Nociceptive Afferent Vagal Input Is Enhanced After Transection of the Aortic Depressor Nerve

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To test the hypothesis that baroreceptor reflexes are involved in the reduced nociceptive responses associated with hypertension, the effects of acute intravenous doses of serotonin (0.75–144 µg/kg) on inhibition of the tail-flick reflex, blood pressure, and heart rate were examined in lightly pentobarbital-anesthetized Sprague-Dawley, Wistar-Kyoto, and spontaneously hypertensive rats before and after bilateral transection of the aortic depressor nerve. Before transection, the ED$_{50}$s for inhibition of the tail-flick reflex in the three strains of rats were 14, 13, and 44 µg/kg, respectively. After bilateral transection of the aortic depressor nerve, all three strains displayed a significant increase in sensitivity toward the serotonin-induced inhibition of the tail-flick reflex (ED$_{50}$s of 7, 7, and 6 µg/kg, respectively). There were no changes in the cardiovascular responses to serotonin after transection of the aortic depressor nerve in Wistar-Kyoto or spontaneously hypertensive rats. In Sprague-Dawley rats, however, the cardiopulmonary cardiovascular afferent-mediated responses were enhanced. In Sprague-Dawley rats, the nociceptive sensitivity to serotonin was unaltered when administered during either the peak increase or decrease in pressure produced by intravenous phenylephrine or nitroprusside, respectively. These results suggest that 1) afferent fibers within the aortic depressor nerve provide strong tonic inhibitory influences on the noxious information conveyed by the vagus in response to intravenous serotonin and 2) these fibers appear to produce their effects by mechanisms that are unrelated to baroreceptor function. (Hypertension 1990;15:797–802)

It has been reported that spontaneously hypertensive rats (SHR) are markedly less sensitive to a cutaneous noxious stimulus than normotensive Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats. It has been suggested that these rats have a genetic disposition toward hypoalgesia because removal of afferent baroreceptor information or long-term reduction in blood pressure does not alter nociceptive sensitivity. Other studies have shown that an acute increase in blood pressure induces a state of hypoalgesia in normotensive rats. It has been suggested that this might possibly be through baroreceptor activation leading to an alteration of central nociceptive control mechanisms.

In previous studies, we have suggested that intravenous serotonin might be a noxious visceral stimulus with effects that are mediated by activation of nociceptive cardiopulmonary vagal afferents. In the present study, we sought 1) to determine if SHR show an elevated threshold to a noxious visceral stimulus (i.e., intravenous serotonin) when compared with normotensive Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats and 2) to test the hypothesis that baroreceptor afferents within the aortic depressor nerve (ADN) provide a tonic inhibitory influence on nociceptive vagal afferent input produced by intravenous serotonin.

**Methods**

Male 16-week-old SD rats (n=16) (Biolabs, St. Paul, Minnesota), WKY rats (n=5), and SHR (n=10) (University of Iowa breeding colony, Iowa City, Iowa) were used in this study. All animals were housed and maintained at a constant temperature of 22°C on a 12-hour light/dark cycle and provided with food and water ad libitum.

Under sodium pentobarbital anesthesia (Nembutal 45 mg/kg) (Abbott Labs., North Chicago, Illinois), the left femoral artery and vein were cannulated for the recording of blood pressure and heart rate and the delivery of drugs, respectively. All wound margins were liberally coated with a local anesthetic ointment (Nupercainal, Ciba Pharmaceuticals, Edison, New Jersey).
Nociceptive Testing

Nociceptive responses were determined with a tail-flick apparatus. Briefly, the apparatus consisted of a beam of focused radiant heat provided by a 50 W projector lamp. Radiant heat was focused on the underside of the tail at one of five sites 8–10 mm apart with the most proximal site approximately 4 cm from the tip. The tail-flick latency was measured to the nearest 0.10 sec as the time from onset of heating of the tail to withdrawal of the tail from the beam. A cut-off latency of 7 seconds was used to avoid tissue damage to the tail. Details of the apparatus and procedure have been reported elsewhere.12

Experimental Protocol

Once the rat was at a steady baseline tail-flick latency of 1.7–2.4 seconds (derived from the three previous trials), serotonin (5-hydroxytryptamine creatinine sulphate, Sigma Chemical Co., St. Louis, Missouri) was administered intravenously (0.75–144 μg/kg), and the effect on inhibition of the tail-flick reflex, blood pressure, and heart rate (HR) were recorded. Each rat received at least four doses from the dosing regime and were tested 3, 33, 63, and 123 seconds after drug administration. Tail-flick latency measurements were continued at 2-minute intervals thereafter until the rat returned to baseline. After either inhibition of the tail-flick reflex by intravenous serotonin or the administration of the maximal dose of serotonin used, a bilateral transection of the ADN was performed, and the dosing and testing regimen was repeated.

A second series of experiments was designed to test whether baroreceptor activation produced by increases in mean arterial pressure (MAP) by using phenylephrine hydrochloride 8 μg/kg (Sigma Chemical Co.), or baroreceptor inactivation produced by decreases in MAP by using sodium nitroprusside 8 or 16 μg/kg (Abbott Labs.), is responsible for the changes in nociceptive sensitivity observed after bilateral transection of the ADN. Naive, lightly-anesthetized SD rats were tested with increasing doses (3–48 μg/kg) of serotonin until maximal inhibition of the tail-flick reflex was produced (blood pressure and HR were continuously recorded). These rats were then given an intravenous dose of phenylephrine hydrochloride 8 μg/kg, and at the peak elevation in MAP, the same doses of serotonin
were readministered and the tail-flick reflex was tested at 3, 33, 63, and 123 seconds. This same procedure was repeated for the maximal decrease in MAP produced by sodium nitroprusside 8 or 16 μg/kg. A bilateral transection of the ADN was subsequently performed, and the serotonin-dosing regimens for normalization of cardiopulmonary afferents as indicated by significantly greater initial hypotension and smallerpressor response after bilateral transection of ADN as compared with Wistar-Kyoto (WKY) rats (middle panels), and spontaneously hypertensive rats (SHR) (right panels). *Significantly different from pretransection depressor and pressor responses to intravenous serotonin.

**Data Acquisition and Statistical Analyses**

All data are presented as mean±SEM. The mean tail-flick latencies are presented as raw data in seconds and are representative of the maximal level of tail-flick inhibition at the dose reported. Data were analyzed by repeated-measures analysis of variance with covariance (ANCOVA) by using orthogonal polynomial curve fitting to determine the slopes of the dose-response curves and the ED₅₀s. Differences between individual means were determined by Student's modified t test with a Bonferroni correction for multiple comparisons using the modified error mean square from the covariance analysis.

**Results**

**Effect of Intravenous Serotonin in Sprague-Dawley, Wistar-Kyoto, and Spontaneously Hypertensive Rats**

Serotonin (3–144 μg/kg) produced a dose-dependent inhibition of the tail-flick reflex in all three strains (Figure 1, panel A). The ED₅₀s (50% of maximal effect) for inhibition of the tail-flick reflex for SD rats (n=10), WKY rats (n=5), and SHR (n=10) were 14, 13, and 44 μg/kg, respectively (Figure 1, panel A). The injection of vehicle alone did not significantly alter tail-flick latency. Intravenous administration of serotonin typically produced a dose-related triphasic effect on blood pressure (Figure 2) and bradycardia, which returned to baseline. At all doses tested, both the SHR and WKY rats showed a greater initial hypotension and bradycardia and a less pronounced pressor response when compared with SD rats. The late hypotensive phase was similar in all three strains. The WKY rats and SHR were similar in their responses to intravenous serotonin except that the SHR showed a less pronounced pressor response.

**Effect of Bilateral Aortic Depressor Nerve Transection**

Bilateral transection of the ADN significantly increased the nociceptive sensitivity to intravenous serotonin in all three strains. The ED₅₀s before transection for SD rats, WKY rats, and SHR were 14, 13, and 44 μg/kg, respectively. After transection, the ED₅₀s shifted to 7, 7, and 6 μg/kg, respectively (Figure 1, panels B, C, and D). There was no significant change in baseline tail-flick latencies after transection. In SD rats, bilateral transection of the ADN significantly enhanced the initial hypotension, significantly reduced the pressor response, and enhanced the bradycardia at all doses of serotonin tested (Figure 2). In contrast, transection of the ADN did...
not alter the cardiovascular responses to the intravenous administration of serotonin in either SHR or WKY rats (Figure 2).

Effect of Pharmacological Alteration of Arterial Pressure

In six SD rats, when serotonin was administered at the peak increases or decreases in MAP produced by phenylephrine hydrochloride or sodium nitroprusside (Figure 3), there was no change in the dose-dependent inhibition of the tail-flick reflex as compared with serotonin alone (Figure 4). There was a significant increase, however, in sensitivity to the nociceptive effect of serotonin after bilateral transection of the ADN (ED₅₀ before and after transection were 11 and 4 μg/kg, respectively) (Figure 4). An acute dose of phenylephrine hydrochloride 8 μg/kg or sodium nitroprusside 8 or 16 μg/kg did not alter baseline tail-flick latencies.

When serotonin was administered at the peak rise in MAP produced by phenylephrine hydrochloride 8 μg/kg (from 116.2±6.5 to 171.7±4.7 mm Hg), there was a significantly greater bradycardia when compared with serotonin alone (Figures 3 and 4). When serotonin was administered at the peak fall in MAP produced by sodium nitroprusside 8 or 16 μg/kg (121.7±3.9 to 74.7±6.2 mm Hg, or 123.5±4.3 to 62.1±5.5 mm Hg, respectively), there was a significantly greater bradycardia to intravenous serotonin when compared with serotonin alone (Figures 3 and 4). After transection of the ADN there was a significantly greater bradycardia (Figure 4), and although not shown, there was a significantly attenuatedpressor response to intravenous serotonin similar to that depicted in Figure 2.

Discussion

The results presented here extend reports from our laboratories suggesting that intravenous administration of serotonin is capable of acting as a noxious visceral stimulus. This conclusion is based on previous findings that intravenous serotonin produces pseudofective responses (abolished by bilateral vagotomy) and inhibits the tail-flick reflex (markedly attenuated by bilateral vagotomy). The finding that SHR are markedly less sensitive to this noxious visceral stimulus as compared with age-matched WKY and SD rats is in general agreement with previous reports that SHR are markedly less sensitive to noxious cutaneous stimuli.

![Figure 3](https://hyper.ahajournals.org/)

**Figure 3.** Recordings of typical blood pressure (upper left panel) and heart rate (lower left panel) responses to intravenous serotonin (12 μg/kg). Middle and right panels show the point at which serotonin was administered (large arrows) during acute increases, or decreases, in blood pressure produced by intravenous administration of either phenylephrine 8 μg/kg (middle panels) (small arrows) or nitroprusside 16 μg/kg (right panels) (small arrows).

![Figure 4](https://hyper.ahajournals.org/)

**Figure 4.** Plottings showing effect of graded doses of intravenous serotonin (SHT), expressed as log scale on x axis (μg/kg), on changes in tail-flick (TF) latency (panel A) or heart rate (HR) (panel B), represented on y axis (seconds or beats/minute [bpm]), in 16-week-old normotensive Sprague-Dawley rats in absence (pretransection, ○) and presence of intravenously administered phenylephrine (+PE 8 μg/kg) (□) or nitroprusside (+NP 8 or 16 μg/kg) (△ and ▲), and after bilateral transection of aortic depressor nerve (posttransection, ●).
It has been reported that afferent fibers in the ADN carry the majority of baroreceptor afferents, and that these fibers alter their rate of firing only in response to changes in arterial pressure. Because acute increases in MAP have been reported to induce hypoalgesia, and transection of baroreceptor afferents abolishes this hypoalgesia, we hypothesized that bilateral transection of the ADN in the rat, by removing a large component of afferent information about changes in pressure, might be capable of attenuating the hypoalgesia observed in SHR. The present results clearly demonstrate that the hypoalgesia is reversed by bilateral transection of the ADN in SHR, as indicated by the sevenfold increase in sensitivity to serotonin. There is also a twofold increase in sensitivity to serotonin in normotensive SD and WKY rats, suggesting that afferent fibers within the ADN might tonically inhibit nociceptive cardiopulmonary afferent transmission in each of the three strains of rat tested.

Whether the shift in nociceptive sensitivity produced by transection of the ADN is caused by alterations in afferent baroreceptor information can be partly answered by examining the cardiovascular responses to the intravenous administration of serotonin before and after transection. In the SHR and WKY rats, there were no qualitative or quantitative differences in cardiovascular responses to serotonin produced by bilateral transection of the ADN, yet the sensitivity of the nociceptive responses to serotonin was dramatically increased.

Clearly, in these two strains, changes in MAP alone were not responsible for the increase in nociceptive sensitivity. In contrast, bilateral transection of the ADN in SD rats significantly enhanced cardiopulmonary reflexes evoked by serotonin, as indicated by the increased initial hypotension and bradycardia and decreased secondary pressor response. This suggests that, at least in the SD rat, afferent information in the ADN normally inhibits cardiopulmonary reflexes.

If changes in baroreceptor activity were responsible for alterations in nociceptive sensitivity in the SD rat, we would expect that pharmacologically induced increases or decreases in MAP might lead to a change in sensitivity to the nociceptive effect of intravenous serotonin. When serotonin was administered at the peak increase or decrease in MAP produced by phenylephrine hydrochloride or sodium nitroprusside, there was a greater degree of reflex bradycardia indicative of increased cardiopulmonary transmission. Therefore, if changes in MAP are responsible for alteration in nociceptive sensitivity produced by serotonin in the SD rats, we would expect that changes in baroreceptor activity produced by pharmacological manipulation with phenylephrine hydrochloride and sodium nitroprusside might result in a shift in the nociceptive sensitivity to intravenous serotonin. Alterations of baroreceptor activity by pharmacologically induced changes in MAP, however, did not alter nociceptive sensitivity in the SD rats, although there was a significant increase in both the hemodynamic and nociceptive sensitivities to the intravenous administration of serotonin after transection of the ADN in these same rats. Thus, transection of the ADN but not changes in MAP that would alter baroreceptor activity cause the increase in nociceptive sensitivity to serotonin. On the basis of these results, we offer the novel hypothesis that the ADN might carry not only baroreceptor information but might also transmit nonbaroreceptor afferent information that tonically inhibits nociceptive information carried by cardiopulmonary afferents activated by intravenous serotonin.

We have found that 1) intravenously administered serotonin produces a dose-dependent inhibition of the tail-flick reflex in SD rats, WKY rats, and SHR; 2) there is no difference in nociceptive sensitivity between SD and WKY rats but SHR show a markedly reduced sensitivity to a noxious visceral stimulus; 3) fibers in the ADN appear to provide a tonic inhibitory influence on vagal afferents carrying nociceptive input activated by intravenous serotonin; and 4) these fibers carried in the ADN appear to produce their inhibitory effects by mechanisms that are unrelated to baroreceptor function.

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