Activation of 5-HT1A receptors attenuates tachycardia induced by restraint stress in rats

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ABSTRACT

In order to better understand the central mechanisms that mediate increases in heart rate during psychological stress, we examined the effects of systemic and intra-medullary (raphe region) administration of the 5-HT1A-receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)-tetraline) on cardiac changes elicited by restraint in Hooded Wistar rats with pre-implanted ECG telemetric transmitters. 8-OH-DPAT reduced the basal heart rate from 356±12 to 284±12 BPM, predominantly via a non-adrenergic, non-cholinergic mechanism. Restraint stress caused tachycardia (an initial transient increase from 318±3 to 492±21 BPM with a sustained component of 379±12 BPM). β-Adrenoreceptor blockade with atenolol suppressed the sustained component, whereas muscarinic blockade with methyl-scopolamine (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-HT1A antagonist WAY-100,635 (100 µg/kg). Given alone, the antagonist had no effect. Systemically injected 8-OH-DPAT (100 µg/kg) attenuated both the sympathetically-mediated sustained component (from +85±19 to +32±9 BPM) and the vagally-mediated transient (from +62±5 to +25±3 BPM). Activation of 5-HT1A receptors in the medullary raphe by microinjection of 8-OH-DPAT mimicked the anti-tachycardic effect of the systemically administered drug but did not affect basal heart rate. We conclude that tachycardia induced by the restraint stress occurs due to a sustained increase in cardiac sympathetic activity associated with a transient vagal withdrawal. Activation of central 5-HT1A receptors attenuates this tachycardia by suppressing both autonomic effects. At least some of the relevant receptors are located in the medullary raphe/parapyramidal area.
Key words: serotonin, psychological stress, heart rate, sympathetic, medullary raphe.
INTRODUCTION

That psychological stress consistently elicits sympathetically-mediated tachycardic responses is firmly established, but the central mechanisms generating rises in cardiac sympathetic activity are still poorly understood (6). In addition to a theoretical interest, this issue is of major clinical importance 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 beta-blockers. So far, few attempts have been made to reach this goal, mainly due to a lack of knowledge of the localization and pharmacological sensitivity of presynaptic cardiomotor neurons. Recent evidence indicates that the final medullary relay for the descending pathways that activate the heart during stress are located in the raphe-parapyramidal area, and that relevant cardiomotor neurons are sensitive to, and could be inhibited by, serotonin-1A (5-HT1A) receptor agonists (13, 23).

Involvement of 5-HT1A receptors in cardiovascular control is well documented and the consensus is that their activation results in central sympatholytic effects (see (10, 14) for reviews). Most of the relevant studies were conducted either in anaesthetized animals or, if in conscious ones, drug effects were studied in the quiet awake state. Two recent studies have demonstrated that activation of 5-HT1A receptors attenuates cardiovascular changes elicited by psychological stresses (13, 20). In both of these studies it was suggested, but not proven, that the anti-tachycardic effects of 5-HT1A agonists were mediated by suppressing 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 (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: i) to determine how cardiac vagal and sympathetic activity contribute to tachycardia observed during restraint stress; ii) to test whether activation of 5-HT1A receptors could prevent this tachycardia and, what mechanisms might be involved; iii) to identify a potential location of relevant 5-HT1A receptors; and, iv) to determine whether these receptors are activated during restraint by intrinsically released 5-HT. In this study, we used the selective 5-HT1A agonist 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)-tetraline].

MATERIALS AND METHODS
Male Hooded Wistar rats weighing 280-320 g were used in all experiments. 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.

Preliminary surgery
Preliminary surgery was conducted under isoflurane (1.5% in 100% oxygen) anaesthesia. Carprofen (5 mg/kg) was used as an analgesic after the surgery. The telemetric ECG radio-transmitters (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 processus 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 inter-aural line at the midline, inserted vertically to the IV ventricle, fixed to the skull using stainless steel screws and dental cement and closed with an obturator. Animals recovered from anaesthesia and were returned to the animal house for at least one week before experimental studies. They were kept on a reverse 12h/12h 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 at least 1h. Drugs were administered either subcutaneously, diluted in 0.5 ml Ringer’s solution (Experiments 1-7 below), or microinjected into the medullary raphe (Experiments 8-10).

**Experiment 1: does systemic treatment with 8-OH-DPAT affect basal heart rate?** In the first group of rats (n=6), either 8-OH-DPAT (100 µg/kg) or, on different days, Ringer’s solution were administered and recordings were obtained for an hour. Similar injections were made in the second (n=6) and third (n=6) groups after β-adrenergic blockade (atenolol 2 mg/kg) or after a combined muscarinic and β-adrenergic blockade (methyl-scopolamine bromide 50 µg/kg plus atenolol 2 mg/kg), respectively.

**Experiment 2: does systemic treatment with 8-OH-DPAT affect stress-induced tachycardia?** On different days, either 8-OH-DPAT (10, 30 or 100 µg/kg) or Ringer’s solution was administered and 15 min later the rats were placed into a restrainer (transparent plastic tube, with 60 mm ID) for 30 min (n=7).
Experiment 3: does systemic treatment with 8-OH-DPAT affect stress-induced tachycardia following 5-HT1A receptor blockade with WAY-100,635? Prior to the restraint, animals received, on different days, the following combination of drugs: a) Ringer/Ringer; b) Ringer/8-OH-DPAT (100 µg/kg); c) WAY-100,635/8-OH-DPAT (both at 100 µg/kg) (n=6).

Experiment 4: does systemic treatment with WAY-100,635 affect stress-induced tachycardia? Prior to restraint, animals received, on different days, either WAY-100,635 (100 µg/kg) or Ringer’s solution (n=6).

Experiment 5: does autonomic blockade affect restraint-induced cardiac responses? Prior to restraint, animals received, on different days: a) Ringer’s solution; b) atenolol (2 mg/kg); c) methyl-scopolamine bromide (50 µg/kg) (n=8).

Experiment 6: does systemic treatment with 8-OH-DPAT affect stress-induced tachycardia following vagal blockade? Prior to restraint, animals received, on different days, the following combination of drugs at 10-min intervals: a) methyl-scopolamine (50 µg/kg)/Ringer; b) methyl-scopolamine (50 µg/kg)/8-OH-DPAT (100 µg/kg) (n=6).

Experiment 7: does systemic treatment with 8-OH-DPAT affect stress-induced tachycardia following β-adrenergic blockade? Prior to restraint, animals received, on different days, the following combination of drugs at 10-min intervals: a) atenolol (2 mg/kg)/Ringer; b) atenolol (2 mg/kg)/8-OH-DPAT (100 µg/kg). N=6.
Experiment 8: does intracerebral microinjection of 8-OH-DPAT affect basal heart rate?

Animals received an intra-medullary microinjection of either 8-OH-DPAT (1 nmol in 100 nl) or, on a different day, the equivalent volume of sterile Ringer’s solution, and recordings were continued for another hour. An injection cannula (OD 0.2 mm stainless steel wire, Small Parts, USA) 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 observing 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 (n=6).

Experiment 9: does intracerebral microinjection of 8-OH-DPAT affect stress-induced tachycardia? Prior to restraint, animals received an intra-medullary microinjection of either 8-OH-DPAT (1 nmol in 100 nl) or, on a different day, the equivalent volume of sterile Ringer’s solution. In another 5 animals (control group), similar injections were performed 2.5 mm more dorsal (8.5 mm below the surface of the skull). Microinjections were performed similarly to the previous experiment (n=8).

Experiment 10: does 5-HT1A receptor blockade with WAY-100,635 prevent anti-tachycardic effects of intra-medullary administered 8-OH-DPAT? Prior to restraint, animals received, on different days, the following combination of drugs: a) Ringer (s.c.) / Ringer (brain microinjection, 100 nl.); b) Ringer (s.c.) / 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl); c) WAY-100,635 (s.c., 100 µg/kg) / 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl) (n=6).
Thus, this study was conducted in 77 rats (57 with systemic administration and 20 with intra-
medullary administration of 8-OH-DPAT). Each animal cohort was used for just one type of
experiment. All experimental procedures were performed at least 48 h apart. To avoid serial
effects, we used a counterbalanced or rotational design. All chemicals were from Sigma (USA).

Visualization of microinjection sites

Medullary microinjection sites were labelled with horseradish peroxidase (HRP) (100 nl of 0.1%
solution), which was administered into the medulla immediately after the termination of the last
experiment using the same injection cannula. Rats were euthanized with Lethabarb and perfused
transcardially with formaldehyde fixative. Brains were removed and sectioned (50 µm thickness).
HRP was visualised by incubating sections in 0.05% solution of di-amino-benzidine 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 A/D converter and
Chart 5.4 software (ADInstruments). Heart rate was calculated from the ECG records using the
same software. After removing artefacts, heart rate was automatically averaged for every minute.
Spectral indices of heart rate variability were computed using the heart rate variability (HRV)
module of the Chart 5.4 software. The low-frequency (LF) 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. For
characterising the recovery of the HR after handling-related tachycardia, we used $T_{1/2}$ – time
period during which HR fell to 50% of the peak increase. The dose-dependence of 8-OH-DPAT–induced effects was assessed using linear regression. Group data was analysed by ANOVA, with Fishers’s protected t-test and with the significance threshold set at the 0.05 level. All data is presented as mean±SEM and, where possible, data values are embedded in our illustrations.

RESULTS

Effect of systemic 8-OH-DPAT on the basal heart rate and on HRV indices
Subcutaneous injection of either 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 the basal, and remained at this low level from about 15 to 40 min post-injection (Fig. 1A). In contrast, after the vehicle, reversion of injection-induced tachycardia was quite slow, so that during the period from 15 to 40 min post-injection HR was still different both from basal level and from the corresponding values after 8-OH-DPAT. There was also a significant difference in the speed of HR decay from the peak: T1/2 was 2.5±0.7 min and 13±1.2 min after vehicle and drug, respectively (p<0.01, n=6). Treatment with 8-OH-DPAT substantially and significantly elevated HRV indices that reflect vagal modulation of the heart rate – RMSSD (1.6±0.1 and 4.8±0.7 ms after vehicle and drug, respectively; p<0.01, n=6) and high-frequency 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 than determined whether 8-OH-DPAT-induced bradycardia could be prevented by beta-adrenergic blockade (Fig. 1B). Administration of atenolol caused short-lasting 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 compared to those after the first injection. While after the vehicle HR still continued to
fall, the 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 compared to the corresponding basal (pre-atenolol) values.

In the next experiment, we tested whether 8-OH-DPAT-induced bradycardia persists after a combined vagal and sympathetic blockade (Fig. 1C). Administration of methyl-scopolamine 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 prior to the third injection. As shown in Fig. 1C, 8-OH-DPAT still produced a substantial bradycardic effect, so that during the maximum of this effect, HR values were significantly different from both 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 doses of 10, 30 and 100 µg/kg) affects restraint-induced cardiac responses. Mean group data are shown in Fig. 2A, and data values are presented in Table 1A. Tachycardia associated with drug or vehicle administration reverted to the basal level within 15 min, so there was no difference for the pre-restraint values between the four conditions. After vehicle, restraint stress caused tachycardia which peaked at about 500 BPM within 1-1.5 min and than 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. In subsequent sections we will thus refer to these data points as “peak restraint” and “steady-state restraint”. Effects of pre-
treatment with 8-OH-DPAT depended on the dose used. After the dose of 10 µg/kg, restraint-induced tachycardia did not differ from the control condition. At the dose of 30 µg/kg, 8-OH-DPAT substantially reduced the steady-state increase in HR, and at the dose of 100 µg/kg, the drug attenuated both initial peak tachycardia and the steady-state increase in the HR (Table 1A). Fig. 2B shows results of the linear regression analysis.

Effect of systemic 8-OH-DPAT on restraint-induced tachycardia after selective blockade of 5-HT1A receptors

In order to determine whether selective blockade of 5-HT1A receptors prevents anti-tachycardic effects of 8-OH-DPAT during stress, we compared restraint-induced changes in HR in three conditions using the following drug combinations: i) Ringer/Ringer; ii) Ringer/8-OH-DPAT; and iii) WAY-100,635/8-OH-DPAT. Pre-restraint, peak and steady-state values did not differ between Ringer/Ringer and WAY-100,635/8-OH-DPAT conditions; both were however substantially and significantly different from the Ringer/8-OH-DPAT condition (Fig. 3 and Table 1B). Thus, selective blockade of 5-HT1A receptors completely abolished the effects of 8-OH-DPAT.

Effects of systemically administered WAY-100,635 on restraint-induced tachycardia.

In order to test whether there are any 5-HT1A receptor-dependent cardiac effects due to intrinsic 5-HT release during stress, we subjected rats to the restraint after injection of either vehicle or WAY-100,635 (100 µg/kg s.c.), a selective 5-HT1A receptor antagonist. We did not find any differences between vehicle and drug conditions (see Fig. 4 for graphs and data values, n=6).

Effects of autonomic blockade on restraint-induced tachycardia.
In the next set of experiments (n=8), we addressed the question of which autonomic components mediate restraint-induced tachycardia. For this purpose, rats were subjected to the restraint 15 min after injection of either atenolol, methyl-scopolamine or vehicle (Fig. 5; data values for HR are also presented in this figure; for the differences in HR values see Table 2). After vehicle, the restraint provoked tachycardic responses similar to those described in the previous sections. In animals with sympathetic blockade, restraint provoked only a small transient tachycardia, and during the second half of restraint, HR did not differ from pre-restraint or basal values. Administration of methyl-scopolamine caused a rapid rise in the HR that remained elevated. Subjecting rats to the restraint after vagal blockade caused initial tachycardia, with HR values significantly higher compared to post-vehicle restraint. After vagal blockade, the increase in the HR for the “steady-state” component (vs. pre-restraint values) was significantly higher compared to Ringer (Table 2). For both vehicle and methyl-scopolamine conditions, “steady-state” values were significantly different compared to pre-restraint (p<0.01).

Effects of systemic 8-OH-DPAT on restraint-induced tachycardia after sympathetic blockade.

In this experiment (n=6), after sympathetic blockade with atenolol, either 8-OH-DPAT or vehicle were administered prior to the restraint (see Fig. 6 for traces and data values). The vehicle injection provoked a small and short-lasting tachycardia, so that prior to 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 both the basal level and from the pre-restraint value in the Vehicle condition. After 8-OH-DPAT, restraint provoked a small transient tachycardic response (+25±3 BPM) that was significantly different
compared to vehicle (+62±5 BPM, p<0.01, n=6). The time course of 8-OH-DPAT-elicited bradycardia was similar to that occurring in the first experiment of this study (ie. the drug alone).

*Effects of systemic 8-OH-DPAT on restraint-induced tachycardia after vagal blockade.*

In 6 animals tested, administration of methyl-scopolamine caused a rapid increase in HR. Effects of a subsequent injection of 8-OH-DPAT did not differ from those of vehicle, so that the pre-restraint values for both conditions were not different (see Fig. 8 for traces and data values). Restraint-induced tachycardia was substantially and significantly attenuated in 8-OH-DPAT-treated animals, in terms of both absolute values (see Fig. 7) and the magnitude of increase (+85±19 BPM after vehicle vs. +32±9 BPM after the drug, 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 not different from corresponding pre-restraint values (although there was a tendency for a fall for the drug condition, with p=0.062).

*Effects of intra-medullary microinjection of 8-OH-DPAT on the basal heart rate.*

Apart from a short-lasting tachycardia associated with handling, microinjection of either Ringer’s solution or 8-OH-DPAT into the medullary raphe had no effect on the basal heart rate (see Fig. 8 for illustrations and data values). Handling-related increase in HR was shorter after 8-OH-DPAT (T_{1/2}=4.2±0.4 min) compared to Ringer (T_{1/2}=11±0.9 min, p<0.05, n=6).

*Effects of intra-medullary microinjection of 8-OH-DPAT on restraint-induced tachycardia.*

To identify the potential location of 5-HT1A receptors responsible for the described above anti-tachycardic effects of 8-OH-DPAT, we microinjected the drug or the vehicle into the raphe/parapyramidal area of the lower brainstem (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 faster after 8-OH-DPAT, so that pre-restraint HR values were significantly different (see Fig. 9A for graphs and data values). After 8-OH-DPAT, restraint-induced tachycardia was attenuated, both in terms of absolute HR values for the transient and steady-state components (Fig. 9A) and in terms of differences between the pre-restraint and the restraint values. For the peak tachycardia, the latter were reduced from +144±11 to +59±7 BPM (n=8, p<0.05), and for the steady-state component from +31±4 to 8±5 BPM. An example of a histologically confirmed injection site is presented in Fig. 9B. In another 5 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-100,635 on anti-tachycardic action of intra-medullary administered 8-OH-DPAT.

In the described above experiment, OH-DPAT was microinjected into the raphe/parapyramidal area at relatively high concentration. In order to prove that the anti-tachycardic effect of the drug represented a specific effect mediated by its interaction with 5-HT1A receptors, in another 6 rats intra-medullary microinjections of 8-OH-DPAT were preceded by systemic administration of either WAY-100,635 (100 µg/kg) or vehicle; systemic vehicle followed by intra-medullary 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-100,635 completely abolished the effect of 8-OH-DPAT, so that the magnitude of the tachycardia did not differ from that following intra-medullary administration of the vehicle. Data values are presented in Fig. 10.
DISCUSSION

There are several novel findings in this study: i) a demonstration that activation of central 5-HT1A receptors evokes bradycardia mediated by cardiac non-β-adrenergic, non-cholinergic neurotransmission mechanisms; ii) that 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; iii) that 8-OH-DPAT substantially attenuates both of these autonomic components, in a dose-dependent manner, and acting via 5-HT1A receptors; and iv) that at least some of these receptors must be located in the medullary raphe/parapyramidal area.

Effects of 8-OH-DPAT on the basal heart rate.

Bradycardic effects of 8-OH-DPAT observed in our rats are in full agreement with previous reports comprehensively reviewed by (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 terms “sympathetic” and “vagal” are used as synonyms for “adrenergic” and “cholinergic”. Our HRV analysis results - specifically the rise in the RMSSD (root-mean-square of the beat-to-beat differences, an index that reflects increased difference between adjacent RR interval) and in the high-frequency 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 due to sympathetic
withdrawal; positive α-adrenergic chronotropy has been demonstrated in both in situ and in vitro rat heart preparations (17, 19, 21).

It is now well recognised that cardiac sympathetic and cardiac vagal nerve terminals, in addition to noradrenaline and acetylcholine, contain numerous other neurotransmitters, defined usually as “NANC” – “non-adrenergic non-cholinergic” (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 both classical and NANC neurotransmitters. We consider that this is an alternative plausible explanation of the 8-OH-DPAT-induced bradycardia reported here. As 8-OH-DPAT is a well known hypothermic agent, it may be that fall in body temperature (not measured in our study) directly contributed to the overall bradycardic effect of the drug. At present we cannot define whether observed bradycardia occurred due to the reduction in the excitatory or due the increase in the inhibitory cardiac drive; clarification of the underlying pharmacological mechanisms and evaluation of potential hypothermia-induced fall in heart rate requires 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 to WAY-100,635, a selective antagonist of these receptors. While we cannot entirely exclude a 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 as this is a putative location of presympathetic cardiomotor neurons activated during stress. Evidence for this was initially presented by Zaretsky et al. (23) who observed substantial attenuation of tachycardia after intra-raphe 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). Our current microinjection results support this finding and confirm that the effect of the drug was indeed due to the activation of 5-HT1A receptors, as it was sensitive to the selective 5-HT1A receptor antagonist. Both systemic administration and intra-medullary microinjection of 8-OH-DPAT also caused more a rapid return of HR to the basal level after handling-related tachycardia compared to vehicle. This is not surprising as handling is also a stressful event, and likely activates the same brain areas as does restraint. While the anti-tachycardic 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 auto-inhibitory 5-HT1A receptors (8).

Intra-medullary microinjection of 8-OH-DPAT had no effect on the basal HR, in contrast to the systemic administration of the drug that caused bradycardia. This is in good accord with the already cited study by Zaretsky et al. (23) who did not observe any 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 anti-tachycardic effect) is mediated at some other location. Additional studies are required to identify this location.
Intriguingly, pre-treatment with WAY-100,635 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 (eg. 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. As adrenals do not contribute to the rise in the 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 sympatholytic. Such an effect is in agreement with previous studies in anaesthetized 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 noradrenaline 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 for the whole duration of the restraint. We do not have a satisfactory explanation for why this occurred; 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 to the control (post-vehicle) condition, indicating that the drug-sensitive component was sustained, at least compared to control.

Our data suggest that a central sympatholytic effect is not the only mechanism responsible for the anti-tachycardiac action of 8-OH-DPAT during restraint stress. Transient vagal withdrawal is the likely mechanism underlying the initial short-lasting restraint-induced tachycardic component, as this component virtually disappeared following the muscarinic receptor blockade. It is unlikely that non-adrenergic transmitters released from the sympathetic nerves contribute to this component, as in this case the time course of their effect must be similar to that of the noradrenaline effect (which is sustained as we have demonstrated here using β-adrenoreceptor 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 anaesthetized animals (14). The potential underlying mechanism could be the same as 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, namely that after methyl-scopolamine, the sustained component of the tachycardia was substantially larger than in control. Clearly, the methyl-scopolamine-resistant sustained component was sympathetically-mediated, 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 sympatho-vagal co-activation 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 as vagal effects could be presynaptic and thus would not be observed when noradrenaline effects are suppressed. Our hypothesis is certainly speculative, and requires more direct evidence.

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

Van den Buuse and Wegener (20) recently reported anti-tachycardic effects of 5-HT1A receptor agonists during another stress paradigm, the open field. The authors found that both tachycardic effects of stress and anti-tachycardic 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 Wistars vs. Wistar Kyoto rats from van den Buuse)

*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 heart rate returned to near-basal level. Additionally, we always paired drug with vehicle, thus excluding any effect of injection-related stress.

Both autonomic blockers used in our study (atenolol and methyl-scopolamine) poorly penetrate the blood-brain barrier, and thus 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 or compromise our results. Systemic administration of 8-OH-DPAT at doses similar to ours reduced arterial pressure in conscious rats at rest (3), and therefore bradycardic effects of the drug observed in our study at resting state (without stress) were clearly not the consequence of baroreflex. Restraint stress causes sustained pressor response in rats (1, 5, 12). While 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 made any contribution to the anti-tachycardic 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: i) tachycardia induced by restraint stress occurs due to sustained increase in cardiac sympathetic activity associated with a transient vagal withdrawal; ii) activation of central 5-HT1A receptors attenuates this tachycardia, by suppressing both autonomic effects; iii) some relevant receptors are located in the medullary raphe/parapyramidal area. While we did not find any evidence of activation of these receptors by intrinsically released serotonin during psychological stress, we believe that our results represent both theoretical and clinical interest. Firstly, 5-HT1A receptors could serve as a marker (though non-specific) for the identification of the raphe-spinal presympathetic cardiomotor neurons. Secondly, presented here evidence for the central cardioprotective effect of 5-HT1A receptor agonists may be clinically relevant, as suppression of excessive cardiac sympathetic tone at its origin, in the brain, could represent an alternative therapeutic strategy for those cardiac patients who do not tolerate or who are insensitive to beta-blockers.

**Acknowledgements:** We would like to thank Ms Sarah Fitzpatrick for the excellent technical support. This study was funded by grants from NHMRC and NHF of Australia.
FIGURE LEGENDS

Fig. 1. Systemic 8-OH-DPAT reduces basal HR when given alone (A), after β-adrenergic blockade with atenolol (B) and after combined β-adrenergic and M-cholinergic blockade with atenolol and methyl-scopolamine. Experiments were conducted in 3 separate groups of rats, n=6 for each. White circles – vehicle; black symbols – 8-OH-DPAT (100 µg/kg s.c.). Mean data values are presented near corresponding traces. * and ** - significantly different from the pre-injection basal level, p<0.05 and p<0.01, respectively. # and ## - significantly different from vehicle for the same time point, p<0.05 and p<0.01, respectively.

Fig. 2. Systemic pre-treatment with 8-OH-DPAT attenuates tachycardia elicited by the restraint stress. A - traces showing changes in the heart rate in animals (n=7) pre-treated, on different days, with either vehicle or three doses of 8-OH-DPAT (10, 30 and 100 µg/kg s.c.). Time of injection and the restraint period are indicated on the time axis. Also marked are the time periods (baseline, pre-restraint, peak restraint and steady-state restraint) during which the data value is statistically assessed. The data values from this experiment are presented in Table 2. B – results of regression analysis of data from (A) showing dose-dependence of 8-OH-DPAT action for both peak (upper graph) and steady-state (bottom graph) components of restraint-induced tachycardia.

Fig. 3. Selective blockade of 5-HT1A receptors with WAY-100,635 (100µg/kg s.c.) prevents anti-tachycardic effects of 8-OH-DPAT (100 µg/kg s.c.) during restraint stress; n=6. Traces show changes in heart rate in animals pre-treated, on different days, with the following drug or vehicle combination: vehicle/vehicle (Control), vehicle/8-OH-DPAT (5-HT1A receptor activation) and WAY-100,635/8-OH-DPAT (5-HT1A receptor agonist after receptor blockade). Time of injection and the restraint period are indicated on the time axis. Also marked are the time periods...
(baseline, pre-restraint, peak restraint and steady-state restraint) during which data values are statistically assessed. The data values from this experiment are presented in Table 2.

Fig. 4. Selective blockade of 5-HT1A receptors with WAY-100,635 (100µg/kg s.c.) did not affect tachycardia elicited by the restraint stress. Traces show changes in heart rate in animals pre-treated, on different days, with either vehicle or WAY-100,635; n=6. Time of injection and the restraint period are indicated on the time axis. Mean data values are presented near corresponding traces.

Fig. 5. Autonomic blockade reveals components of restraint-elicited tachycardic responses. Traces show changes in heart rate in animals pre-treated, on different days, with vehicle (○), β-adrenergic blocker atenolol (△, 2 mg/kg s.c.) or muscarinic cholinergic blocker methylscopolamine (□, 50 µg/kg s.c.); n=8. Time of injection and the restraint period are indicated on the time axis. Mean data values are presented near corresponding traces. * and ** - significantly different from the corresponding pre-restraint level, p<0.05 and p<0.01, respectively. Horizontal dashed lined highlight pre-restraint values of the HR. Note substantial difference during the second half of the restraint between the three conditions.

Fig. 6. Systemically administered 8-OH-DPAT attenuates transient stress-induced tachycardia persisting after β-adrenergic blockade with atenolol (2 mg/kg). Traces show changes in heart rate in animals pre-treated, on different days, with either Atenolol/Ringer (○) or Atenolol/8-OH-DPAT (●) combination; n=6. Drugs were administered subcutaneously. Time of injection and the restraint period are indicated on the time axis. Mean data values are presented near corresponding traces. ## - significantly different from vehicle for the same time point, p<0.01.
Fig. 7. Systemically administered 8-OH-DPAT attenuates stress-induced tachycardia persisting after muscarinic cholinergic blockade with methyl-scopolamine (50 µg/kg). Traces show changes in heart rate in animals pre-treated, on different days, with either methyl-scopolamine/Ringer (○) or methyl-scopolamine/8-OH-DPAT (●) combinations; n=6. Drugs were administered subcutaneously. Time of injection and the restraint period are indicated on the time axis. Mean data values are presented near corresponding traces. ## - significantly different from vehicle for the same time point, p<0.01.

Fig. 8. Intra-raphe microinjection of 8-OH-DPAT does not affect basal heart rate. Traces show changes in heart rate in animals treated, on different days, with either vehicle (○) or 8-OH-DPAT (1nmol in 100nl; ●); n=6. Note faster return to the baseline after the drug. Time of injection is indicated on the time axis.

Fig. 9. Intra-raphe microinjection of 8-OH-DPAT attenuates tachycardia elicited by the restraint stress. A - traces show changes in heart rate in animals pre-treated, on different days, with either vehicle or 8-OH-DPAT (1nmol in 100nl; n=8). Time of injection and the restraint period are indicated on the time axis. Mean data values are presented near corresponding traces. * and ** - significantly different from the pre-injection basal level, p<0.05 and p<0.01, respectively; # - significantly different from vehicle for the same time point, p<0.05. B – illustration of the intramedullary injection site (dark-field photograph of the coronal section cut through the lower brainstem). Brown area surrounded by the dashed line contains the HRP reaction product (see Methods). Abbreviations: Amb – nucleus ambiguus; ECu – external cuneate nucleus; Gi –
gigantocellular reticular nucleus; NTS – nucleus of the solitary tract; Py – pyramid; spV – spinal tract of the trigeminal nerve; SpV – spinal nucleus of the trigeminal nerve.

Fig. 10. Systemically administered WAY-100,635 (100 µg/kg) prevents anti-tachycardic effects of intra-medullary microinjected 8-OH-DPAT. Traces show changes in heart rate in animals pre-treated, on different days, with the following combination of drugs: ○ - Ringer (s.c.) / Ringer (brain microinjection, 100 nl); ● - Ringer (s.c.) / 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl); (●) - WAY-100,635 (s.c., 100 µg/kg) / 8-OH-DPAT (brain microinjection, 1 nmol in 100 nl). Time of injections and the restraint period are indicated on the time axis. Mean data values are presented near corresponding traces. * and ** - significantly different from the pre-injection basal level, p<0.05 and p<0.01, respectively; # and ## - significantly different from two other conditions (Ringer/Ringer and WAY-100,635/8-OH-DPAT) for the same time point, p<0.05 and p<0.01, respectively.
REFERENCES


Table 1. Restraint-induced tachycardia and effects of 5-HT1A 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>A. Effects of different doses of 8-OH-DPAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>318±3</td>
<td>353±15</td>
<td>492±21**</td>
<td>379±12** (+27±13)</td>
</tr>
<tr>
<td>8-OH-DPAT 10 µg/kg</td>
<td>311±3</td>
<td>321±4</td>
<td>501±10** (+181±16)</td>
<td>365±3* (+43±15)</td>
</tr>
<tr>
<td>8-OH-DPAT 30 µg/kg</td>
<td>307±2</td>
<td>320±4</td>
<td>450±23** (+132±17)</td>
<td>320±2## (+2±18)</td>
</tr>
<tr>
<td>8-OH-DPAT 100 µg/kg</td>
<td>310±2</td>
<td>337±5</td>
<td>419±17*** (+81±14##@)</td>
<td>314±2## (-23±14##@@)</td>
</tr>
<tr>
<td><strong>B. WAY-100,635 prevents effects of 8-OH-DPAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle + Vehicle</td>
<td>327±3</td>
<td>387±16</td>
<td>514±8** (+187±21)</td>
<td>386±14* (+58±17)</td>
</tr>
<tr>
<td>Vehicle + 8-OH-DPAT</td>
<td>318±3</td>
<td>334±12**</td>
<td>400±23*** (+84±16##*)</td>
<td>318±18### (+1±15##@)</td>
</tr>
<tr>
<td>WAY-100,635 + 8-OH-DPAT</td>
<td>331±2</td>
<td>414±13</td>
<td>508±6** (+175±19)</td>
<td>392±6* (+60±17)</td>
</tr>
</tbody>
</table>

Data are presented as mean±S.E.M. (BPM). For each set of data, top line shows absolute values (Table 1 A and B), and bottom line shows the difference between the pre-restraint value and peak or steady-state value during restraint (Table 1A). Because pre-restraint level was different between conditions in Table 1B, the bottom lines show here the difference between the basal level and peak or steady-state value during restraint. ** - significantly different from corresponding basal level value, p<0.01; * - significantly different from vehicle, p<0.05; ## - significantly different from vehicle, p<0.01; " - significantly different from the lowest dose of 8-OH-DPAT, p<0.05; "" - significantly different from the lowest dose of 8-OH-DPAT, p<0.01; """" - significantly different from Vehicle/Vehicle condition, p<0.05; """" - significantly different from Vehicle/Vehicle condition, p<0.01; " - significantly different from WAY-100,635 + 8-OH-DPAT condition, p<0.05; """" - significantly different from WAY-100,635 + 8-OH-DPAT condition, p<0.01.
Table 2. Effects of autonomic blockade on restraint-induced tachycardia.

<table>
<thead>
<tr>
<th></th>
<th>Delta (peak/pre-restraint)</th>
<th>Delta (steady-state/pre-restraint)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringer</td>
<td>124±11</td>
<td>25±6</td>
</tr>
<tr>
<td>Atenolol</td>
<td>28±4**</td>
<td>1±4**</td>
</tr>
<tr>
<td>Methyl-scopolamine</td>
<td>97±11##</td>
<td>52±6##</td>
</tr>
</tbody>
</table>

Data are presented as differences between peak during restraint and pre-restraint values (left column) and between “steady-state” restraint and pre-restraint values (right column). ** - significantly different (p<0.01) from both Ringer and Methyl-scopolamin conditions; ## - significantly different (p<0.05) from both Ringer and Atenolol conditions.
- Methyl-scopolamine
- Ringer
- Atenolol

Heart rate (BPM)

Time (min)

- 324±4
- 315±6
- 328±4

- 326±3
- 349±12
- 352±7**
- 374±9*
- 427±11
- 472±9**
- 525±6**
- 478±5**

drug injected

restraint
The graph shows heart rate (BPM) over time (min) with data points for Vehicle and 8-OH-DPAT microinjection.

- **Vehicle**
  - Initial value: 333 ± 8 BPM
  - Final value: 318 ± 11 BPM

- **8-OH-DPAT**
  - Initial value: 320 ± 9 BPM
  - Final value: 307 ± 6 BPM

A black arrow indicates the time of Vehicle or 8-OH-DPAT microinjection.