Cardiovascular mechanisms activated by microinjection of baclofen into NTS of conscious rats

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Landulpho, Carlos Daniel Almeida Pitanga, Ana Carolina Rodrigues Dias, and Eduardo Colombari. Cardiovascular mechanisms activated by microinjection of baclofen into NTS of conscious rats. *Am J Physiol Heart Circ Physiol* 284: H987–H993, 2003. First published November 27, 2002; 10.1152/ajpheart.00447.2002.—The peripheral mechanisms responsible for pressor response produced by microinjections of baclofen ($GABA_B$ agonist) into the nucleus tractus solitarii (NTS) of conscious rats were studied. Bilateral microinjections of baclofen (10–1,000 pmol/100 nl) produced a dose-related increase in mean arterial pressure (MAP) and heart rate. The maximal response was observed after 15 min. Intravenous injection of prazosin decreased MAP to control levels. Subsequent treatment with Manning compound (vasopressin receptor antagonist; iv) produced an additional decrease in MAP. In a different group of rats, vasopressin antagonist was injected first and MAP was significantly decreased; however, it remained elevated compared with prebaclofen injection levels. Subsequent treatment with prazosin abolished the baclofen-induced pressor response. Reductions in baclofen-induced pressor response with prazosin treatment were followed by a reflex tachycardia in animals that received a 100 pmol/100 nl dose of baclofen. The tachycardia was not observed with a dose of 1,000 pmol/100 nl. The pressor response induced by microinjection of baclofen into the NTS of conscious rats may be produced by both increases in sympathetic tonus and vasopressin release.

$GABA_B$ agonist; prazosin; blood pressure; vasopressin; cardiovascular control; baroreflex

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THE MAJORITY OF CARDIOVASCULAR and respiratory afferents relay to the central nervous system (CNS) via the vagus and glossopharyngeal nerves and terminate in the nucleus tractus solitarii (NTS) (12, 17). The NTS is a major site of reflex integration, and it is also richly innervated from regions of the CNS concerned directly or indirectly with cardiorespiratory control (17), changing both the activity of the sympathetic nervous system (SNS) and vasopressin (VP) release (27).

The role of GABA in the NTS has been investigated in the last decade. The baroreceptive region of the NTS contains a high density of GABA-containing nerve terminals (2, 11, 16, 20, 24, 25) and a high density of both $GABA_A$ and $GABA_B$ receptors (4, 10, 25). Previous studies (3, 9, 22, 29, 31) demonstrated that stimulation of $GABA_B$ receptors in this region of the NTS elicits a marked pressor response in anesthetized rats. In conscious rats, systemic administration of baclofen causes a sustained hypertension and tachycardia (23).

Anatomic studies showed projections from the NTS to neurons of the paraventricular nucleus of the hypothalamus to and from the NTS to the spinal cord (6, 18, 32). Previous results showed an important participation of VP in the pressor response produced by microinjection of muscimol ($GABA_A$ agonist) and nipecotic acid (inhibitor of GABA uptake) into the NTS of anesthetized rats (7). Moreover, extensive chemical or electrolytic lesions of the NTS demonstrated that, in addition to VP, the SNS is involved in the pressor response (1).

The mechanisms related to the hypertension observed after baclofen injection into the NTS are not yet clear. Several mechanisms can be considered for this hypertension, the possibility of an increase in sympathetic tonus or release of vasopressin, or both. In this study we sought to determine the cardiovascular effects induced by bilateral microinjections of baclofen into the NTS of conscious rats and the mechanisms involved in these effects.

METHODS

Male Wistar rats (300–350 g) were used in the present study. Rats were allowed free access to food and water. All experimental protocols were approved by the Institutional Ethical Committee.

Rats were anesthetized with pentobarbital sodium (40 mg/kg ip; Sigma, St. Louis, MO) and placed in a stereotaxic apparatus (model 960; David Kopf, Tujunga, CA). Bilateral guide cannulas were implanted in the direction of the NTS in accordance with a previously described technique (8). In brief, a small window was opened caudal to lambda, through which a pair of 15-mm-long stainless steel guide cannulas (22 gauge) were introduced in a perpendicular way 14.5 mm caudal to the bregma, 0.5 mm lateral to the midline, and 5.7 mm below the skull surface of the bregma. The bottom of the guide cannulas was placed in the cerebellum 1.0 mm above
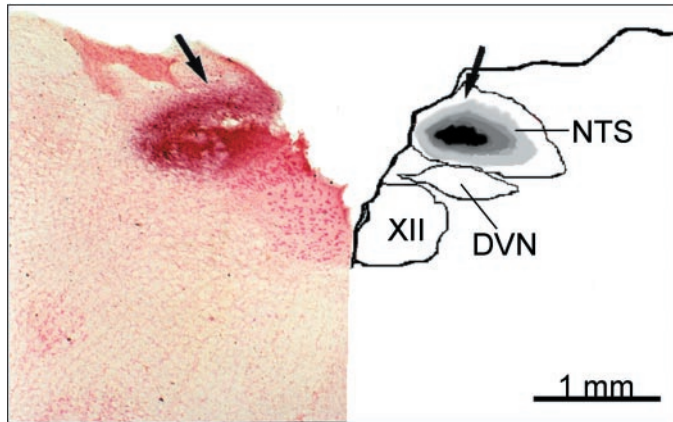
Fig. 1. Photomicrography and schematic representation of a coronal section of the brain stem showing typical bilateral microinjection sites in the nucleus tractus solitarii (NTS). Arrows indicate center of microinjections; DVN, dorsal motor nucleus of vagus; XII, hypoglossal nucleus.

the dorsal surface of the brain stem. Guide cannulas were fixed to the skull with screws and methacrylate and then closed with an occluder until the beginning of the experiment. The needle (33 gauge) used for microinjection into the NTS was 2.5 mm longer than the guide cannulas and was connected with a polyethylene-10 (PE-10) tubing to a 1-µl syringe (Hamilton, Reno, NV). Five days after placement of the NTS cannulas, rats were anesthetized with halothane (Halocarbon), and PE-50 catheters (Clay Adams, Parsippany, NJ) were introduced through the femoral artery and vein to measure pulsatile arterial pressure (PAP) and heart rate (HR) and for drug administration, respectively. Both catheters were tunneled subcutaneously and exteriorized through the back of the neck. PAP and mean arterial pressure (MAP) were determined with a pressure transducer (Statham P23Db) connected to a low-level DC preamplifier in a polygraph (Grass model 7D). HR was derived from arterial pulse wave by a cardiotachometer. Animals were permitted a 24-h period to recover before the experiments.

First protocol. The dose-related effects of baclofen (10, 50, 100, or 1,000 pmol/100 nl; Sigma) on MAP and HR were studied. In this protocol MAP and HR were measured in awake rats immediately before and 15 min after microinjections into the NTS. Different groups of rats were used for each dose. All NTS sites were previously characterized by L-glutamate microinjection (50 mM).

Second protocol. MAP and HR were determined immediately before and 15 min after bilateral microinjections of the GABA_B receptor agonist baclofen (100 or 1,000 pmol/100 nl) into the NTS. Fifteen minutes after baclofen, an α_1-adrenergic antagonist (prazosin, 1 mg/kg iv) and a VP antagonist {1-β-mercapto-β,β-cyclopentamethylene propionic acid 2-[0-(methyl) tyrosine] arginine vasopressin; Manning compound, 10 µg/kg iv} that were administrated with a 15-min time interval between drugs. A control group of animals was maintained to verify the effect of the vasopressin antagonist on basal MAP and HR. Bilateral NTS sites were characterized by L-glutamate microinjection (50 mM). After that, vehicle (saline 0.9%) was microinjected bilaterally into the NTS, followed by intravenous injection of the VP antagonist (10 µg/kg).

Third protocol. MAP and HR were determined immediately before and 15 min after bilateral microinjections of the GABA_B receptor agonist baclofen (100 or 1,000 pmol/100 nl)
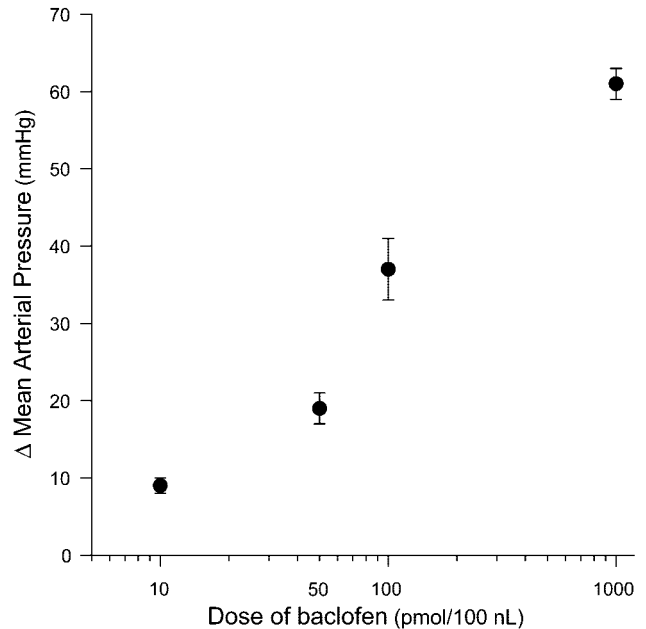
into the NTS. After 15 min, the VP antagonist (Manning compound, 10 µg/kg iv), followed by the α_1-adrenergic antagonist (prazosin, 1 mg/kg iv), were administered with a 15-min time interval between drugs.

Immediately after the experiments, Evans blue (2%) was microinjected into the same site as the microinjections as a marker for histological analysis. Animals were then killed with an overdose of pentobarbital sodium (~200 mg/kg), and 10% buffered Formalin was introduced by intracardiac perfusion. Brains were removed and stored in buffered Formalin for 2 days before 40-µm serial coronal sections were cut and stained by neutral red. Successful microinjection placements into the NTS (Fig. 1) were confirmed for all animals included in this study. In rats in which the microinjections of baclofen were inappropriately placed into adjacent areas to the NTS, no significant changes in arterial pressure and HR were observed (data not shown).

Data were obtained by subtracting the peak of the response to the treatment from the basal level. The dose-

Fig. 2. Changes in mean arterial pressure (ΔMAP) elicited by bilateral microinjection of increasing doses of baclofen (10, 50, 100, and 1,000 pmol/100 nl; $n = 5, 7, 7$, and 8, respectively) into the NTS of conscious rats. The effect was dose dependent ($P < 0.05$). Values were measured 15 min after microinjections.

Table 1. *Dose response to bilateral microinjections of baclofen into NTS*

Doses of Baclofen, pmol/100 nl	ΔMAP, mmHg	ΔHR, beats/min
0	1±2	−8±4
10	9±5*	4±1
50	18±3*	7±1
100	37±4*	−5±2
1,000	66±3*	84±5*

Values are means ± SE maximum change (Δ) after injection. A different group of animals was used for each dose (saline, $n = 6$; 10 pmol, $n = 5$; 50 pmol, $n = 7$; 100 pmol, $n = 7$; and 1,000 pmol, $n = 8$), which received bilateral microinjections of baclofen in a volume of 100 nl of saline solution. *Significantly different compared with control ($P < 0.05$).
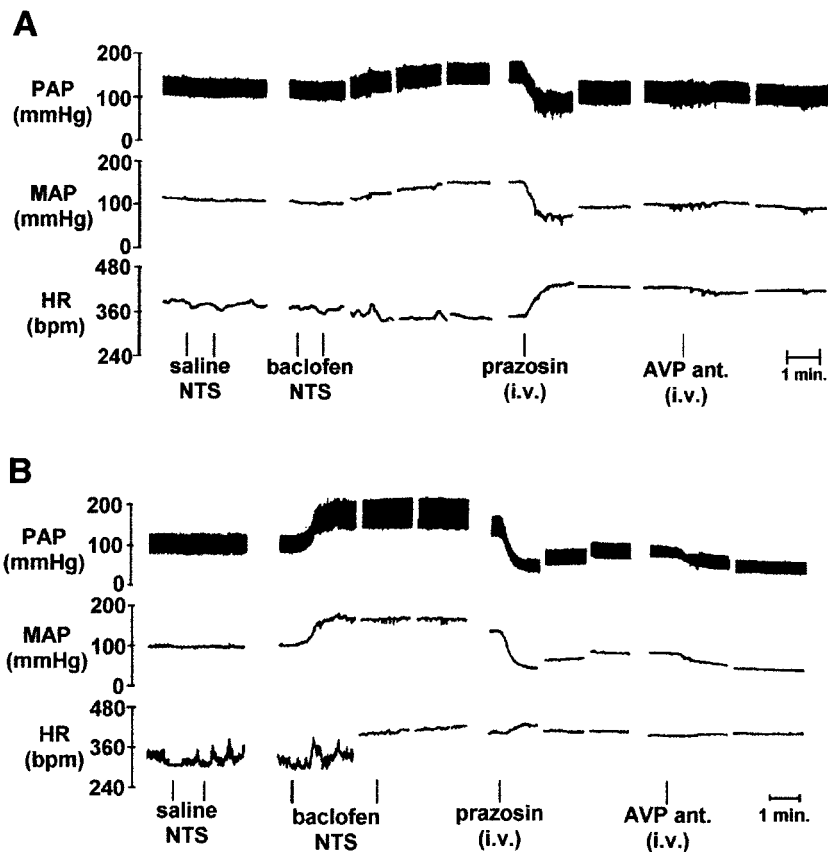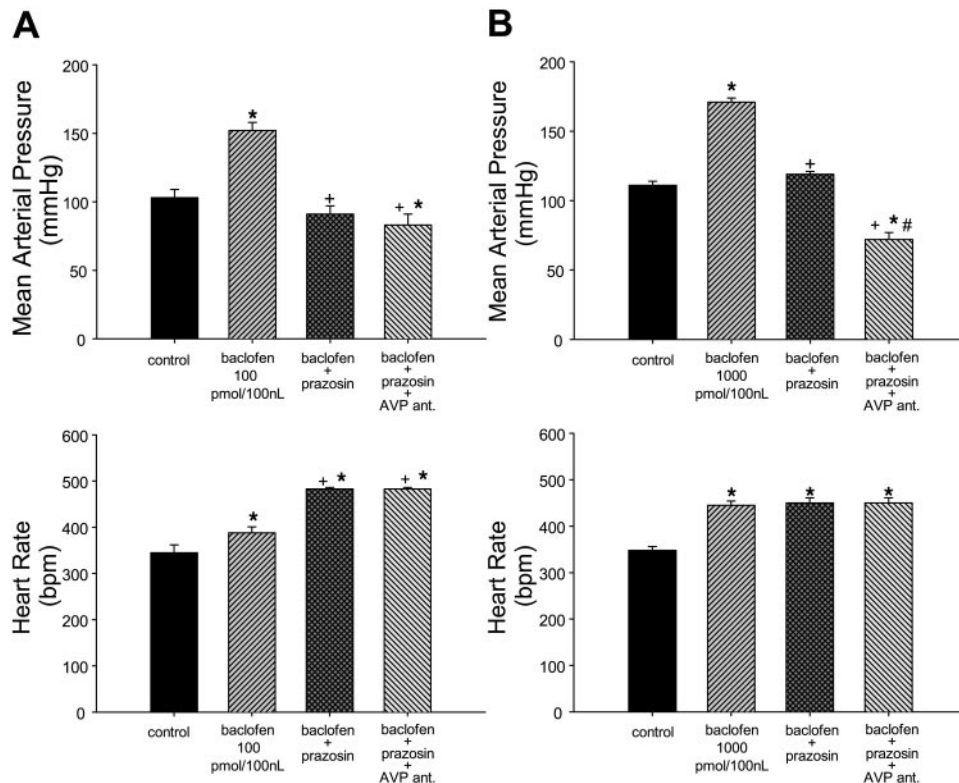
Fig. 3. Effects of bilateral microinjection of 100 pmol/100 nl (*A*) and 1,000 pmol/100 nl (*B*) baclofen into the NTS, followed by intravenous injection of prazosin (1 mg/kg) and vasopressin antagonist (AVP ant; Manning compound, 10 μg/kg) on pulsatile arterial pressure (PAP), MAP, and heart rate [HR, beats/min (bpm)]. Interval of time between treatments was 15 min.

Fig. 4. Effects of bilateral microinjection of 100 pmol/100 nl (*A*; $n = 6$) and 1,000 pmol/100 nl (*B*; $n = 6$) baclofen into the NTS and intravenous injection of prazosin (1 mg/kg) and vasopressin antagonist (Manning compound, 10 μg/kg) on MAP and HR. *Different compared with control ($P < 0.05$); †different from baclofen ($P < 0.05$).
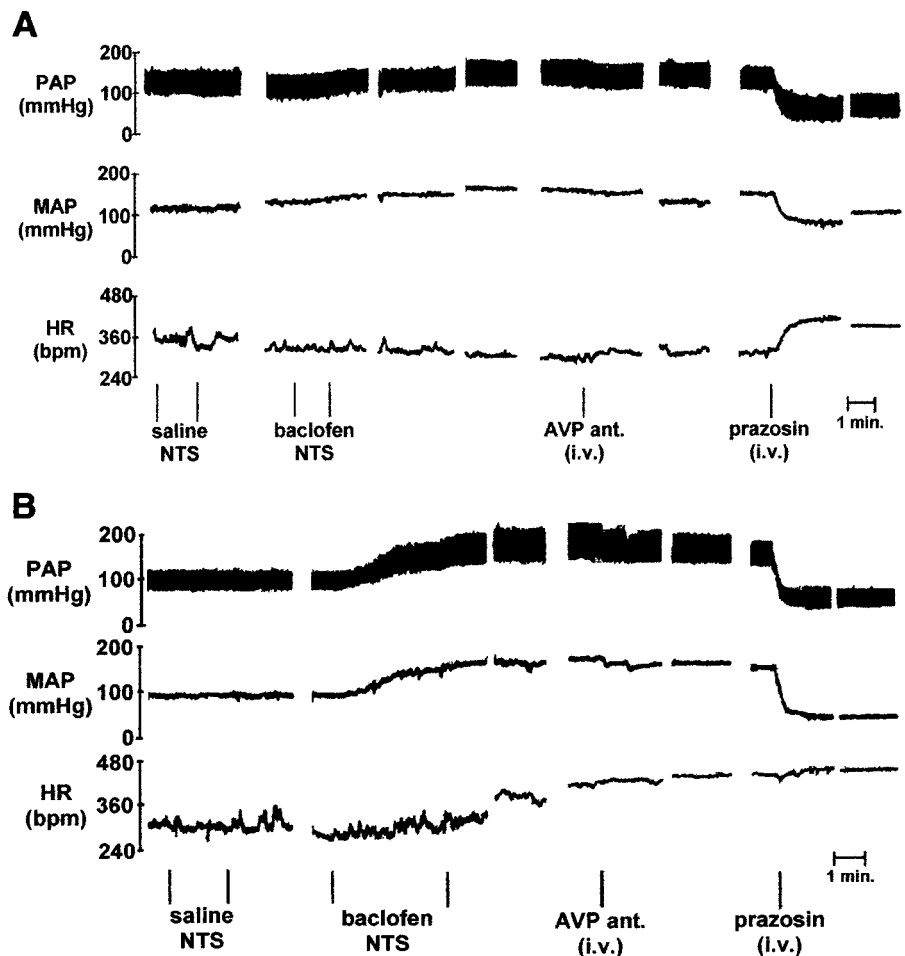
Fig. 5. Effects of bilateral microinjection of 100 pmol/100 nl (*A*) and 1,000 pmol/100 nl (*B*) baclofen into the NTS, followed by intravenous injection of prazosin (1 mg/kg) and vasopressin antagonist (Manning compound, 10 µg/kg) on PAP, MAP, and HR. Time between treatments was 15 min.

response curve is presented as change of the mean ± SE. Other results are shown as absolute means ± SE.

Results were analyzed by one-way analysis of variance (first protocol) and by one-way analysis of variance with repeated measures (second and third protocols). Post hoc determination of differences was established by Newman-Keuls correction for multiple comparisons, with $P < 0.05$ being regarded as significant.

RESULTS

Effects of bilateral microinjections of baclofen into NTS on MAP and HR. Bilateral microinjection of baclofen into the NTS of conscious rats (10, 50, or 100 pmol/100 nl) produced dose-dependent increases in MAP [9 ± 5 ($n = 5$), 18 ± 3 ($n = 7$), and 37 ± 4 ($n = 7$) mmHg, respectively; $P < 0.05$, Fig. 2] but had no effect in HR (Table 1). Bilateral microinjection of 1,000 pmol/100 nl baclofen increased both MAP (66 ± 3 mmHg, $n = 8$; $P < 0.05$) and HR (84 ± 5 beats/min, $n = 8$; $P < 0.05$) (Table 1; Fig. 2). The pressor response induced by these treatments was sustained for at least 1 h, except in the group microinjected with 1,000 pmol/100 nl baclofen, which animals developed respiratory depression after 30 min.

Effects of administration of prazosin and Manning compound on MAP and HR after bilateral microinjec-

tions of baclofen into NTS of conscious rats. Bilateral microinjection of baclofen (100 pmol/100 nl) increased MAP from 103 ± 6 to 152 ± 6 mmHg and HR from 345 ± 17 to 388 ± 13 beats/min ($n = 6$; $P < 0.05$). Fifteen minutes after NTS microinjection, intravenous injection of the α_1-adrenergic receptor antagonist prazosin (1 mg/kg) lowered MAP from 152 ± 6 to 91 ± 6 mmHg and increased HR to 483 ± 3 beats/min ($P < 0.05$). Fifteen minutes after prazosin treatment, injection of the vasopressin receptor antagonist Manning compound (10 µg/kg iv) further decreased MAP from 91 ± 6 to 83 ± 8 mmHg ($n = 6$; $P < 0.05$). However, no additional change was observed in HR (see Fig. 4*A*). Figure 3*A* shows a representative tracing from an animal of this group.

The same protocol was performed in a second group of animals ($n = 6$) that were bilaterally microinjected with 1,000 pmol/100 nl baclofen in the NTS. In these rats, MAP increased from 111 ± 3 to 171 ± 3 mmHg and HR increased from 348 ± 8 to 445 ± 9 beats/min ($P < 0.05$). After intravenous microinjection of prazosin MAP decreased from 171 ± 3 to 119 ± 2 mmHg ($P < 0.05$), and a further decrease was observed after intravenous injection of Manning compound (from 119 ± 2 to 72 ± 5 mmHg; $P < 0.05$). No changes in HR were
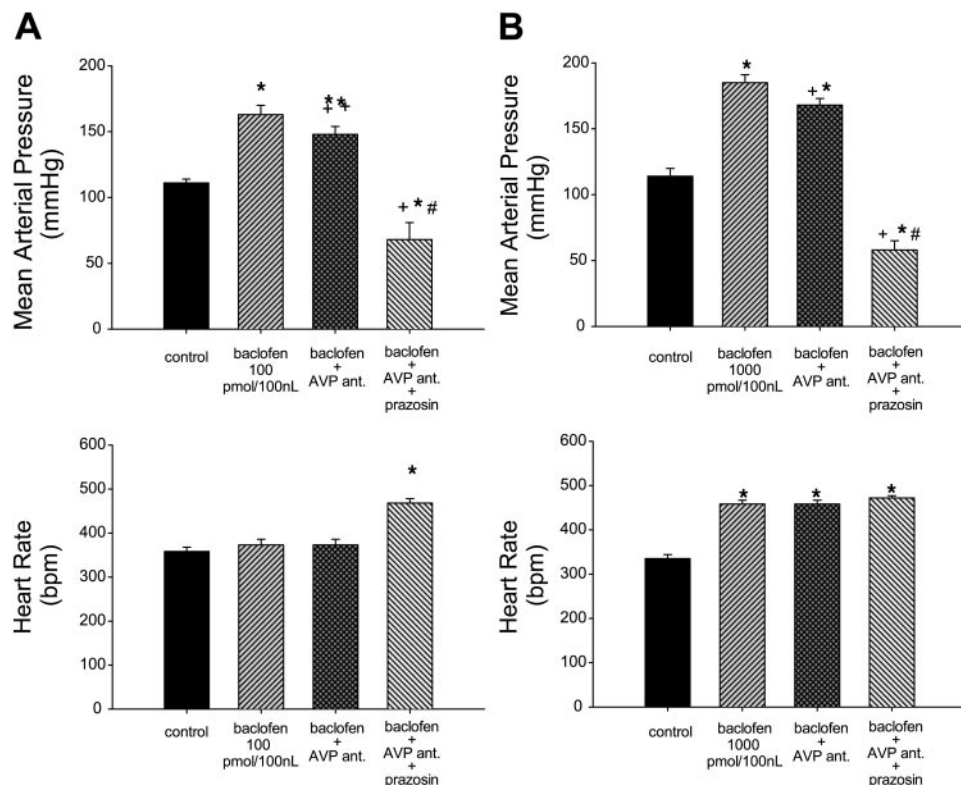
Fig. 6. Effects of bilateral microinjection of 100 pmol/100 nl (*A*; *n* = 6) and 1,000 pmol/100 nl (*B*; *n* = 6) baclofen into the NTS and intravenous injection of prazosin (1 mg/kg) and vasopressin antagonist (Manning compound, 10 μg/kg) on MAP and HR. *Different compared with control (*P* < 0.05); †different from baclofen (*P* < 0.05); #different from baclofen + prazosin (*P* < 0.05).

observed after prazosin and Manning compound treatments (Fig. 4*B*). Figure 3*B* shows a representative tracing from an animal of this group.

In a control experiment, VP antagonist injection after bilateral microinjection of saline into the NTS did not cause significant changes in MAP (basal 116 ± 4 mmHg, after saline 116 ± 4 mmHg, and after Manning compound 114 ± 4 mmHg; *P* > 0.05).

MAP and HR effects of sequential systemic administration of Manning compound and prazosin after bilateral microinjection of baclofen into NTS of conscious rats. Bilateral microinjection of baclofen (100 pmol/100 nl) increased MAP from 111 ± 3 to 163 ± 7 mmHg (*n* = 6; *P* < 0.05); however, HR did not change (358 ± 10 vs. 373 ± 13 beats/min; see Fig. 6*A*). Fifteen minutes after baclofen microinjection, rats received Manning compound (10 μg/kg iv), which lowered MAP from 163 ± 7 to 148 ± 6 mmHg (*P* < 0.05). Fifteen minutes after Manning compound treatment, prazosin (1 mg/kg iv) decreased MAP from 148 ± 6 to 68 ± 1 mmHg and elicited a significant increase in HR from 375 ± 13 to 468 ± 10 beats/min (*n* = 6, *P* < 0.05; see Fig. 6*A*). Figure 5*A* shows a representative tracing from an animal of this group.

The same protocol was repeated on a group of six rats that received bilateral microinjection of 1,000 pmol/100 nl of baclofen in the NTS. Baclofen microinjection increased MAP from 114 ± 6 to 185 ± 6 mmHg and HR from 335 ± 9 to 458 ± 9 beats/min (*P* < 0.05). The following intravenous administration of Manning compound decreased MAP from 185 ± 6 to 168 ± 5 mmHg (*P* < 0.05), and the consecutive administration

of prazosin decreased MAP from 168 ± 5 to 58 ± 7 mmHg (*P* < 0.05). HR remained at the same level after the first treatment (baclofen). Tachycardia was absent in this group, which showed a much greater fall in MAP (from 168 ± 5 to 58 ± 7 mmHg, *P* < 0.05; Fig. 6*B*). Figure 5*B* shows a representative tracing from an animal of this group.

DISCUSSION

The present study shows that the $GABA_B$ receptor agonist baclofen microinjected into the NTS increases arterial pressure in conscious rats. Other studies (14, 15) showed that GABA microinjections into the NTS increase blood pressure in anesthetized rats. These studies also reported that microinjections of the $GABA_A$ antagonist bicuculine produce hypotension in anesthetized rats (14, 15).

Bilateral microinjection of muscimol ($GABA_A$ agonist) (7), or electrolytic and chemical lesions (1) or bilateral microinjections of baclofen into the NTS (9), elicits tachycardia in anesthetized animals. Our study demonstrated that a $GABA_B$-selective agonist also elicits a pressor response in conscious rats. Only animals that received microinjections of baclofen at the highest dose (1,000 pmol/100 nl) displayed tachycardia, whereas the other doses did not demonstrate consistent changes in HR. An exception was that, in the second protocol, 100 pmol/100 nl baclofen produced a significant tachycardia. This result could be due to the highest variance observed in HR basal level. We would expect that such an effect could result from baroreflex

inhibition in addition to an increased sympathetic tonus elicited by baclofen. The fact that the prazosin-induced fall in blood pressure in rats pretreated with the highest dose of baclofen was not accompanied by tachycardia suggests that impairment of baroreflex activity may be involved in these differences in HR responses.

The dose-related increase in MAP after bilateral microinjection of baclofen into the NTS indicates that the inhibition of this system by microinjection of baclofen results in a pressor response. One of the mechanisms that produce the pressure response is the release of VP (7, 13). Studies in anesthetized rats (7) demonstrated an increase in plasma VP during the increase of arterial pressure elicited by microinjection of muscimol into the NTS. This hypertension elicited by muscimol was reversed by intravenous injection of a vasopressin antagonist (7). Chemical or electrolytic lesions of the NTS produce hypertension that cannot be abolished solely by vasopressin antagonists but can be abolished by lesions or application of glycine into the rostroventrolateral medulla, suggesting that bilateral lesions of the NTS increased sympathetic tonus and also the release of vasopressin (1). In the ongoing study, baclofen-induced hypertension was reversed by peripheral blockade of α_1-adrenoreceptors and additional systemic administration of a vasopressin antagonist further lowered MAP below control levels.

To test the hypothesis that both the release of VP and an increase in sympathetic tonus occur simultaneously when baclofen is microinjected into the NTS rather than the administration of prazosin lowering AP and stimulating VP release, a group of animals received an intravenous injection of VP antagonist first. This produced a significant but small decrease in the baclofen-induced hypertension. The remaining hypertension was then completely reversed by intravenous injection of prazosin. Besides demonstrating an involvement of VP in baclofen-induced hypertension, these data suggest that baclofen-induced hypertension is more dependent on sympathetic tonus activation.

The possible mechanisms for mediation of the hypertension elicited by baclofen within the NTS are not completely understood; however, there is evidence that GABA$_B$ receptors within the NTS modulate arterial baroreflexes (5, 27, 30). A recent study (33) suggested a presynaptic mechanism contributing to the inhibition of aortic depressor nerve inputs by GABA$_B$ receptors within the NTS. This same study also observed that monosynaptic neurons in the NTS were less sensitive to GABA$_B$-mediated inhibition than polysynaptic neurons (33). However, the aortic depressor nerve-evoked discharge of some NTS neurons was insensitive to baclofen, suggesting the existence of subpopulations of monosynaptic neurons with differing sensitivities to GABA$_B$ inhibition (33). In our present findings, we observed dose-dependent pressor responses to microinjection of baclofen. Doses of 100 pmol/100 nl elicited a pressor response without HR changes. In a subsequent protocol, peripheral blockade of α_1-adrenoreceptors virtually eliminated baclofen (100 pmol/100 nl) induced

hypertension and resulted in a possible reflex tachycardia (see Fig. 3). Alternatively, dose-dependent effects of baclofen have been described in in vitro studies of NTS (5). Low doses of baclofen produced presynaptic inhibitory effects, whereas higher doses produced a mixture of pre- and postsynaptic inhibition. In this model, we might speculate that, with a higher dose of baclofen, we would observe enhanced hypertension and significant tachycardia. Treatment in those animals with prazosin and VP receptor antagonist completely reversed hypertension and resulted in no change in HR. Considering that we are using microinjections, we may speculate that different doses of baclofen could be altering the activity of different subpopulations of neurons in the NTS.

These data indicate the involvement of the two systems studied after the inhibition of the NTS by bilateral microinjections of baclofen in conscious rats: *1*) the SNS and *2*) the release of VP. According to our results, the main system responsible for the hypertension that followed NTS inhibition is the SNS once systemic administration of prazosin was able to completely reverse the hypertension.

In conclusion, our results show that the hypertension induced by baclofen microinjected into the NTS of conscious rats was produced by increases in sympathetic tonus and may involve the release of VP. These data suggest that NTS neurons exert a tonic inhibitory influence on the SNS and possible mechanisms related to VP release.

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