheres of stress and affective disorders research where cholinceptor supersensitivity might be a bridge or link between phenomena clinicians have long regarded as related. Further, they suggest that swim stress may provide an animal model of cholinergic system supersensitivity that is useful in affective disorders research. These data may be relevant to neuroendocrine changes in chronically stressed animals. Cholinergic agonists induce the release of adrenocorticotropic hormone (ACTH) from pituitary explants (Hillhouse et al. 1975). Implantation of atropine crystals into the hypothysis blocks stress-induced secretion of cortisol (Kapanski and Smelik 1973). Nemeroff (1985) recently reported that stress produces the release of corticotropin-releasing factor (CRF) in vivo. It is conceivable that stress mobilizes a cholinergic mechanism that activates the hypothalamic–pituitary–adrenal axis.

Cholinergic dysfunction may be involved in the pathophysiology of depression (Janowsky et al 1972). Stresses increase the probability that an affective disorder patient will become depressed (Lloyd 1980) or manic (Kennedy et al. 1983). A capacity of stress to activate cholinergic pathways might contribute to this.

There are limitations to a study involving five animals; although the effects of stress were robust and consistent, replication using not only swim stress, but also other forms of stress, will now be required.

References
Hillhouse EW, Burden J, Jones MT (1975): The effects of various putative neurotransmitters on the


