Activation of 5-HT$_{1A}$ receptors in the medullary raphe reduces cardiovascular changes elicited by acute psychological and inflammatory stresses in rabbits

Eugene Nalivaiko, Youichirou Ootsuka, and William W. Blessing
Department of Human Physiology, Centre for Neuroscience, Flinders University, Adelaide, Australia

Submitted 16 December 2004; accepted in final form 25 March 2005

Nalivaiko, Eugene, Youichirou Ootsuka, and William W. Blessing. Activation of 5-HT$_{1A}$ receptors in the medullary raphe reduces cardiovascular changes elicited by acute psychological and inflammatory stresses in rabbits. *Am J Physiol Regul Integr Comp Physiol* 289: R596–R604, 2005. First published March 31, 2005; doi:10.1152/ajpregu.00845.2004.—The present strategy for the prevention of excessive sympathetic neural traffic to the heart relies on the use of beta-blockers, drugs that act at the heart end of the brain-heart axis. In the present study, we attempted to suppress cardiac sympathetic nerve activity by affecting the relevant cardiomyoneurons in the brain using the selective serotonin-1A (5-HT$_{1A}$) receptor agonist 8-hydroxy-2-(di-n-propylamino)tetrain (8-OH-DPAT). In conscious, unrestrained rabbits, instrumented for recordings of heart rate, arterial pressure, or cardiac output, we provoked increases in cardiac sympathetic activity by psychological (loud sound, pinprick, and air jet) or inflammatory (0.5 mg/kg iv lipopolysaccharide) stresses. Pinprick and air-jet stresses elicited transient increases in heart rate (+50 ± 7 and +38 ± 4 beats/min, respectively) and in mean arterial pressure (+16 ± 2 and +15 ± 3 mmHg, respectively). Lipopolysaccharide injection caused sustained increases in heart rate (from 210 ± 3 to 268 ± 10 beats/min) and in arterial pressure (from 74 ± 3 to 92 ± 4 mmHg). Systemically administered 8-OH-DPAT (0.004–0.1 mg/kg) substantially attenuated these responses in a dose-dependent manner. Drug effects were prevented by a selective 5-HT$_{1A}$ receptor antagonist, WAY-100635 (0.1 mg/kg iv). Similarly to systemic administration, microinjection of 8-OH-DPAT (500 nl of 10 mM solution) into the medullary raphe-parapyramidal region caused antitachycardic effects during stressful stimulation and during lipopolysaccharide-elicted tachycardia. This is the first demonstration that activation of 5-HT$_{1A}$ receptors in the medullary raphe-parapyramidal area causes suppression of neurally mediated cardiovascular changes during acute psychological and immune stresses.

TACHYCARDIA OFTEN ACCOMPANIES psychological stress and bacterial infection. In both instances, increases in the activity of cardiac sympathetic nerves substantially contribute to cardiac acceleration. In predisposed patients, stress-related cardiac sympathetic activation may lead to potentially fatal arrhythmias (36, 38). Tachycardia during a febrile response does not usually represent immediate cardiac threat, but the presence of inflammatory markers is associated with cardiac morbidity and mortality (37, 39), and malignant cardiac effects of a proinflammatory state are likewise sympathetically mediated (40). At present, only one group of pharmacologically active substances, the beta-blockers, are available for preventing or reducing undesirable effects of increased sympathetic outflow at the level of the heart. It thus seems reasonable to suggest [as Lown and colleagues did long ago (3)] that prevention of increases in cardiac sympathetic activity at its source, in the brain, may prove to be another prospective approach for protecting the heart from the excessive sympathetic activity. So far, only $\alpha_2$-adrenoreceptor agonists are used as centrally acting sympatholytic drugs (14). They have a potent hypotensive activity, and thus their use as central cardioprotective agents is limited. It would thus be of major clinical importance to develop a selective cardiac sympatholytic drug that does not have this hypotensive effect.

The ability of serotonin (5-HT) receptor ligands to modify cardiac control via the central nervous system is now well established (see Refs. 23 and 35 for reviews). Of particular interest are central 5-HT$_{1A}$ receptors located on or near presynaptic neurons in the lower brain stem (see discussion). Their participation in cardiac control may be readily tested by use of selective ligands. In anesthetized rats, microinjection of the selective 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetrain (8-OH-DPAT) to the medullary raphe reverses centrally elicited tachycardia and reverses the increase in cardiac sympathetic activity (26). It is presently unknown whether activation of 5-HT$_{1A}$ receptors may reduce or prevent stress-related cardiac changes in conscious animals. In the present study, we address these issues. We tested whether systemic administration of 8-OH-DPAT alters changes in cardiac output, heart rate, and arterial pressure elicited by sudden stressful stimuli. We found that tachycardia during the acute febrile response is sympathetically mediated and tested whether it may be attenuated by 8-OH-DPAT. We confirmed specificity of 8-OH-DPAT action using WAY-100635, a selective 5-HT$_{1A}$ antagonist (12). Using direct brain microinjections, we proved that 8-OH-DPAT action is mediated, at least in part, via the medullary raphe-parapyramidal area.

MATERIALS AND METHODS

Preliminary surgery. Experiments were carried out on 26 New Zealand White rabbits weighing 2.5–3 kg. All efforts were made to reduce animal pain or discomfort. Experiments were conducted in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Flinders University Animal Welfare Committee. During preliminary surgery under Hypnorm (0.3 ml/kg im fentanyl-fluanisone)-Hynovel (2 mg/kg im midazolam) anesthesia, telemetric arterial pressure probes (TA11PA-C40, DSI) were implanted into the peritoneal cavity in 14 animals. The probe’s catheter was inserted in the right femoral artery via a small incision and advanced to the abdominal aorta. The artery was repaired, and blood flow was restored. In another six rabbits, ultrasound Doppler flow probes (6 mm OD, Iowa Doppler Products)
were implanted around the root of the ascending aorta for measuring stroke volume and cardiac output. In an additional six rabbits, under isoflurane anesthesia (1–2% in O2), a stainless steel guide cannula was implanted into the fourth ventricle of the brain, without touching the medullary surface, using a stereotactical approach developed in our laboratory. The cannula was fixed to the skull with screws and dental cement. During the same surgical session, a telemetric arterial pressure probe (TA11PA-C40, DSI) was implanted in the abdominal aorta, as described above.

**Experimental protocols.** On the day of the experiment, the rabbit was placed in a small experimental cage and remained undisturbed for 30 min. Experimental protocols for the first group (group 1; telemetric pressure probes only, n = 6) and the second group (group 2; aorta Doppler probes, n = 6) are schematically presented in Fig. 1. After the first two intravenous injections, we assessed the effects of the drugs on basal cardiovascular parameters. Because 8-OH-DPAT action is relatively short lasting (33), 30 min later we repeated the injections and assessed their effects on cardiovascular changes elicited by standardized stressful stimuli [loud sound (96 dB, 5 kHz, 0.5 s); pinprick; and 5-min air-jet stress]. Each animal was subjected to five experiments, using the following combinations of intravenously administered substances: 1) Ringer-Ringer; 2) Ringer-8-OH-DPAT (4 μg/kg); 3) Ringer-8-OH-DPAT (20 μg/kg); 4) Ringer-8-OH-DPAT (100 μg/kg); and 5) WAY-100635 (100 μg/kg)-8-OH-DPAT (100 μg/kg). In the third experimental group (group 3; telemetric pressure probes, n = 8), we assessed the effects of systemic 8-OH-DPAT on pressor and tachycardic effects of bacterial lipopolysaccharide (LPS). LPS (0.6 μg/kg iv) was administered after a 30-min observation period, and, 2 h later, when hypertension and tachycardia were fully developed, we injected the following on different days: 1) 8-OH-DPAT (300 μg/kg) followed 10 min later by WAY-100635, 2) atenolol (1 mg/kg) followed 10 min later by methyl-scopolamine (50 μg/kg), and 3) methyl-scopolamine (50 μg/kg) followed 10 min later by 8-OH-DPAT (300 μg/kg). To avoid serial effects, we used a rotational design in our experiments. To prevent LPS-induced desensitization, at least 1 wk was allowed between experiments. In the fourth experimental group (group 4; brain cannulas plus telemetric pressure probes, n = 6 animals), we assessed the effects of local intramedullary microinjections of 8-OH-DPAT on pressor- and LPS-elicited pressor and tachyphylactic effects. On 2 experimental days, after the 30-min observation period, animals were subjected to a 6-min air-jet stimulus. In the middle of the air-jet stimulation (3 min), we injected into the raphe-parapyramidal area, in a counterbalanced manner, either 300 nl of Ringer solution or 300 nl of 8-OH-DPAT (10 mM). On the third experimental day, animals received systemic administration of LPS (0.6 μg/kg). Two hours later, we microinjected Ringer solution (300 nl) into the raphe-parapyramidal area, followed 10 min later by 8-OH-DPAT (300 nl of 10 mM solution). All brain microinjections were performed via fine silica microtubing (200 μm OD) connected to a hematocrit capillary, using a hand-held syringe. Injection volume was controlled by observing meniscus movement in the capillary. The silica tubing was inserted into the guide cannula and advanced to the raphe area just before the injection and was removed immediately afterward. Horseradish peroxidase was included in the injectate. At the end of the experiment, animals were deeply anesthetized with Nembutal and perfused transcardially with a fixative. Brains were removed, sectioned at 50 μm, and processed for visualization of the horseradish peroxidase product. All drugs were obtained from Sigma-Aldrich (Australia) and were dissolved in Ringer solution before injection (except WAY-100635, which was first dissolved in DMSO and then diluted 1:4 in Ringer solution).

**Data analyses.** To determine stroke volume, we computed averaged aorta flow waveform during one heartbeat, calculated the area under the curve, and multiplied it by a cross-sectional area of the aorta (measured postmortem). Cardiac output was computed by multiplying stroke volume by heart rate. Gross movements of the animals were detected using a piezoelectric sensor attached to the cage.

To assess the effects on basal cardiovascular parameters and the animal’s movements in experiments with stressful stimuli, data were collected and averaged for 10 min just before the first Ringer-WAY-100635 injection and from 5 to 25 min after 8-OH-DPAT or vehicle injection. To assess the effects on stress-evoked responses, we compared 10–15 s of data just before the stimulus presentation with the maximal poststimulus effects (usually lasting for just a few seconds except in the air-jet stress, in which we measured effects at steady-state levels during the 5th min). In experiments with LPS, mean values were measured for 10 min pre- and postinjection. We analyzed data using StatView 5.0 (SAS Institute). ANOVA with repeated measures and Fisher’s protected t-test were used to determine the significance of differences in measured variables. Linear regression was used to assess dose dependence.

**RESULTS**

Effects of systemic 8-OH-DPAT on basal cardiovascular parameters. Basal levels of the heart rate did not differ in groups 1 and 2 (213 ± 7 and 207 ± 6 beats/min, respectively), and thus heart rate data from both groups were combined (210 ± 5 beats/min). Basal mean arterial pressure was 77 ± 5 mmHg, basal stroke volume was 1.7 ± 0.1 ml, and basal cardiac output was 350 ± 9 ml/min. Ringer injections caused small and short-lasting increases in heart rate, cardiac output, and arterial pressure, which returned to baseline within 1–1.5 min. Stroke volume remained unaffected.

Subsequently administered 8-OH-DPAT produced small and variable changes in heart rate and arterial pressure but did not affect stroke volume. After the dose of 20 μg/kg, there was a tendency for a depressor effect and bradycardia (decrease/no change/increase were observed in 3/3/0 and 5/6/1 animals, respectively), whereas after the dose of 100 μg/kg, the trend was the opposite (1/1/4 and 1/4/7, respectively). These changes, however, were not significant in either case. The drug also produced a substantial increase in motor activity (Fig. 2). This usually comprised single movements (changing posture, moving a limb, tapping the floor of the cage), separated by intervals. This effect was dose dependent, with the number of motor episodes per 5 min after the 20 and 100 μg/kg doses (20 ± 4 and 52 ± 8, respectively) being significantly different from each other and from the control (P < 0.01). Increase in motility became evident within 1 min after 8-OH-DPAT injection and lasted for 15–20 min. Administration of WAY-100635 before 8-OH-DPAT (100 μg/kg) prevented the cardiovascular and locomotor effects of the agonist.

Effects of systemic 8-OH-DPAT on cardiovascular responses elicited by psychological stresses. Raw data traces of
control stress-elicited responses are shown in Fig. 3, and grouped data values are presented in Fig. 4 and Table 1. All cardiovascular responses occurred with a short latency (0.7–1.3 s). In control animals, the loud sound elicited a short-lasting bradycardia associated with small increases in arterial pressure. Pinprick stimuli consistently provoked tachycardia and larger pressor responses. Responses to the air jet were more complex: just after the onset, tachycardia occurred in 9 of 12 animals, with the development of bradycardia in the other 3 animals.

In all instances, this was associated with increases in arterial pressure. Initial tachycardia- or bradycardic responses were short lasting. Heart rate tended to return to the baseline level within 15–20 s and then gradually increased toward the end of the air-jet stimulus, at which point all animals displayed moderate tachycardia and hypertension. Alerting stimuli produced only small (<5% of basal level) and inconsistent changes in stroke volume (data not shown).

8-OH-DPAT substantially attenuated tachycardic responses to the pinprick and to the air-jet stress (Fig. 4). Drug action was dose dependent, as revealed by a linear regression analysis. Moreover, invariably tachycardic control responses to the pinprick and air-jet stimuli reverted to bradycardia in 3 of 12 cases after the administration of 8-OH-DPAT at doses of 20 and 100 μg/kg. Sound-elicited bradycardia was not affected by 8-OH-DPAT, as were small changes in the stroke volume (not shown).

8-OH-DPAT attenuated pressor responses to the pinprick and air-jet stresses and tended to reduce the pressor effects of the loud sound. Pretreatment with WAY-100635 prevented the effects of 8-OH-DPAT (100 μg/kg) on tachycardic and pressor responses to pinprick and steady-state air-jet stress (Fig. 4 and Table 1). After

![Fig. 2. Effects of 8-OH-DPAT (20 μg/kg iv) on arterial pressure (AP), animal’s motility, and heart rate (HR) in conscious rabbits. A control injection of Ringer solution was made 5 min before administration of 8-OH-DPAT. BPM, beats/min.](http://ajpregu.physiology.org/)

![Fig. 3. AP and HR responses to loud sound, pinprick, and air-jet stress in conscious rabbits. A: control responses (after Ringer solution). B: responses after 8-OH-DPAT (100 μg/kg iv) obtained on different day. Arrows indicate moments when stimuli were delivered, and dotted lines correspond to the duration of the air-jet stress. Note different time scale during air-jet stresses.](http://ajpregu.physiology.org/)
Way-100635 administration, no bradycardia was observed after the pinprick or during the air-jet stress.

Effects of systemic 8-OH-DPAT on cardiovascular responses elicited by LPS. Intravenous administration of LPS caused sustained increases in heart rate (from 210 ± 3 to 268 ± 10 beats/min, \( P < 0.01 \)) and arterial pressure (from 74 ± 3 to 92 ± 4 mmHg, \( P < 0.01 \)), as illustrated in Fig. 5. Both variables reached steady-state levels within 1.5–2 h after LPS injection. Sympathetic blockade with atenolol performed at this stage (\( n = 8 \)) resulted in a prompt return of the heart rate to the basal level (Fig. 6A), with atenolol-induced decreases being proportional to the LPS-induced increases (Fig. 6A, right). Subsequent vagal blockade with methyl-scopolamine caused a substantial and significant tachycardic effect (Fig. 6A). Autonomic blockade had no effect on the arterial pressure.

When 8-OH-DPAT was administered during LPS-induced tachycardia, it caused a rapid reduction in the heart rate in all animals (\( n = 8 \), Figs. 5 and 6B). In five rabbits, the effect was clearly biphasic, with a larger initial transient component, whereas in the other three, the heart rate remained low, close to the initial basal level. 8-OH-DPAT-provoked falls in heart rate correlated with the previous increases in this parameter (Fig. 6B, right). Mean arterial pressure initially rose by 5–7 mmHg in six of eight rabbits and then assumed an oscillatory pattern, clearly associated with major body movements. Overall, increases in mean arterial pressure were not significant. In all cases, 8-OH-DPAT caused dramatic increases in the animal's motility, as shown on the motion sensor (middle trace in Fig. 5). Subsequent administration of Way-100635 led to a rapid increase in heart rate, which in most cases exceeded the

Table 1. Effects of 8-OH-DPAT on stress-elicited changes in heart rate and arterial pressure

<table>
<thead>
<tr>
<th></th>
<th>Control (Ringer)</th>
<th>4 ( \mu )g/kg</th>
<th>20 ( \mu )g/kg</th>
<th>100 ( \mu )g/kg</th>
<th>100 ( \mu )g/kg 8-OH-DPAT + 100 ( \mu )g/kg WAY-100635</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Sound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta ) Heart rate, beats/min</td>
<td>-26±4</td>
<td>-30±4</td>
<td>-19±5</td>
<td>-22±8</td>
<td>-26±6</td>
</tr>
<tr>
<td>( \Delta ) Arterial pressure, mmHg</td>
<td>+5±1</td>
<td>+4±1</td>
<td>+2±2</td>
<td>+1±2</td>
<td>+2±1</td>
</tr>
<tr>
<td>B. Pinprick</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta ) Heart rate, beats/min</td>
<td>+50±7</td>
<td>+34±11</td>
<td>+11±7**</td>
<td>+7±8**#</td>
<td>+38±8§§</td>
</tr>
<tr>
<td>( \Delta ) Arterial pressure, mmHg</td>
<td>+16±2</td>
<td>+11±3</td>
<td>+6±3*</td>
<td>+5±1**#</td>
<td>+9±3#</td>
</tr>
<tr>
<td>C. Air-jet stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta ) Heart rate, beats/min</td>
<td>+38±4</td>
<td>+30±5</td>
<td>+15±8**</td>
<td>+9±3**#</td>
<td>+37±6§§</td>
</tr>
<tr>
<td>( \Delta ) Arterial pressure, mmHg</td>
<td>+15±3</td>
<td>+15±2</td>
<td>+14±2</td>
<td>+7±2**##</td>
<td>+19±3§§</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( \Delta \). Change in values (poststimulus minus prestimulus). 8-OH-DPAT, 8-hydroxy-2(di-\( \alpha \)-propylamino)tetralin. Different from control \(* P < 0.05 \) and \(** P < 0.01 \); different from previous dose(s) \#P < 0.05 and \##P < 0.01; different from same dose (100 \( \mu \)g/kg) without antagonist §§P < 0.05 and §§§P < 0.01.)
sustained level before 8-OH-DPAT injection, and to an immediate cessation of hypermotility (Figs. 5 and 6B).

When, on different days, vagal blockade with methyl-scopolamine was performed during sustained LPS-evoked tachycardia (n = 6), heart rate increased further (Fig. 6C). 8-OH-DPAT, administered at this stage, attenuated tachycardia (Fig. 6C), evoked hypermotility, and did not affect mean arterial pressure. Importantly, between-animal differences in heart rate were substantially diminished after the drug, as evidenced by the reduced SE values (Fig. 6C). Cardiovascular responses to the first, second, and third administrations of LPS (with 1-wk intervals in between) were reproducible and of a similar magnitude.

Effects of intraraphe administration of 8-OH-DPAT on cardiovascular responses elicited by psychological stresses. Here, we describe results obtained in rabbits with preimplanted brain cannulas and telemetric pressure probes. Tachycardic responses to air-jet stimulus in this group did not differ from those described above, if Ringer solution was injected to the raphe area in the middle of the stimulation (Fig. 7, top). Heart rate consistently increased toward the end of the air-jet stimulus in all animals (from 199 ± 14 to 234 ± 10 beats/min, P < 0.05, n = 6). In contrast, after 8-OH-DPAT microinjection, heart rate started to fall within 1 min, so that by the end of the stimulation it did not differ from the prestimulus level but was significantly different from the tachycardia observed in vehicle-injected animals. Pressor responses to air-jet stimulation (+14–16 mmHg) were similar to those in animals without brain cannulas. Neither Ringer nor 8-OH-DPAT microinjections affected arterial pressure. The only difference noted was that, at the termination of the air-jet stimulus, pressure tended...
to return to basal levels more rapidly in Ringer-injected animals (Fig. 7, bottom).

Pinprick stimulus provoked substantial tachycardic responses after vehicle administration (+35 ± 9 beats/min), whereas, after 8-OH-DPAT microinjection, tachycardia was substantially smaller (7 ± 4 beats/min, *P < 0.05, n = 6). Associated small rises in arterial pressure (2–7 mmHg) did not differ between the two conditions.

Effects of intraraphe administration of 8-OH-DPAT on cardiovascular responses elicited by LPS. LPS administration resulted in substantial and sustained rises in heart rate and arterial pressure in all animals 1.5–2 h after injection (Fig. 8). Intraraphe microinjection of Ringer solution performed in this state did not affect arterial pressure and transiently (~1 min) reduced heart rate in two of five cases. Subsequent (10 min later) intraraphe microinjection of 8-OH-DPAT substantially and significantly reduced tachycardia, with heart rate returning to levels close to pre-LPS, and provoked small and delayed falls in arterial pressure (values shown in Fig. 8).

The location of intraraphe microinjection of 8-OH-DPAT is illustrated in Fig. 9. Such injections never caused hypermotility (n = 11, data not shown).

**DISCUSSION**

This is the first demonstration, in any species, that activation of central 5-HT1A receptors causes strong suppression of neurally mediated cardiovascular changes during acute psychological stress and during acute inflammatory response and that relevant 5-HT1A receptors are located in the medullary raphe-parapyramidal area. Using these two very different animal models, we found that stimulation of 5-HT1A receptors with 8-OH-DPAT substantially reduces sympathetically mediated tachycardia and increases in cardiac output. These effects were prevented or reversed by the selective 5-HT1A antagonist WAY-100635. Here, we first discuss the effects of systematically administered drugs and then concentrate on their site of action revealed in experiments with intramedullary microinjections.

**8-OH-DPAT has no effects on basal cardiovascular parameters.** Most studies of the cardiovascular action of 8-OH-DPAT have been performed in anesthetized animals, where consistent, substantial, and dose-dependent bradycardic and depressor effects were found (10, 11, 13, 16, 19, 24). In the few studies conducted in conscious animals, these effects were much less consistent or absent (11, 16, 20), similar to our present observations. A plausible explanation for this discrepancy is that many general anesthetics elevate sympathetic tone, thus providing a substrate for a central sympatholytic action of 8-OH-DPAT. This finding once again emphasizes the importance of conducting experiments in conscious, nonsedated animals.

**5-HT1A receptors and cardiac sympathetic outflow during psychological stress.** Cardiovascular responses to alerting stimuli occurred with a short (~1-s) latency and quickly recovered, suggesting that they were neurally mediated [as also was shown in our previous studies (28, 30)]. It is possible that circulating catecholamines contributed to the sustained changes seen during the air-jet stress. Tachycardic responses after the pinprick stress are largely mediated and during the air-jet stress are at least in part mediated via sympathetic activation rather than via vagal withdrawal (28). Therefore, attenuation of stress-elicited tachycardia by 8-OH-DPAT was most likely due to the reduction in activity of cardiac sympathetic nerves. Conversion of tachycardic pinprick- and air-jet-elicited responses into bradycardia in some animals by 8-OH-DPAT suggests that in these instances sympathetic blockade unmasked small vagally mediated components, also seen after propranolol administration (28). In rabbits, bradycardia elicited by acoustic stimulation is vagally mediated (28). In the present experiments, 8-OH-DPAT did not modify bradycardic responses elicited by the acoustic stimulation, suggesting that the
drug did not affect parasympathetic cardiomotoneurons. We cannot rule out some vagal contribution to reduced tachycardia after pinprick and air-jet stimuli.

8-OH-DPAT attenuated pressor responses to pinprick and air-jet stimuli. These pressor changes were caused by the combination of increases in cardiac output and by peripheral vasoconstriction, as evidenced here as well as in previous studies in rabbits (43, 46). Arterial pressure still rose even in those instances when tachycardic responses were converted to bradycardia by 8-OH-DPAT, indicating that peripheral vasoconstriction overrode falls in cardiac output. We found only minor influences of stressful stimuli on the stroke volume, so that heart rate was clearly the major factor defining cardiac output. We thus conclude that 8-OH-DPAT alters stress-elicited increases in heart rate (and hence in cardiac output) to a larger extent than it affects total peripheral resistance (with the exception discussed below).

5-HT1A receptors and cardiac sympathetic outflow during acute febrile reaction. Tachycardia associated with the febrile/inflammatory reaction is a very common clinical phenomena known for millennia. Paradoxically, its pathophysiology is poorly understood, possibly because, being a relatively non-threatening condition, it has not attracted much scientific attention. To our best knowledge, no animal studies have examined the neural substrate of rise in heart rate during the acute febrile response elicited by a natural, peripherally acting stimulus, and in only one human study sympathetic origin of tachycardia was assessed and confirmed (9). Our results with peripheral beta-adrenergic blockade fully support this human data, and indicate that activation of cardiac adrenoreceptors is a major event leading to tachycardia after the administration of the bacterial endotoxin. This finding is supported by the direct relationship between tachycardic action of LPS and brady- cardiac effects of atenolol, and by the fact that the vagal tone after LPS was still substantial. We used a low dose of LPS that does not evoke toxic shock (32). In our previous work (4), our group demonstrated that in the identical experimental conditions LPS causes a robust and reliable rise in body temperature of 1.5°C; however, we did not measure body temperature in the present experiments. Cardiovascular responses to the repetitive administration of LPS were not desensitized, proving that we have chosen an appropriate dose and interval between injections (1, 2).

The most important and novel finding in our study of the acute febrile reaction is the ability of 8-OH-DPAT to attenuate LPS-evoked tachycardia. This effect was reversed by WAY-100635 and was thus mediated via 5-HT1A receptors. The brady- cardiac action of 8-OH-DPAT was more pronounced in those animals where LPS-induced tachycardia was larger, suggesting that activation of 5-HT1A receptors led to a suppression of LPS-evoked sympathetically mediated cardioacceleration. Persistence of the 8-OH-DPAT effects after vagal blockade fully supports this idea. Relatively rapid (minutes) suppression of tachycardia by 8-OH-DPAT indicates that the drug effect was not due to alterations in circulating catecholamines, possibly elevated after LPS (41), but rather due to the suppression of sympathetic neural outflow to the heart. This also explains why administration of 8-OH-DPAT reduced between-animal variability in heart rate in rabbits after vagal blockade.

Our results thus indicate that tachycardia during the acute febrile reaction is not a result of direct temperature-induced changes in the frequency of the cardiac pacemaker; it is mediated predominantly by cardiac sympathetic nerves.

Location of relevant 5-HT1A receptors. After finding that systemic administration of 8-OH-DPAT possesses substantial anti-tachycardic effects during psychological and immune stresses and that the drug action is mediated via 5-HT1A receptors, we addressed the question of localization of these receptors. Local microinjections of 8-OH-DPAT into the medullary raphe-parapyramidal area resulted in reduction of tachycardia elicited by the same stimuli as used in experiments with intravenous 8-OH-DPAT. Similarly to these previous experiments, falls in heart rate were rapid and substantial, suggesting that activation of 5-HT1A receptors in the caudal ventromedial medulla most likely represents a major mechanism whereby systemic 8-OH-DPAT produces its action. It is of course entirely possible that activation of forebrain 5-HT1A receptors, responsible for the anxiolytic action of 8-OH-DPAT, contributed to its anti-tachycardic effects. Additionally, it may be that the small delayed depressor effect (Fig. 8) was due to a slow diffusion of the drug from the injection site to the presympathetic vasomotor neurons located in the rostral ventrolateral medulla.

The principal difference noted between systemic and local administration of 8-OH-DPAT was the lack of hypermotility in the latter case. This is not surprising since the local dose was more than 500 times smaller than the systemic dose, 8-OH-DPAT-elicted hypermotility was dose dependent and was reversed by WAY-100635, indicating that it was also mediated by activation of 5-HT1A receptors (in a presently unknown location). Motor abnormalities have been reported previously after administration of 5-HT1A agonists to conscious animals (15, 20), and thus the changes described here correspond to the rabbit version of serotonin syndrome. It is possible that after intravenous 8-OH-DPAT, hypermotility-elicited cardiovascular effects interfered with those caused by direct inhibition of raphe-spinal cardiomotoneurons (see below).

Raphe/parapyramidal region in the lower brain stem controls cardiac function. Our results represent an advance in the understanding of neural mechanisms of cardiovascular effects of 5-HT1A agonists. That this class of drugs acts via the central nervous system was demonstrated in early studies (13, 16). In experiments that have followed, presympathetic neurons in the lower brain stem have been identified as potential targets of the drug action (8, 18, 21, 31, 45). In a recent study conducted in anesthetized rats, microinjection of 8-OH-DPAT into the medullary raphe region reversed centrally elicited tachycardia and increase in cardiac sympathetic nerve activity (26, 42). In conscious rats, direct inhibition of raphe neurons by muscimol attenuated air-jet stress-elicited tachycardia (48). Our finding logically extends this line of knowledge by providing direct evidence that activation of 5-HT1A receptors in the raphe-parapyramidal area reduces stress-elicited tachycardia in conscious animals.

The medullary raphe is the major source of descending 5-HT projections to the spinal sympathetic neurons. Many bulbospinal neurons located in the raphe-parapyramidal area are serotonergic and express inhibitory 5-HT1A autoreceptors on their perikarya and dendrites (17). Retrograde viral tracing revealed that this same area contains presympathetic cardiomyonurons.
(44); their neurotransmitter phenotype is presently unknown. If all or some of these raphe-spinal cardiometoneurons are serotonergic, it may be that in our experiments 8-OH-DPAT attenuated tachycardic responses by inhibiting, via 5-HT1A autoreceptors, these cardiac premotoneurons.

Bulbo-spinal neurons located in the raphe-parapyramidal area participate in the control of several other autonomic functions. Recent studies indicate that the ventral medullary raphe contains presympathetic vasomotoneurons innervating the cutaneous vascular bed (5, 6, 29). Presym pathetic neurons controlling thermogenesis in the brown adipose tissue are also located in this area (27). Importantly, these functions (cardioacceleration, thermogenesis, cutaneous vasoconstriction) are activated during various stresses, as well as during pharmacological activation of the major hypothalamic “defense” area: the dorsomedial hypothalamus (22, 29, 47). Our data are in good accord with recent findings in anesthetized animals (7, 42) that the integrity of the raphe pallidus area is essential for the expression of dorsomedial hypothalamus-induced tachycardia and hyperthermia.

Thus a concept is presently emerging that the medullary raphe is a major brain stem center integrating sympathetic outflows during stressful situations. Interestingly, all of these neural outflows appear to be inhibited by the same type of serotonin receptor (5-HT1A) in the raphe region (4, 26, 33), as also found in the present experiments.

Relevance for human studies. The present strategy for the prevention of excessive sympathetic neural traffic to the heart relies on beta-blockers, drugs that act at the heart end of the brain-heart axis. In the present study, we demonstrated that cardiac sympathetic nerve activity may be suppressed by affecting the relevant cardiometoneurons in the brain stem using the selective 5-HT1A receptor agonist 8-OH-DPAT. Our findings of central cardiac sympatholytic effects of 5-HT1A agonists may be clinically relevant in several respects. First, the combination of anxiolytic and cardiac sympatholytic properties may be beneficial for patients with panic disorders (who are at risk of cardiac arrhythmias). Second, recent clinical reports present evidence that fever exacerbates cardiac arrhythmias in patients with long QT syndrome and Brugada syndrome (25, 34). Our results with LPS, in conjunction with previous human studies (9), suggest that these arrhythmias may be precipitated by a fever-related increase in cardiac sympathetic activity. If so, central sympatholytic properties of 5-HT1A agonists would provide a major advantage: by reducing cardiac sympathetic tone, they would protect the heart, and by reducing cutaneous sympathetic vascular activity (see above), they would cause an increase in heat dissipation with subsequent fall in body temperature. Finally, the reduction of cardiac sympathetic tone in the brain, at the site of its origin, may prove useful for patients who require beta-adrenergic blockade but are insensitive to beta-blockers.

Conclusion. Pharmacological activation of 5-HT1A receptors in the medullary raphe-parapyramidal area attenuates tachycardia elicited by psychological stress and as part of the active febrile reaction to the proinflammatory agent LPS. 5-HT1A agonists thus appear to be appropriate candidates for the development of centrally acting sympatholytic cardioprotective drugs, especially for clinical situations associated with high fever.

ACKNOWLEDGMENTS

We are grateful to Candice Morgan and Robyn Flook for technical help.

GRANTS

This study was supported by grants from the National Heart Foundation of Australia, National Health and Medical Research Council, and Flinders Medical Centre Foundation.

REFERENCES


AJP-Regul Integr Comp Physiol • VOL 289 • AUGUST 2005 • www.ajpregu.org


