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Activation of 5-HT$_{1A}$ receptors attenuates tachycardia induced by restraint stress in rats

Sukonthar Ngampramuan,1 Mathias Baumert,2 Mirza Irfan Beig,3 Naipinhin Kotchabhakdi,1 and Eugene Nalivaiko3

1Neuro-Behavioural Biology Centre, Institute of Science and Technology for Research and Development, Mahidol University, Salaya Nakron Pathom, Thailand; 2Centre for Biomedical Engineering, School of Electrical and Electronic Engineering, University of Adelaide, and 3Department of Human Physiology and Centre for Neuroscience, Flinders University, Adelaide, Australia

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Ngampramuan S, Baumert M, Beig MI, Kotchabhakdi N, Nalivaiko E. Activation of 5-HT$_{1A}$ receptors attenuates tachycardia induced by restraint stress in rats. Am J Physiol Regul Integr Comp Physiol 294: R132–R141, 2008. First published October 24, 2007; doi:10.1152/ajpregu.00464.2007.—To better understand the central mechanisms that mediate increases in heart rate (HR) during psychological stress, we examined the effects of systemic and intramedullary (raphe region) administration of the serotonin-1A (5-HT$_{1A}$) receptor agonist 8-hydroxy-2-(dil-n-propylamino)tetraline (8-OH-DPAT) on cardiac changes elicited by restraint in hooded Wistar rats with preimplanted ECG telemetric transmitters. 8-OH-DPAT reduced basal HR from 356 ± 12 to 284 ± 12 beats/min, predominantly via a nonadrenergic, noncholinergic mechanism. Restraint stress caused tachycardia (an initial transient increase from 318 ± 3 to 492 ± 21 beats/min with a sustained component of 379 ± 12 beats/min). β-Adrenoceptor blockade with atenolol suppressed the sustained component, whereas muscarinic blockade with methylscopolamine (50 μg/kg) abolished the initial transient increase, indicating that sympathetic activation and vagal withdrawal were responsible for the tachycardia. Systemic administration of 8-OH-DPAT (10, 30, and 100 μg/kg) attenuated stress-induced tachycardia in a dose-dependent manner, and this effect was suppressed by the 5-HT$_{1A}$ antagonist WAY-100635 (100 μg/kg). Given alone, the antagonist had no effect. Systemically injected 8-OH-DPAT (100 μg/kg) attenuated the sympathetically mediated sustained component (from +85 ± 19 to +32 ± 9 beats/min) and the vagally mediated transient (from +62 ± 5 to +25 ± 3 beats/min). Activation of 5-HT$_{1A}$ receptors in the medullary raphe by microinjection of 8-OH-DPAT mimicked the antitachycardic effect of the systemically administered drug but did not affect basal HR. We conclude that tachycardia induced by restraint stress is due to a sustained increase in cardiac sympathetic activity associated with a transient vagal withdrawal. Activation of central 5-HT$_{1A}$ receptors attenuates this tachycardia by suppressing autonomic effects. At least some of the relevant receptors are located in the medullary raphe-parapyramidal area.

serotonin; psychological stress; heart rate; sympathetic; medullary raphe

THAT PSYCHOLOGICAL STRESS consistently elicits sympathetically mediated tachycardic responses is firmly established, but the central mechanisms generating increases in cardiac sympathetic activity remain poorly understood (6). In addition to a theoretical interest, this issue is of major clinical importance, inasmuch as the ability to suppress potentially deleterious increases in cardiac sympathetic activity at its origin, in the brain, would be a valuable alternative to widely used β-blockers. Few attempts have been made to reach this goal, mainly because of a lack of knowledge of the localization and pharmacological sensitivity of presympathetic cardiometer neurons. Recent evidence indicates that the final medullary relay for the descending pathways that activate the heart during stress is located in the raphe-parapyramidal area and that relevant cardiometer neurons are sensitive to, and could be inhibited by, serotonin-1A (5-HT$_{1A}$) receptor agonists (13, 23).

Involvement of 5-HT$_{1A}$ receptors in cardiovascular control is well documented, and the consensus is that their activation results in central sympatholytic effects (see Refs. 10 and 14 for reviews). Most of the relevant studies were conducted in anesthetized animals; in conscious animals, drug effects were studied in the quiet awake state. Two recent studies have demonstrated that activation of 5-HT$_{1A}$ receptors attenuates cardiovascular changes elicited by psychological stresses (13, 20). In both of these studies, it was suggested, but not proven, that the antitachycardic effects of 5-HT$_{1A}$ agonists were mediated by suppression of the stress-elicited activation of cardiac sympathetic nerves.

Restraint is a well-established experimental paradigm for provoking psychological stress in rats. Restraint consistently elicits a robust rise in arterial pressure and heart rate (HR) (1, 5, 12). However, the autonomic mechanisms mediating restraint-induced tachycardia have not been characterized, and the effects of 5-HT agonists on restraint-induced cardiac effects have not been tested.

We have therefore pursued the following aims in this study: 1) to determine how cardiac vagal and sympathetic activity contribute to tachycardia induced by restraint stress, 2) to test whether activation of 5-HT$_{1A}$ receptors could prevent this tachycardia and to determine the mechanisms that might be involved, 3) to identify a potential location of relevant 5-HT$_{1A}$ receptors, and 4) to determine whether these receptors are activated during restraint by intrinsically released 5-HT. In this study, we used the selective 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT).

MATERIALS AND METHODS

Male hooded Wistar rats (280–320 g body wt) were used in all experiments. All efforts were made to reduce animal pain or discomfort. Experiments were conducted in accordance with the European Commission (77/237/EEC) and the United Kingdom Home Office (80/860/EEC) guidelines for the care and handling of animals. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: E. Nalivaiko, Dept. of Human Physiology, Flinders Medical Centre, Bedford Park 5042 SA, Australia (e-mail: eugene.nalivaiko@flinders.edu.au).

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Community Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Flinders University Animal Welfare Committee.

Preliminary Surgery

Preliminary surgery was conducted under isoflurane (1.5% in 100% oxygen) anesthesia. Carprofen (5 mg/kg) was used as an analgesic after the surgery. Telemetric ECG radio transmitters (model TA11CA-F40, Data Sciences International) were implanted into the peritoneal cavity. Electrodes were placed according to the method described by Sgoifo (16); on the internal surface of the xiphoid process and in the mediastinum along the trachea at the level of the left ventricle. These placements permit recovery of 95–99% of heartbeats, even in vigorously moving animals. In some animals, during the same surgical session, a stainless steel guide cannula was stereotaxically positioned 2.8 mm caudal to the interaural line at the midline, inserted vertically to the IV ventricle, fixed to the skull with stainless steel screws and dental cement, and closed with an obturator. Animals recovered from anesthesia and were returned to the animal house for ≥1 wk before experimental studies. They were kept on a reverse 12:12-h light-dark cycle.

Experimental Protocol

Experiments were carried out between 9 AM and 2 PM. ECG probes were switched on, and the animals remained in their home cages for ≥1 h. Drugs were administered subcutaneously, diluted in 0.5 ml of Ringer solution (experiments 1–7), or microinjected into the medullary raphe (experiments 8–10).

Experiment 1: does systemic treatment with 8-OH-DPAT affect basal HR? In the first group of rats (n = 6), 8-OH-DPAT (100 μg/kg) or, on different days, Ringer solution was administered and recordings were obtained for 1 h. Similar injections were made in the second (n = 6) and third (n = 6) groups after β-adrenergic blockade with atenolol (2 mg/kg) or a combined muscarinic and β-adrenergic blockade with methylscopolamine bromide (50 μg/kg) + atenolol (2 mg/kg), respectively.

Experiment 2: does systemic treatment with 8-OH-DPAT affect stress-induced tachycardia? On different days, 8-OH-DPAT (10, 30 or 100 μg/kg) or Ringer solution was administered, and 15 min later the rats (n = 7) were placed in a restrainer (60-mm-ID transparent plastic tube) for 30 min.

Experiment 3: does systemic treatment with 8-OH-DPAT affect stress-induced tachycardia after 5-HT1A receptor blockade with WAY-100635? Before the restraint, animals (n = 6) received, on different days, the following combination of drugs: 1) Ringer solution + Ringer solution, 2) Ringer solution + 8-OH-DPAT (100 μg/kg), or 3) WAY-100635 + 8-OH-DPAT (both at 100 μg/kg).

Experiment 4: does systemic treatment with WAY-100635 affect stress-induced tachycardia? Before restraint, animals (n = 6) received, on different days, WAY-100635 (100 μg/kg) or Ringer solution.

Experiment 5: does autonomic blockade affect restraint-induced cardiac responses? Before restraint, animals (n = 8) received, on different days: 1) Ringer solution, 2) atenolol (2 mg/kg), or 3) methylscopolamine bromide (50 μg/kg).

Experiment 6: does systemic treatment with 8-OH-DPAT affect stress-induced tachycardia after vagal blockade? Before restraint, animals (n = 6) received, on different days, the following combination of drugs at 10-min intervals: 1) methylscopolamine (50 μg/kg) + Ringer solution or 2) methylscopolamine (50 μg/kg) + 8-OH-DPAT (100 μg/kg).

Experiment 7: does systemic treatment with 8-OH-DPAT affect stress-induced tachycardia after β-adrenergic blockade? Before restraint, animals (n = 6) received, on different days, the following combination of drugs at 10-min intervals: 1) atenolol (2 mg/kg) + Ringer solution or 2) atenolol (2 mg/kg) + 8-OH-DPAT (100 μg/kg).

Experiment 8: does intracerebral microinjection of 8-OH-DPAT affect basal HR? Animals (n = 6) received an intramedullary microinjection of 8-OH-DPAT (1 nmol in 100 nl) or, on a different day, the equivalent volume of sterile Ringer solution, and recordings were continued for 1 h. An injection cannula (0.2-mm-OD stainless steel wire; Small Parts) was inserted 11 mm below the surface of the skull. Injections were made using a hand-held syringe, and the injection volume was assessed by observation of the meniscus in a glass capillary attached to the injection cannula. Injections were made slowly (~20 s), and the cannula remained in place for 1 min after injection.

Experiment 9: does intracerebral microinjection of 8-OH-DPAT affect stress-induced tachycardia? Before restraint, animals received an intramedullary microinjection of 8-OH-DPAT (1 nmol in 100 nl) or, on a different day, the equivalent volume of sterile Ringer solution. Another five animals (control group) were injected similarly at a site that was 2.5 mm more dorsal (8.5 mm below the surface of the skull). Microinjections were performed as described for experiment 8 (n = 1).

Experiment 10: does 5-HT1A receptor blockade with WAY-100635 prevent antiarrhythmic effects of intramedullary microinjection of 8-OH-DPAT? Before restraint, animals (n = 6) received, on different days, the following combination of drugs: 1) Ringer solution (subcutaneously) + Ringer solution (brain microinjection, 100 nl), 2) Ringer solution (subcutaneously) + 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl), or 3) WAY-100635 (100 μg/kg sc) + 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl).

Thus this study was conducted in 77 rats (57 with systemic and 20 with intramedullary administration of 8-OH-DPAT). Each animal cohort was used for just one type of experiment. All experimental procedures were performed ≥48 h apart. To avoid serial effects, we used a counterbalanced or rotational design. All chemicals were obtained from Sigma.

Visualization of Microinjection Sites

Medullary microinjection sites were labeled with horseradish peroxidase (100 nl of 0.1% solution), which was administered into the medulla immediately after the termination of the last experiment via the same injection cannula. Rats were euthanized with pentobarbital sodium (Lethabarb) and perfused transcardially with formaldehyde fixative. Brains were removed and cut into 50-μm-thick sections. Horseradish peroxidase was visualized by incubation of sections in 0.05% solution of diaminobenzidine for 10 min, with the subsequent addition of a 0.01% solution of hydrogen peroxide. Sections were dried, dehydrated in alcohol, mounted on slides, stained with neutral red, and photographed.

Data Acquisition and Analysis

Analog ECG signals were digitized at 400 Hz and acquired using a PowerLab analog-to-digital converter and Chart 5.4 software (ADInstruments). HR was calculated from the ECG records using the same software. After removal of artifacts, HR was automatically averaged for every minute. Spectral indexes of HR variability were computed using the HR variability (HRV) module of the Chart 5.4 software. The low-frequency band was set at 0.15–1.0 Hz and the high-frequency (HF) band at 1.0–3.0 Hz. HF power is a measure of vagally mediated respiratory sinus arrhythmia. We also computed the root mean square of the beat-to-beat interval differences (RMSSD), a standard HRV index reflecting fast vagal modulation of the interbeat intervals. To characterize the recovery of HR after handling-related tachycardia, we used the time period during which HR fell to 50% of the peak increase (t50). The dose dependence of 8-OH-DPAT-induced effects was assessed using linear regression. Group data were analyzed by ANOVA, with Fisher’s protected t-test and with the significance threshold set at the 0.05 level. Values are means ± SE.
RESULTS

Effect of Systemic 8-OH-DPAT on Basal HR and HRV Indexes

Subcutaneous injection of vehicle or 8-OH-DPAT (100 μg/kg) caused transient tachycardia of similar magnitude. After the drug, HR fell within 10–15 min to a level significantly lower than basal and remained at this low level from ~15 to 40 min after injection (Fig. 1A). In contrast, after the vehicle, reversion of injection-induced tachycardia was quite slow; therefore, from 15 to 40 min after injection, HR was still different from the basal level and the corresponding values after 8-OH-DPAT. There was also a significant difference in the speed of HR decay from the peak: t_{1/2} = 2.5 ± 0.7 and 13 ± 2.12 min after vehicle and drug, respectively (P < 0.01, n = 6). 8-OH-DPAT substantially and significantly elevated HRV indexes that reflect vagal modulation of the HR RMSSD (1.6 ± 0.1 and 4.8 ± 0.7 ms after vehicle and drug, respectively, P < 0.01, n = 6) and HF power of the HRV (0.9 ± 0.2 and 4.9 ± 0.3 ms² after vehicle and drug, respectively, P < 0.05, n = 6).

We then determined whether 8-OH-DPAT-induced bradycardia could be prevented by β-adrenergic blockade (Fig. 1B). Administration of atenolol caused short-duration tachycardia, so that 15 min later, just before the injection of vehicle or 8-OH-DPAT, HR did not differ from basal values for both conditions. This second injection provoked tachycardic responses that were smaller than those following the first injection. Although after the vehicle, HR continued to fall, bradycardia after the drug was more prominent, with a clear downward deflection (Fig. 1B). We compared HR during the period of maximal action of 8-OH-DPAT (detected in the previous experiment); the values were significantly different between the two conditions and, also, for each of them, were lower than the corresponding basal (pre-atenolol) values.

Next, we tested whether 8-OH-DPAT-induced bradycardia persists after a combined vagal and sympathetic blockade (Fig. 1C). Administration of methylscopolamine caused sustained tachycardia; injection of atenolol 10 min later caused a fall in HR after small injection-related tachycardia. The time course of HR change did not differ for both conditions before the third injection. As shown in Fig. 1C, 8-OH-DPAT still produced a substantial bradycardic effect, so that at the peak of this effect, HR values were significantly different from corresponding values after the vehicle and from the basal (pre-scopolamine) values.

Effect of Systemic 8-OH-DPAT on Cardiac Responses Elicited by Restraint Stress

In this experiment (n = 7), we tested whether activation of 5-HT1A receptors with 8-OH-DPAT (administered systemically at 10, 30, and 100 μg/kg) affects restraint-induced cardiac responses. Mean group data are shown in Fig. 2A, and values are presented in Table 1. Tachycardia associated with drug or vehicle administration reverted to the basal level within 15 min, so the prerestraint values were not different between the four conditions. After vehicle, restraint stress caused tachycardia, which peaked at ~500 beats/min within 1–1.5 min and then started to decline, approaching the steady-state level within 10–15 min and remaining at this level (or sometimes slowly declining) until the end of the restraint. We will thus refer to these data points as “peak restraint” and “steady-state restraint.” Effects of pretreatment with 8-OH-DPAT depended on the dose. After 10 μg/kg 8-OH-DPAT, restraint-induced tachycardia did not differ from the control condition. At 30 μg/kg, 8-OH-DPAT substantially reduced the steady-state increase in HR, and at 100 μg/kg, 8-OH-DPAT attenuated initial peak tachycardia and the steady-state increase in HR (Table 1). Figure 2B shows results of the linear regression analysis.
Effects of Autonomic Blockade on Restraint-Induced Tachycardia

In the next set of experiments \((n = 8)\), we addressed the following question: Which autonomic components mediate restraint-induced tachycardia? Rats were subjected to the restraint 15 min after injection of atenolol, methylscopolamine, or vehicle (Fig. 5, Table 2). After vehicle, the restraint provoked tachycardic responses similar to those described above. In animals with sympathetic blockade, restraint provoked only a small transient tachycardia, and during the second half of restraint, HR did not differ from prerestraint or basal values. Administration of methylscopolamine caused a rapid rise in the HR that persisted. Subjecting rats to the restraint after vagal blockade caused initial tachycardia, with HR significantly higher than in restrained animals injected with vehicle. After vagal blockade, the increase in HR for the “steady-state” component (vs. prerestraint values) was significantly greater than after Ringer solution (Table 2). For vehicle and methylscopolamine conditions, steady-state values were significantly different from prerestraint values \((P < 0.01)\).

Effects of Systemic 8-OH-DPAT on Restraint-Induced Tachycardia After Sympathetic Blockade

After sympathetic blockade with atenolol, 8-OH-DPAT or vehicle was administered before the restraint \((n = 6;\) Fig. 6). The vehicle injection provoked a small and short-duration tachycardia, so that before the restraint, HR did not differ from the basal level. In vehicle-treated animals, restraint elicited only an initial transient tachycardic component of moderate amplitude. Injection of 8-OH-DPAT caused slow and long-lasting bradycardia, so that before the restraint, HR was significantly different from the basal level and the prerestraint value in vehicle-treated animals. After 8-OH-DPAT, restraint provoked a small transient tachycardic response \((+25 \pm 3 \text{ beats/min})\) that was significantly different from the response to vehicle injection \((+62 \pm 5 \text{ beats/min}, P < 0.01, n = 6)\). The time course of 8-OH-DPAT-elicited bradycardia was similar to that in experiment 1 (i.e., drug alone).

Effects of Systemic 8-OH-DPAT on Restraint-Induced Tachycardia After Vagal Blockade

In six animals, administration of methylscopolamine caused a rapid increase in HR. Effects of a subsequent injection of 8-OH-DPAT did not differ from those of vehicle injection, so that the prerestraint values for both conditions were not different (see Fig. 8). Restraint-induced tachycardia was substantially and significantly attenuated in 8-OH-DPAT-treated animals, in terms of absolute values (Fig. 7) and the magnitude of the increase \((+85 \pm 19 \text{ and } +32 \pm 9 \text{ beats/min after vehicle and drug, respectively, } P < 0.01, n = 6)\). After reaching peak values, HR began to fall, with a time course similar for both conditions, so that steady-state values were also significantly different from each other, but they were not different from corresponding prerestraint values (although there was a tendency for a fall for the drug condition, with \(P = 0.062)\).

Effects of Intramedullary Microinjection of 8-OH-DPAT on Basal HR

Apart from a short-lasting tachycardia associated with handling, microinjection of Ringer solution or 8-OH-DPAT into
Table 1. Restraint-induced tachycardia and effects of 5-HT₁₅A receptor ligands

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Pre restraint</th>
<th>Peak restraint</th>
<th>Steady-State restraint</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute value</td>
<td>318±3</td>
<td>353±15</td>
<td>492±21b</td>
<td>379±12b</td>
</tr>
<tr>
<td>Δ</td>
<td>35±3</td>
<td>10±15</td>
<td>12±11</td>
<td>9±11</td>
</tr>
<tr>
<td><strong>8-OH-DPAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μg/kg</td>
<td>311±3</td>
<td>321±4</td>
<td>501±10b</td>
<td>365±3</td>
</tr>
<tr>
<td>Absolute value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>10±14</td>
<td>+18±2</td>
<td>+12±2</td>
<td>+10±2</td>
</tr>
<tr>
<td>30 μg/kg</td>
<td>307±2</td>
<td>320±4</td>
<td>450±23b</td>
<td>320±2</td>
</tr>
<tr>
<td>Absolute value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>6±15</td>
<td>+13±2</td>
<td>+1±2</td>
<td>+2±2</td>
</tr>
<tr>
<td>100 μg/kg</td>
<td>310±2</td>
<td>337±5</td>
<td>419±17d,f</td>
<td>314±2d,e</td>
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</tr>
<tr>
<td>Δ</td>
<td>9±12</td>
<td>+8±14</td>
<td>-2±12</td>
<td>-3±12</td>
</tr>
</tbody>
</table>

**Prevention of 8-OH-DPAT effects by WAY-100635**

|                  |          |              |                |                        |
| Vehicle + vehicle|          |              |                |                        |
| Absolute value   | 327±3    | 387±16       | 514±8b         | 386±14a                |
| Δ                 | 10±13     | +17±21       | +3±17          | +15±17                 |
| Vehicle + 8-OH-DPAT|        |              |                |                        |
| Absolute value   | 318±3    | 334±12g,i    | 400±23h,i      | 318±18h,i              |
| Δ                 | 11±18     | +10±16h,i    | +1±15h,i       | +2±15h,i               |
| WAY-100,635 + 8-OH-DPAT| |              |                |                        |
| Absolute value   | 331±2    | 414±13       | 508±6b         | 392±26c                |
| Δ                 | 11±17     | +15±19       | +6±17          | +6±17                  |

Values are means ± SE, expressed in beats/min. 5-HT₁₅A, serotonin-1A. For each set of data, Δ values represent difference between pre-restraint and peak or steady-state during restraint [for effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)] and difference between baseline and peak or steady-state value during restraint (for prevention of 8-OH-DPAT effects by WAY-100635). Significantly different from corresponding basal level value: *P < 0.05; **P < 0.01. Significantly different from vehicle: †P < 0.05; ‡P < 0.01. Significantly different from lowest dose of 8-OH-DPAT: §P < 0.05; ¶P < 0.01. Significantly different from vehicle + vehicle: ⅠP < 0.05; ⅡP < 0.01. Significantly different from WAY-100635 + 8-OH-DPAT: ⅢP < 0.05; ⅣP < 0.01.

Effects of Intramedullary Microinjection of 8-OH-DPAT on Restraint-Induced Tachycardia

To identify the potential location of 5-HT₁₅A receptors responsible for the above-described antitachycardic effects of 8-OH-DPAT, we microinjected the drug or the vehicle into the raphe-parapyramidal area of the lower brain stem (n = 8). The procedure of animal handling during injection caused transient tachycardia of similar magnitude, but the return to the basal level (or, in some animals, even slightly below this level) was relatively slow due to the presence of 8-OH-DPAT. The combination of a vehicle (control) or WAY-100,635 (5-HT₁₅A receptor agonist) had no effect on basal HR (Fig. 8). Handling-related increase in HR was shorter after 8-OH-DPAT than Ringer solution (t₁₀₀ = 4.2 ± 0.4 and 11 ± 0.9 min, respectively, P < 0.05, n = 6).

**Fig. 3.** Selective blockade of serotonin 1A (5-HT₁₅A) receptors with WAY-100635 (100 μg/kg sc) prevents antitachycardic effects of 8-OH-DPAT (100 μg/kg sc) during restraint stress (n = 6). Traces show changes in HR in animals pretreated, on different days, with the following drug or vehicle combination: vehicle + vehicle (control), vehicle + 8-OH-DPAT (5-HT₁₅A receptor activation), or WAY-100635 + 8-OH-DPAT (5-HT₁₅A receptor agonist after receptor blockade). Values are presented in Table 2.

**Fig. 4.** Selective blockade of 5-HT₁₅A receptors with WAY-100635 (100 μg/kg sc) did not affect tachycardia elicited by restraint stress. Traces show changes in HR in animals pretreated, on different days, with vehicle or WAY-100635 (n = 6). Mean values are presented near corresponding traces.
faster after 8-OH-DPAT, so that prerestraint HR values were significantly different (Fig. 9A). After 8-OH-DPAT, restraint-induced tachycardia was attenuated, in terms of absolute HR values for the transient and steady-state components (Fig. 9A) and differences between prerestraint and restraint values. For the peak tachycardia, the latter were reduced from +144 ± 11 to +59 ± 7 beats/min (n = 8, P < 0.05) and for the steady-state component from +31 ± 4 to 8 ± 5 beats/min. An example of a histologically confirmed injection site is presented in Fig. 9B. In another five animals, control microinjections were performed 2.5 mm more dorsally. We did not find any effects of 8-OH-DPAT in this control group (data not shown).

**Effects of Systemic WAY-100635 on Antitachycardic Action of Intramedullary Injection of 8-OH-DPAT**

In the above-described experiment, OH-DPAT was microinjected into the raphe-parapyramidal area at relatively high concentration. To prove that the antitachycardic effect of the drug represented a specific effect mediated by its interaction with 5-HT1A receptors, in another six rats intramedullary microinjections of 8-OH-DPAT were preceded by systemic administration of WAY-100635 (100 μg/kg) or vehicle; systemic vehicle followed by intramedullary vehicle served as a control. As illustrated in Fig. 10, if the drug was given after the vehicle, it substantially and significantly attenuated restraint-induced tachycardia, similar to the previous experiment. Pre-treatment with WAY-100635 completely abolished the effect of 8-OH-DPAT, so that the magnitude of the tachycardia did not differ from that after intramedullary administration of the vehicle.

**DISCUSSION**

The novel findings of this study are as follows. 1) Activation of central 5-HT1A receptors evokes bradycardia mediated by cardiac non-β-adrenergic, noncholinergic neurotransmission mechanisms. 2) A sustained component of tachycardia elicited by the restraint stress in rats is mainly due to sympathetic activation, whereas vagal withdrawal contributes to the initial larger transient component. 3) 8-OH-DPAT substantially attenuates both of these autonomic components, in a dose-
dependent manner and acting via 5-HT1A receptors. At least some of these receptors must be located in the medullary raphe-parapyramidal area.

**Effects of 8-OH-DPAT on Basal HR**

Bradyecardic effects of 8-OH-DPAT observed in our rats are in full agreement with previous reports comprehensively reviewed by others (10, 14). In earlier studies, it was clearly demonstrated that the action of the drug is not peripheral (7). Traditional interpretation of cardiac effects induced by 5-HT1A agonists is that these are caused by sympathetic withdrawal and/or vagal activation (10, 14), where the terms “sympathetic” and “vagal” are used as synonyms for “adrenergic” and “cholinergic.” Our HRV analysis results, specifically the rise in the RMSSD (an index that reflects increased difference between adjacent R-R intervals) and in the HF power (reflecting vagally mediated respiratory sinus arrhythmia), indicate that, indeed, 8-OH-DPAT modified the activity of vagal neurons. However, the fact that the drug still caused a substantial fall in HR after combined β-adrenergic and muscarinic receptor blockade indicates that activity of some other cardiac receptors must be involved in the genesis of 8-OH-DPAT-elicited bradycardia. One possibility is that an additional α-adrenergically mediated tachycardic component was eliminated as a result of sympathetic withdrawal; positive α-adrenergic chronotropy has been demonstrated in in situ and in vitro rat heart preparations (17, 19, 21).

It is well recognized that cardiac sympathetic and cardiac vagal nerve terminals, in addition to norepinephrine and acetylcholine, contain numerous other neurotransmitters, defined usually as “nonadrenergic noncholinergic” (NANC) (15). Acting centrally, 8-OH-DPAT modifies the activity of cardiac autonomic nerves (11), and this must lead to the alteration of cardiac release of classical and NANC neurotransmitters. We

Fig. 8. Microinjection of 8-OH-DPAT into raphe does not affect basal HR. Traces show changes in HR in animals (n = 6) treated, on different days, with vehicle or 8-OH-DPAT (1 nmol in 100 nl). Note faster return to baseline after treatment.

Fig. 9. Microinjection of 8-OH-DPAT into raphe attenuates tachycardia elicited by restraint stress. A: changes in HR in animals (n = 8) pretreated, on different days, with vehicle or 8-OH-DPAT (1 nmol in 100 nl). Mean values are presented near corresponding traces. Significantly different from preinjection basal level: *P < 0.05; **P < 0.01. #Significantly different from vehicle for the same time point, P < 0.05. B: intramedullary injection site (dark-field photograph of coronal section cut through lower brain stem). Brown area surrounded by dashed line contains horseradish peroxidase reaction product. Amb, nucleus ambiguous; ECu, external cuneate nucleus; Gi, gigantocellular reticular nucleus; NTS, nucleus of the solitary tract; Py, pyramid; spV, spinal tract of trigeminal nerve; SpV, spinal nucleus of trigeminal nerve.
consider that this is an alternative plausible explanation for the 8-OH-DPAT-induced bradycardia reported here. Inasmuch as 8-OH-DPAT is a well-known hypotensive agent, it may be that fall in body temperature (not measured in our study) directly contributed to the overall bradycardic effect of the drug. We cannot define whether bradycardia occurred as a result of the reduction in the excitatory or the increase in the inhibitory cardiac drive; clarification of the underlying pharmacological mechanisms and evaluation of potential hypotermia-induced fall in HR require further studies.

Site and Mechanism of Action of 8-OH-DPAT

8-OH-DPAT completely abolished the steady-state component of the restraint-induced tachycardia and substantially suppressed the initial transient component. These effects were dose dependent, with a concentration range similar to those reported previously (10, 14). The effect of the drug was mediated via 5-HT1A receptors, as confirmed by the sensitivity of the effect of WAY-100635, a selective antagonist of these receptors. Although we cannot entirely exclude an NANC-dependent bradycardic action of 8-OH-DPAT in reducing stress-induced tachycardia, its dominant effect appears to be due to a central sympatholytic action. This follows from our finding that the major component of the stress-induced tachycardia is sympathetically mediated.

5-HT1A receptors are widely expressed in the brain, including areas involved in cardiac control during stress (9, 22). Of those, the medullary raphe-parapyramidal area is of major interest, inasmuch as this is a putative location of presympathetic cardiac motor neurons activated during stress. Evidence for this was initially presented by Zaretsky et al. (23), who observed substantial attenuation of tachycardia after intraraphe injection of muscimol in stressed rats. In our recent study in rabbits, we found that microinjection of 8-OH-DPAT in this area attenuated tachycardic responses to the air-jet stress (13). The microinjection results of the present study support this finding and confirm that the effect of the drug was indeed due to the activation of 5-HT1A receptors, inasmuch as it was sensitive to the selective 5-HT1A receptor antagonist. Return of HR to the basal level after handling-related tachycardia was more rapid with systemic administration and intramedullary microinjection of 8-OH-DPAT than with vehicle. This is not surprising, inasmuch as handling is also a stressful event and likely activates the same brain areas as does restraint. Although the antitachycardic action of 8-OH-DPAT during stress could be mediated by limbic structures involved in emotional processing, our microinjection experiments indicate that, at least in part, the drug’s action may be realized in the medullary raphe, via autoinhibitory 5-HT1A receptors (8).

Intramedullary microinjection of 8-OH-DPAT had no effect on the basal HR, in contrast to the systemic administration of the drug, which caused bradycardia. This is consistent with the study by Zaretsky et al. (23), who observed no changes in the basal HR after pharmacological inhibition of the raphe region by muscimol. Our finding suggests that the bradycardic effect of 8-OH-DPAT (as opposed to its antitachycardic effect) is mediated at some other location. Additional studies are required to identify this location.

Intriguingly, pretreatment with WAY-100635 alone did not affect basal HR or stress-induced tachycardia, similar to a number of other studies where the antagonist prevented effects of agonists but was without effect when given alone (18). This suggests that, during stress, there is no intrinsic release of 5-HT in the vicinity of 5-HT1A receptors involved in cardiac control. Thus the functional significance of these receptors remains unclear.

Autonomic Mechanisms Involved in Cardiac Control During Stress and Modulation of These Mechanisms by 8-OH-DPAT

Our study is the first pharmacological dissection of cardiac responses during restraint, a widely used stress paradigm. Restraint-induced tachycardia is well documented (1, 5, 12), but effects of autonomic blockade on cardiac changes during restraint have not been assessed. We found that β-adrenergic blockade completely abolished the steady-state tachycardia and substantially reduced the initial transient tachycardic component. Inasmuch as adrenals do not contribute to the rise in HR during restraint (1), our findings indicate that increase in the cardiac sympathetic nerve activity, with subsequent activation of β-adrenoreceptors, is the predominant mechanism mediating restraint-induced tachycardia. The sustained tachycardic component was reduced by 8-OH-DPAT, and thus the drug effect could be defined as cardiac sympathetic. Such an effect is in agreement with previous studies in anesthetized animals (10, 14), extends the previous knowledge to the conscious state, and, most importantly, indicates that 5-HT1A receptor activation efficiently suppresses the stress-induced rise in cardiac sympathetic activity.

8-OH-DPAT strongly suppressed the stress-induced tachycardic component that persisted after muscarinic receptor blockade. As discussed above, restraint-related tachycardia (and especially its steady-state component) is sympathetically mediated, and, thus, provided that the drug action is central, we must conclude that it attenuated stress-induced elevation of

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**Fig. 10.** Systemically administered WAY-100635 (100 μg/kg) prevents antitachycardic effects of 8-OH-DPAT microinjected into medulla. Traces show changes in HR in animals pretreated, on different days, with the following combination of drugs: Ringer solution (subcutaneously) + 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl), Ringer solution + 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl), or WAY-100635 (100 μg/kg sc) + 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl). Mean values are presented near corresponding traces. Significantly different from preinjection basal level: *P < 0.05; **P < 0.01. Significantly different from Ringer solution + Ringer solution and WAY-100635 + 8-OH-DPAT for the same time point: #P < 0.05; ##P < 0.01.
norepinephrine release from cardiac sympathetic nerves. We cannot define here the 8-OH-DPAT-sensitive component as steady state, because, surprisingly, in this experiment, HR was not maintained at a high level throughout the restraint. We do not have a satisfactory explanation for this; it may be that the second drug injection in this experiment affected adaptation during restraint. What is most important here is that, during restraint, 8-OH-DPAT caused a near-parallel downward shift in HR compared with the control (postvehicle) condition, indicating that the drug-sensitive component was sustained, at least compared with control.

Our data suggest that a central sympatholytic effect is not the only mechanism responsible for the antitachycardic action of 8-OH-DPAT during restraint stress. Transient vagal withdrawal is the likely mechanism underlying the initial short-lasting restraint-induced tachycardic component, inasmuch as this component virtually disappeared after the muscarinic receptor blockade. It is unlikely that nonadrenergic transmitters released from the sympathetic nerves contribute to this component, inasmuch as, in this case, the time course of their effect must be similar to that of the norepinephrine effect (which is sustained, as we have demonstrated here using 8-adenoreceptor blockade). The fact that 8-OH-DPAT substantially reduced the atenolol-insensitive transient component indicates that activation of 5-HT1A receptors in the brain may also modify activity of cardiac vagal neurons, counteracting their inhibition during stress. This idea is supported by previous experiments in anesthetized animals (14). The potential underlying mechanism could be the same as that described for the gastric vagal neurons, namely, their disinhibition by 8-OH-DPAT-induced presynaptic suppression of GABA release (2).

A comparison of stress-induced responses after autonomic blockade revealed another interesting phenomenon: after methylscopolamine, the sustained component of the tachycardia was substantially larger than in control. Clearly, the methylscopolamine-resistant sustained component was sympathetically mediated, inasmuch as it was completely suppressed by atenolol. It must be then that, in the control situation, this steady-state component was gradually reduced by some mechanism. One potential explanation is that, in the course of restraint, the initial vagal withdrawal may change to a gradual restitution of vagal activity; such sympathovagal coactivation has been recently reported in rats during conditioned fear (4). The lack of slowly developing bradycardia after β-adrenergic blockade does not necessarily contradict this suggestion, inasmuch as vagal effects could be presynaptic and, thus, would not be observed when norepinephrine effects are suppressed. Our hypothesis is certainly speculative and requires more direct evidence.

The antitachycardic effect of 8-OH-DPAT reported here is in full agreement with our previous study in rabbits, where we demonstrated that the drug suppresses tachycardia elicited by air-jet stress (13). These tachycardic responses in rabbits were quite modest (<50 beats/min), and thus the value of the present study is not only in extending our previous observation to a new species, i.e., the rat, but also in demonstrating that activation of 5-HT1A receptors may counteract a quite vigorous rise in HR.

Van den Buuse and Wegener (20) recently reported anti-tachycardic effects of 5-HT1A receptor agonists during another stress paradigm, the open field. They found that tachycardic effects of stress and antitachycardic effects of 5-HT1A agonists were strain dependent. Our results are in good accord with this report in terms of similar sensitivity to 8-OH-DPAT of two lines derived from the Wistar strain (our hooded Wistar rats and the Wistar-Kyoto rats of van den Buuse and Wegener).

Methodological Issues

We certainly acknowledge that subcutaneous administration of drugs is a stressful procedure: it consistently evoked tachycardic responses due to handling and injection-induced pain. These effects are, however, relatively short term, and we provided enough time between injection and stress onset, so that HR returned to near-basal level. Additionally, we always paired drug with vehicle, thus excluding any effect of injection-related stress.

Because both autonomic blockers used in our study (atenolol and methylscopolamine) poorly penetrate the blood-brain barrier, it is likely that their effects were predominantly peripheral.

Aiming to conduct the study in the least invasive manner and focusing on cardiac effects, we did not measure arterial pressure in our rats. We believe that lack of the pressure data does not diminish the value of our results, nor does it compromise our results. Systemic administration of 8-OH-DPAT at doses similar to ours reduced arterial pressure in conscious rats at rest (3); therefore, bradycardic effects of the drug observed in our study at rest (without stress) were clearly not the consequence of baroreflex. Restraint stress causes sustained pressor response in rats (1, 5, 12). Although effects of 8-OH-DPAT on the restraint-induced pressor responses have not been studied, the drug consistently suppressed rises in the arterial pressure elicited by open field and air-jet stresses (13, 20). It is therefore also unlikely that baroreflex contributed to the antitachycardic effects of 8-OH-DPAT during restraint; if anything, the negative chronotropic action of the drug could counteract, and overcome, the potential tachycardic effect of the baroreflex.

Significance and Perspectives

Our results indicate, for the first time, that 1) tachycardia induced by restraint stress occurs due to sustained increase in cardiac sympathetic activity associated with a transient vagal withdrawal, 2) activation of central 5-HT1A receptors attenuates this tachycardia, by suppressing both autonomic effects, and 3) some relevant receptors are located in the medullary raphe-parapyramidal area. Although we did not find any evidence of activation of these receptors by intrinsically released 5-HT during psychological stress, we believe that our results represent theoretical and clinical interest. 1) 5-HT1A receptors could serve as a marker (though nonspecific) for the identification of the raphe-spinal presynaptic cardiomotor neurons. 2) Evidence for the central cardioprotective effect of 5-HT1A receptor agonists may be clinically relevant, inasmuch as suppression of excessive cardiac sympathetic tone at its origin, in the brain, could represent an alternative therapeutic strategy for cardiac patients who do not tolerate or are insensitive to β-blockers.

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ACTIVATION OF 5-HT1A RECEPTORS REDUCES TACHYCARDIA IN STRESS

GRANTS

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