Inhibition of the cardiovascular response to stress by systemic 5-HT_{1A} activation: sympathoinhibition or anxiolysis?

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Vianna DML, Carrive P. Inhibition of the cardiovascular response to stress by systemic 5-HT_{1A} activation: sympathoinhibition or anxiolysis? Am J Physiol Regul Integr Comp Physiol 297: R495–R501, 2009. First published June 10, 2009; doi:10.1152/ajpregu.00232.2009.—5-HT_{1A} agonists given systemically are known to produce anxiolytic effects. In addition, a growing body of research is showing that those compounds also have central sympathoinhibitory properties. Since emotional arousal gives rise to sympathetic activation, it is not clear whether systemic treatment with a 5-HT_{1A} agonist reduces the sympathetic response to emotional stress primarily by a direct action on sympathetic-related sites in the brain or indirectly through reducing anxiety. To test this, we compared the effect of intraperitoneal injections of 8-hydroxy-2-(di-n-propylamino)tetrinal (8-OH-DPAT; 0.05 and 0.25 mg/kg), a preferential 5-HT_{1A} agonist, or vehicle on the cardiovascular responses to four stressors known to produce sympathetic activation, three being emotional stressors, and one physiological. In conscious rats, 30-min exposure to either a neutral context, a fear-conditioned context, or to restraint stress led to increases in heart rate and blood pressure, which were attenuated by 8-OH-DPAT. In contrast, the same treatment did not reduce the cardiovascular response to 30-min cold exposure (4°C). The results suggest that 8-OH-DPAT acts preferentially on limbic, rather than central, autonomic sites. Hence, doses of 5-HT_{1A} agonists, which are just sufficient to produce anxiolysis, are not enough to cause true sympathoinhibition.

sympathetic responses; freezing; anxiety; cold defense; tachycardia

NOT LONG AFTER ITS DISCOVERY, the 5-HT_{1A} receptor (31) was suggested to be responsible for the anxiolytic action of buspirone (10). Since then, a great deal of research has firmly established that 5-HT_{1A} receptor activation can induce anxiolysis (2, 6, 33).

Quite independently, research in the central control of the autonomic nervous system, done mostly in the unconscious animal, has also shown that systemic 5-HT_{1A} receptor stimulation can give rise to sympathoinhibition (19). This sympathoinhibition does not affect the baroreflex or chemoreflex (16), but it does suppress responses evoked from the dorsomedial hypothalamus (DMH; 15, 16), a region implicated in limbic and thermoregulatory functions (4). In line with this, activation of 5-HT_{1A} receptors in the medullary raphe, a major efferent target from the DMH, can inhibit sympathetic responses to intravenous leptin (22), skin cooling (23, 29), inflammation (26), restraint (27), and air jet stress (26).

Since sympathetic responses to all of these challenges can be attenuated at the level of the medullary raphe through 5-HT_{1A} stimulation, and since systemic 5-HT_{1A} activation can also reduce sympathetic responses to cold exposure (28, 30), inflammatory (26), air jet (26), open field (34), restraint (27), and social stress (25), it has been widely assumed that systemically administered 5-HT_{1A} agonists reduce all those sympathetic responses by acting on the medullary raphe (25, 27).

It is certainly likely that high enough systemic doses of 5-HT_{1A} agonists would lead to inhibition of the medullary raphe and, hence, affect the many responses mediated by it. But there remains the possibility that systemic doses that are just sufficient to produce anxiolysis may reduce the sympathetic drive indirectly, by an action at more rostral, purely limbic sites. If this were true, then doses that would be just enough to decrease the sympathetic responses to psychological stress would not affect challenges of a more physical nature in the same way.

To test this possibility, we exposed rats to a series of tests known to evoke sympathetically mediated cardiovascular responses, either through different kinds of emotional stress (Exploration, Conditioned Fear, and Restraint) or through a physical stress (Cold Exposure). We hypothesized that doses of 8-hydroxy-2-(di-n-propylamino)tetrinal (8-OH-DPAT) just sufficient to block or reduce the heart rate (HR) and mean arterial pressure (MAP) responses to Exploration, Conditioned Fear, and Restraint would not reduce HR and MAP responses to cold exposure. Our results show that at the doses used, 8-OH-DPAT reduced the sympathetic responses to all emotional stressors, but not to Cold Exposure.

METHODS

Animals

The subjects were 16 experimentally naïve male Wistar rats (400–550 g) obtained from the colony of specific pathogen-free rats maintained by the University of New South Wales. The animals were housed in individual plastic home boxes (65×40×22 cm) with ad libitum food and water. The room in which they were housed and tested was maintained at a constant temperature of 22–25°C and kept on a normal 12:12-h light/dark cycle (lights off from 7 PM to 7 AM). All procedures were approved by the Animal Ethics Committee of the University of New South Wales and conformed to the rules and guidelines on animal experimentation in Australia. The animals were handled regularly to habituate to the experimenters.

Surgery

Rats were anaesthetized with a mixture of ketamine and xylazine (100 mg/kg and 50 mg/kg ip, respectively) and implanted intraperitoneally with radiotelemetric probes (model PA-C40, n = 11 or model C50-PXT, n = 4; Data Sciences International, St. Paul, MN), as described previously (9, 36). Both probe types record pulsatile blood pressure from the descending aorta. In addition, C50-PXT probes record body temperature. The animals were then given 1 wk to recover from the implantation.

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Drugs

Animals were injected with the preferential 5-HT$_{1A}$ agonist 8-OH-DPAT (Sigma) 30 min prior to testing at 0.25 mg/kg (high dose), 0.05 mg/kg (low dose), and vehicle only (ip). Tests occurred on separate days, and the order of testing was counterbalanced.

Behavioral Tests

Up to four different tests associated with an increase in cardiovascular activity were used: Exploration, Conditioned Fear to context, Restraint, and Cold Exposure. All the tests were done during the light phase of the cycle. Prior to the tests, the animals were at rest in their home cages.

Exploration. Exploratory behavior was evoked by exposing the animals for 30 min to a clean empty plastic home box with no bedding, food, or water.

Fear. Conditioned Fear was evoked by reexposing the animals for 30 min to an aversive context (a foot-shock box) in which the animal had previously received electric foot shocks. The foot-shock boxes were 23-cm long×21-cm wide×60-cm tall (open at the top), and were cleaned before and after use with 0.05% acetic acid. The fear conditioning was done according to a standard procedure in the lab, as previously described (36). Briefly, the animals were given four foot-shock sessions (40 min each, 4 shocks per session, 1 mA/1 s each) on separate days and over the course of 5–8 days. No foot shock was given during the test.

Restraint. Restraint stress lasted for 30 min and was carried out in restrainers made of plastic-coated wire (20-cm long×6-cm wide×7-cm tall).

Cold Exposure. Cold Exposure lasted 30 min and was done in a small refrigerator set at 4°C as previously described (18). The floor of the Plexiglas box (20-cm long×23-cm wide×40-cm tall) in which the animal stood was covered with clean bedding at the temperature of the refrigerator.

Data Collection and Analysis

Radiotelemetry. Four parameters were recorded from the telemetric probes. Two were cardiovascular (MAP and HR), one was thermal [intraperitoneal body temperature (TBody) when using the C50-PXT probes], and one was behavioral [body movement (activity)]. The ART Gold software (Data Sciences International, St. Paul, MN) sampled MAP, HR, and TBody every 30 s from 3-s time windows and cumulated activity for every 30-s period.

Behavior. During the Conditioned Fear test, an experimenter sitting in the experimental room manually recorded the time spent freezing and each time an ultrasonic vocalization (USV) was emitted. Freezing was sampled every 2 s and defined as a complete absence of movement while the animal assumed a characteristic tense posture. Head movement and lying down were scored as not freezing. USV were detected with a bat detector (model Mini-3; Ultrasound Device) tuned to the 20–25 kHz frequency range. Both parameters were entered manually using handheld buttons connected to the ART system, which automatically cumulated those parameters every 30 s in synchrony with the telemetric data.

All parameters were later averaged over 1-min periods.

Statistical Analysis

The data were analyzed using repeated-measures ANOVA. The repeated measure was time (from minute 1 to minute 29 of the 30-min period) and the independent factor was drug dose. Statistical significance was set at $P < 0.05$.

**Figure 1.** Time course of changes in heart rate (HR), mean arterial pressure (MAP), and activity evoked by exploration of a neutral context (clean, empty box) after injections of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; 0, 0.05, or 0.25 mg/kg ip). Data are means ± SE.
RESULTS

Prior to the tests, the animals were at rest, usually asleep. Baseline values for HR and MAP were on average 345 beats/min and 104 mmHg, respectively (Fig. 1–4). TBody was recorded in only five of the sixteen rats and had an average baseline value of 37.1°C (data not shown).

Saline injection produced an ~10-min surge of activity associated with increases in HR and MAP (Figs. 1–4). The response was similar immediately after the 8-OH-DPAT injections, except that the cardiovascular responses were attenuated. HR was particularly reduced with the high dose. TBody increased by 0.5°C after saline and 0.2°C after the low dose and fell by 0.9°C after the high dose of 8-OH-DPAT, on average, over the 30 min after the injections. These were considered good indications that the drug was acting.

Exploration

Exposure to a clean empty home cage evoked a vigorous exploratory response, which lasted about 20 min, peaking at the third minute after the test started (Fig. 1). This was associated with concomitant increases in HR and MAP (peaking at +125 beats/min and +23 mmHg, respectively). 8-OH-DPAT reduced all variables measured ($F_{2,27} > 3.75; P < 0.041$). Post hoc comparisons indicated that the low dose significantly decreased HR only (by 28%; $F_{1,14} = 9.66; P = 0.008$; gray circles), while the high dose significantly decreased all variables, virtually abolishing the HR (−92%) and MAP (−79%) responses to the change of cage and significantly reducing activity (−64%; $F_{1,13} > 8.26; P < 0.013$; dark circles).

Fear

Reexposure to a context previously paired with foot shocks evoked the typical conditioned fear response previously described (36, 37) (Fig. 2). Behaviorally, the response was characterized by an immobile freezing posture that lasted for most of the reexposure session (see freezing and activity traces). Freezing immobility was also associated with the

![Graph](http://ajpregu.physiology.org/)

Fig. 2. Time course of changes in HR, MAP, freezing, ultrasonic vocalizations, and activity evoked by conditioned fear to context (foot-shock box) after injections of 8-OH-DPAT (0, 0.05 or 0.25 mg/kg ip). Data are means ± SE.

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emission of 22 kHz USV. The cardiovascular response included a marked increase in HR and MAP, which remained stable throughout the reexposure (on average, +80 beats/min and +28 mmHg above baseline).

Treatment with 8-OH-DPAT had a clear effect on both HR and MAP responses to fear \((F_{2,20} = 5.32; P < 0.014)\). Moreover, freezing and USV were also reduced \((F_{2,20} = 6.07; P < 0.009)\), but activity was not significantly changed \((F_{2,20} = 0.92; P = 0.41)\). A closer look at Fig. 2 reveals that the low dose did not have a significant effect on any of those variables \((F_{1,13} < 1.15; P > 0.30)\). In contrast, the high dose reduced the HR response by 49%, the MAP response by 61%, freezing by 67%, and completely abolished USV \((F_{1,13} > 10.63; P < 0.006)\).

**Restraint**

Animals submitted to restraint stress displayed a strong cardiovascular response as reported previously \((9)\), consisting of rapid increases in HR and MAP, which peaked within the first 10 min (+190 beats/min and +40 mmHg from baseline at peak, respectively), and bouts of vigorous struggling (Fig. 3). After 8-OH-DPAT, both HR and MAP were reduced \((F_{2,21} > 3.75; P < 0.040)\). Activity was unchanged \((F_{2,21} = 0.42; P = 0.66)\). Post hoc analysis revealed that the low dose significantly reduced only HR \((F_{1,14} = 9.84; P = 0.007)\) by 17%, whereas the high dose attenuated both HR and MAP \((F_{1,14} > 6.34; P < 0.025)\) by 54 and 48%, respectively.

**Cold Exposure**

Exposure to a 4°C environment evoked strong cardiovascular and behavioral responses (Fig. 4). Animals in this condition were very active throughout the exposure session, and had average increases of +140 beats/min in HR and +22 mmHg in MAP. None of these measures were affected by 8-OH-DPAT \((F_{2,24} < 2.81; P > 0.80)\).

**DISCUSSION**

This study shows that systemic doses of 8-OH-DPAT that are sufficient to greatly reduce the cardiovascular and behavioral responses to different forms of psychological stress in the rat are ineffective against cold exposure. Briefly, the low dose (0.05 mg/kg) had a minor impact on HR during Exploration and Restraint, whereas the high dose (0.25 mg/kg) had a substantial impact on all variables measured during Exploration, Fear, and Restraint. In contrast, 8-OH-DPAT did not affect any of the variables measured during Cold Exposure.

Is there evidence that our drug was working during the Cold Exposure experiment? Looking at Fig. 4 we can see that indeed, the high dose of 8-OH-DPAT reduced the HR and MAP responses to the injection procedure, as observed in the other three tests and as reported previously \((25)\). Similarly, in the first 10 min after the end of the Cold Exposure, one can see that the animals treated with the high dose were less active, which again is what was observed after the three other tests.

**Fig. 3.** Time course of changes in HR, MAP, and activity evoked by restraint stress (restrainer) after injections of 8-OH-DPAT (0, 0.05, or 0.25 mg/kg ip). Data are means ± SE.

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Finally, 8-OH-DPAT treatment is classically associated with falls in core temperature (3, 11, 12, 14). Two animals that were submitted to the Cold Exposure test carried C50-PXT probes, which record body temperature. In both of these, core temperature fell by 1.5°C in the 30 min following injection of the high dose, while saline and low-dose injections led to slight increases in the 0.2–0.4°C range, which is again similar to what was observed before the other tests. Hence, the drug was acting, but could block neither the cardiovascular nor the behavioral effects of Cold Exposure as it did for the other stressors.

To our knowledge, this is the first report on the impact of systemic administration of 8-OH-DPAT on HR and MAP responses to cold. Ootsuka and Blessing (28, 30) have shown that higher doses (0.5 mg/kg sc and 0.1 mg/kg iv, respectively) could block brown adipose tissue thermogenic and cutaneous vasoconstrictor responses to cold. There is, thus, a possibility that HR and/or MAP could have been altered with a higher dose or if intravenous administration had been adopted. As explained below, this would mean that the sites affected by the present high dose have a lower threshold than the sites that mediate the cardiovascular response to cold.

The cardiovascular effects of systemic injections of 8-OH-DPAT are known to derive from a central action (5, 8). An action on autoreceptors located in medullary raphe neurons is usually assumed (25, 27). The medullary raphe has been recognized as a critical relay for the cardiac, thermogenic, and cutaneous vasoconstrictor responses to both psychological, cold, and inflammatory stressors (for a review, see Ref. 4), and it has been shown that microinjection of 8-OH-DPAT in the medullary raphe can block these responses in anesthetized (23) and awake animals (26, 29, 30). The medullary raphe receives input from a network that includes the preoptic area and the DMH, which are both involved in psychological and cold stress responses (4).

Since the medullary raphe seems to mediate most sympathetic responses to both psychological and cold stress, as also does the preoptic area and DMH, neither are likely to be the sites where 8-OH-DPAT acted in the present study, where it attenuated psychological but not cold stress-related responses. As 8-OH-DPAT microinjected directly into the medullary raphe can block responses produced by cold (29), it seems that the present doses were under the threshold needed to activate 5-HT_{1A} receptors in this area. It is likely that the present dose...
influenced areas involved in limbic rather than thermoregulatory functions. Apart from its sympathoinhibitory effect, 8-OH-DPAT is known for its anxiolytic effect on a number of animal stress paradigms and also in humans (2). The observed reduction of HR and BP during Exploration, Restraint, and Conditioned Fear, but not Cold Exposure strongly suggests that this sympathoinhibitory effect is due to anxiolysis, rather than a direct effect on a pathway that is common to both psychological and cold stressors. In line with this is the observation that the behaviorally suppressive effects of 8-OH-DPAT appeared at the same dose at which the sympathoinhibitory effect became apparent.

Although not systematically scored, flat back and head weaving, behavioral signs of the characteristic 8-OH-DPAT-provoked serotonin syndrome, were observed after the high dose. Moreover, we observed a decrease in locomotion during Exploration, which is an effect sometimes seen after 8-OH-DPAT treatment (14, 21) when the animals are not previously habituated to the testing environment (7). Nevertheless, Cold Exposure produced similar levels of activity in all treatment groups, which suggests that their ability to move was not impaired.

Previous research (20) has implicated the dorsal and median raphe nuclei as the most likely sites for the anxiolytic action of 8-OH-DPAT. Those were the sites where microinjections of 8-OH-DPAT most consistently evoked an anxiolytic effect at a relatively low dose (2, 20). Most importantly, microinjection of 8-OH-DPAT into the median raphe has been shown to disrupt contextual fear expression (1), which is in line with the present results. These effects are probably due to stimulation of pre-synaptic/somatodendritic 5-HT1A receptors in rostral raphe neurons, resulting in a reduction of their firing rate (32) and a consequent decrease in the serotonergic neurotransmission in the forebrain, where the effect of serotonin is generally thought to be anxiogenic via activation of non-5HT1A receptors (13). Interestingly, the dorsal raphe nucleus also targets the dorsal-lateral periaqueductal gray (35), where serotonin has an anti-aversive effect via activation of postsynaptic 5-HT1A receptors (13, 17). Similarly to the present findings, microinjection of 8-OH-DPAT in this region attenuates the pressor and tachycardic responses to air jet stress (38), while inhibition with muscimol has no effect on the sympathetic responses to cold exposure (23). In light of this, the effects of 8-OH-DPAT, as seen in the present study, could have been mediated by an action on the rostral raphe nuclei and possibly on other structures, such as the periaqueductal gray, but not on the medullary raphe.

**Perspectives and Significance**

Systemic 5-HT1A activation reduces sympathetic responses to psychological stress. Our results show that this effect cannot be generalized to other kinds of stress-elicited sympathetic responses. Larger doses of 5-HT1A agonists would lead to general sympatho-inhibition through a different mechanism of action. Nalivaiko (24) proposed the medullary raphe as the critical site where 8-OH-DPAT would inhibit sympathetic responses to psychological stress. Indeed, direct injection of 8-OH-DPAT in the medullary raphe attenuates those responses (26). However, it would be interesting to know whether it is possible to block the effect of systemic 5-HT1A activation with a 5-HT1A antagonist injected into the medullary raphe. The present results suggest that, in those circumstances, systemic 8-OH-DPAT would still reduce the sympathetic response to psychological stress through its anxiolytic action. But that might be a good thing. By leaving the cardiovascular response to cold intact at anxiolytic doses, 5-HT1A agonists could potentially be used as antiarrhythmic drugs (25) without having a major impact on thermoregulation.

**GRANTS**

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**REFERENCES**


