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Participation of AT_1 and AT_2 Receptors in the Differential Interaction between Angiotensin II or III and *Alpha*-2 Adrenoceptors in the Nucleus Reticularis Gigantocellularis in Cardiovascular Regulation and Antinociception in Rats[1]

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ABSTRACT

We evaluated possible interactions between angiotensin II (AII) or angiotensin III (AIII) and the *alpha*-2 adrenoceptors of the nucleus reticularis gigantocellularis (NRGC) in the medulla oblongata that are involved in cardiovascular regulation and antinociception, as well as the angiotensin receptor subtypes involved, using Sprague-Dawley rats that were anesthetized with pentobarbital sodium. The efficacy of guanabenz, which acts on the *alpha*-2 adrenoceptors in the NRGC to elicit hypotension, bradycardia and antinociception, based on tail-flick responses to noxious thermal stimuli (50°C), was used as our experimental index. Bilateral microinjection of AII or AIII into the NRGC, at equimolar doses (40 pmol) that did not alter base-line systemic arterial pressure, heart rate or tail-flick latency, significantly and site-specifically attenuated the cardiovascular suppression elicited by guanabenz (100 μg/kg i.v.). This attenuation was appreciably antagonized by coadministration of the AT_2 receptor antagonist PD-123319 (1.6 nmol). Concomitant examination of tail-flick responses revealed discernible inhibition by AII (40 pmol), but potentiation by AIII (40 pmol), of guanabenz-induced antinociception. These differential modulating effects of AII and AIII were, however, antagonized by comicroinjection of losartan (1.6 nmol) into the bilateral NRGC. Our results suggest that both AII and AIII produced a reduction, *via* AT_2 receptors, of the activity of *alpha*-2 adrenoceptors in the NRGC that are involved in central cardiovascular regulation. On the other hand, antinociception induced by activation of *alpha*-2 adrenoceptors in the NRGC was suppressed by AII and potentiated by AIII, although AT_1 receptors may play a major role in both interactions.

It is well established that the renin-angiotensin system in the central nervous system plays an important role in blood pressure regulation and body fluid homeostasis (Wright and Harding, 1992). In addition to the effector peptide AII, the C-terminal heptapeptide AII fragment des-Asp-AII or AIII has been demonstrated to be an active member of the brain angiotensins (Wright and Harding, 1992). The cloning and sequencing of angiotensin receptors (Murphy *et al.*, 1991; Sasaki *et al.*, 1991) and recent discovery of specific nonpeptide angiotensin antagonists devoid of agonist activity (Timmermans *et al.*, 1993) have led to the definition of at least two subtypes of angiotensin receptors. The receptor subtype that is associated with losartan and appears to mediate essentially all of the known effects of AII is designated AT_1. The other receptor subtype, which is associated with PD-123177 and its structural analogs, is designated AT_2. The physiologic role for the AT_2 receptor subtype has not yet been convincingly demonstrated (Timmermans *et al.*, 1993; Wright and Harding, 1994). Whether AII and AIII share the same subtype(s) of angiotensin receptors in the same physiologic process also requires further elucidation.

The NRGC is a major nucleus in the medullary reticular formation that actively participates in central regulation of cardiovascular functions (Chan, 1984). Previous studies from our laboratory demonstrated that activation of postsynaptic *alpha*-2 adrenoceptors in the NRGC by guanabenz may underlie the hypotensive and negative chronotropic and inotropic effects of this *alpha*-2 adrenoceptor agonist (Lim *et al.*, 1985; Chan and Lin, 1988; Lim *et al.*, 1988b; Chen and Chan, 1989). We further reported (Lee *et al.*, 1990; Yin *et al.*, 1990; Chan *et al.*, 1991) that AIII attenuates the cardiovascular suppressant actions of guanabenz by acting on the postsyn-

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ABBREVIATIONS: aCSF, artificial cerebrospinal fluid; AII, angiotensin II; AIII, angiotensin III; HR, heart rate; MSAP, mean systemic arterial pressure; NRGC, nucleus reticularis gigantocellularis; SAP, systemic arterial pressure.

aptic *alpha*-2 adrenoceptors in the NRGC. Whether similar modulatory action is exerted by AII is presently unknown.

In addition to its role in central cardiovascular regulation, NRGC participates in central processing of nociceptive signals (Chan, 1979a,b). Likewise, activation of the *alpha*-2 adrenoceptors at the NRGC results in antinociception (Lai and Chan, 1982; Chan and Chan, 1987; Lim *et al.*, 1988a). At the same time, several reports (Haulica *et al.*, 1986; Shimamura *et al.*, 1987; Kalyuzhnyi and Fedoseeva, 1990; Yien *et al.*, 1993) suggest the participation of central AII or AIII in the antinociceptive process. Little information, however, is presently available regarding the interaction between AII or AIII and *alpha*-2 adrenoceptors at the NRGC in antinociception.

To fill the void in our present knowledge, as outlined above, the current study was undertaken to seek answers to four crucial questions concerning possible interactions between AII or AIII and *alpha*-2 adrenoceptors in the NRGC. First, does AII, similarly to AIII (Yin *et al.*, 1990), promote a modulatory action on *alpha*-2 adrenoceptors in the NRGC that are involved in cardiovascular regulation? Second, does AII or AIII exert an influence on the *alpha*-2 adrenoceptors in the NRGC that are involved in antinociception? Third, are there differences in efficacy and action of AII and AIII in both modulatory processes? Fourth, what are the subtypes of angiotensin receptors involved? Our results indicated that AII and AIII produced equipotent reductions, *via* AT_2 receptors, in the activity of *alpha*-2 adrenoceptors in the NRGC that are involved in central cardiovascular regulation. However, antinociception induced by activation of *alpha*-2 adrenoceptors in the NRGC was suppressed by AII and potentiated by AIII, although AT_1 receptors may play a major role in both interactions.

Materials and Methods

General procedures. The procedures used in this study were approved by the Experimental Animal Committee of the National Yang-Ming University. Experiments were carried out in adult male Sprague-Dawley rats (weighing 210–280 g). They were housed in an animal room with a 12/12-hr light/dark cycle (light on from 8:00 A.M. to 8:00 P.M.) and an ambient temperature of 22 ± 1°C, with food and water available *ad libitum*.

Animals were initially anesthetized with pentobarbital sodium (50 mg/kg i.p.). The trachea was intubated to maintain airway patency and to facilitate ventilation. The right and left femoral veins were routinely cannulated for administration of drugs and infusion of anesthetics. The right femoral artery was also cannulated for the measurement of SAP.

After the completion of preparatory surgery, the animal was fixed in a stereotaxic apparatus (Kopf 900, Tujunga, CA) and was allowed to spontaneously breathe room air. Anesthetic maintenance was provided by i.v. infusion of pentobarbital sodium at 20 mg/kg/hr. This anesthetic management offered maintained anesthesia while preserving the capacity for cardiovascular regulation (Yang *et al.*, 1996). The arterial catheter was connected to a pressure transducer (Statham P23XL, Valley View, CA) and in turn to a universal amplifier (Gould G-20-4615-58, Valley View, CA) by which SAP signals were amplified and filtered (frequency range, direct current to 100 Hz). HR was determined with a biotachometer (Gould G-20-4615-65) triggered by the arterial pulses. During the experiment, SAP, MSAP and HR were continuously displayed on a polygraph (Gould RS3400). The rectal temperature was maintained at 37°C with a heating pad, and exposed neural tissues were bathed with warm mineral oil.

Drug administration. Intravenous administration of guanabenz or saline was delivered *via* the cannulated femoral vein, with the bolus maintained at a constant volume of 250 μl. Direct bilateral microinjection of chemical agents was made *via* a stereotaxically positioned 27-gauge needle connected to a 0.5-μl Hamilton microsyringe. The stereotaxic coordinates used for NRGC were as follows: 5.0 to 6.5 mm posterior to the lambda, 0.6 to 0.8 mm lateral to the midline and 7.0 to 8.5 mm from the surface of the cerebellum. Regardless of the contents, the total volume of injection was restricted to 100 nl and was administered over at least 1 min, to limit the extent of diffusion but allow for complete diffusion of the solution.

Assessment of antinociceptive effect. Tail-flick responses to noxious thermal stimuli were used as our experimental index for nociception (Yien *et al.*, 1993; Yang *et al.*, 1995). For this purpose, the distal portion of the tail (5 cm from the tip of the tail) was immersed in a water bath maintained at 50°C. The time taken for the tail to withdraw from the water bath was defined as the tail-flick latency. A cut-off latency was set at 20 sec to prevent thermal injury to the tail. The averaged value of three tail- flick responses, taken at 10-min intervals, was used as the base line.

Effect of bilateral microinjection of AII or AIII into the NRGC on guanabenz-promoted cardiovascular suppression and the subtype of angiotensin receptors involved. The temporal effect of guanabenz (100 μg/kg i.v.) on SAP and HR was followed for 60 min immediately after AII (40 pmol) or AIII (40 pmol) was microinjected bilaterally into the NRGC. Possible physical effects of microinjection were controlled for by injecting the same volume (100 nl) of aCSF into the bilateral NRGC. To elucidate the subtype of angiotensin receptors involved in the interaction between AII or AIII and guanabenz, these procedures were repeated in animals that had been pretreated with comicroinjection of AII or AIII with either the nonpeptide AT_1 receptor antagonist losartan (1.6 nmol) or the AT_2 receptor antagonist PD-123319 (1.6 nmol). The doses used for AII, AIII, guanabenz, losartan and PD-123319 were adopted from preliminary results or previous studies that used these agents for the same purposes as in the present study (Lee *et al.*, 1990; Yin *et al.*, 1990; Chan *et al.*, 1991; Yien *et al.*, 1993).

Effect of bilateral microinjection of AII or AIII into the NRGC on guanabenz-induced antinociception and the subtype of angiotensin receptors involved. We also assessed the possibility that AII or AIII also modulates guanabenz-induced antinociception and the involvement of AT_1 and AT_2 receptors in this interaction. For each of the drug treatment schemes described above, the temporal changes in tail-flick latency were evaluated, at 10-min intervals, for 60 min. It should be mentioned that this evaluation was carried out concurrently with the examination of cardiovascular responses.

Peptides and chemicals. AII and AIII were obtained from Sigma Chemical Co. (St. Louis, MO). Guanabenz, losartan and PD-123319 were kindly provided by Wyeth Laboratory, DuPont-Merck Pharmaceutical Co. and Parke-Davis Pharmaceutical Research, respectively. Guanabenz was freshly prepared with saline. AII, AIII, losartan or PD-123319 was prepared with aCSF, divided into aliquots, stored and thawed immediately before the experiment. Saline and aCSF served as the respective vehicle controls for i.v. administration and microinjection.

Histology. The brain of each animal was removed at the end of the experiment and was fixed in 10% formaldehyde in 30% sucrose-saline for at least 48 hr. The location of the microinjection needle was verified on 25-μm frozen sections stained with Cresyl violet.

Statistical analysis. All values are presented as mean ± S.E. When the maximal value for guanabenz-induced hypotension and bradycardia was used for data analysis, differences in SAP or HR between treatment groups were assessed using one-way analysis of variance, followed by the Student-Newman-Keuls multiple-range test for *post hoc* comparison against the control group. The temporal effect of each pretreatment on guanabenz-induced cardiovascular suppression or antinociception was evaluated using two-way analy-

sis of variance with repeated measures, followed by the Student-Newman-Keuls test for comparisons of individual means at comparable time points. Data were considered to be significantly different at $P < .05$.

Results

Effect of bilateral microinjection of AII into the NRGC on guanabenz-promoted cardiovascular suppression and the subtype of angiotensin receptors involved. In animals that received bilateral microinjection of aCSF into the NRGC, i.v. administration of guanabenz (100 μg/kg), similarly to our previous observations (Lim *et al.*, 1985; Chan and Lin, 1988; Lee *et al.*, 1990; Yin *et al.*, 1990), produced an initial but transient increase in SAP because of its peripheral sympathomimetic action (fig. 1). This was followed by discernible and prolonged hypotension, accompanied by bradycardia (fig. 1), that endured >60 min. When coupled with i.v. injection of saline, microinjection of AII (40 pmol) alone into the bilateral NRGC did not result in appreciable effects on the cardiovascular parameters (MSAP, +2.3 ± 1.9 mm Hg; HR, −5.5 ± 5.3 bpm). Nonetheless, microinjection of the same dose of AII significantly attenuated (fig. 1) the hypotensive and negative chronotropic actions of guanabenz (100 μg/kg). It should be mentioned that the implied reduction in *alpha*-2 adrenoceptor activity in the NRGC was site-specific, because local application of AII into loci outside the NRGC (see fig. 6) failed to produce appreciable antagonistic action on the circulatory depressive effects of the aminoguanidine compound (fig. 2).

To further delineate the subtype of angiotensin receptors involved in our observed modulatory interaction between AII and *alpha*-2 adrenoceptors, the octapeptide was comicroinjected into the bilateral NRGC with either losartan (1.6 nmol) or PD-123319 (1.6 nmol). Whereas blockade of the AT_1 receptors in the NRGC elicited minimal effects (fig. 2), blockade of the AT_2 receptors discernibly blunted the AII-promoted attenuation of cardiovascular suppressive actions of guanabenz (fig. 2).

Effect of bilateral microinjection of AIII into the NRGC on guanabenz-promoted cardiovascular suppression and the subtype of angiotensin receptors involved. The results of microinjection of AIII into the bilateral NRGC on guanabenz-promoted cardiovascular suppression (figs. 1 and 3) were essentially similar to those obtained with AII (figs. 1 and 2) and in our previous studies (Lee *et al.*, 1990; Yin *et al.*, 1990; Chan *et al.*, 1991). Whereas AIII (40 pmol) by itself produced minimal effects on base-line cardiovascular parameters (MSAP, +1.8 ± 2.9 mm Hg; HR, −1.9 ± 3.6 bpm), the same pretreatment significantly antagonized the circulatory suppressive actions of guanabenz (100 μg/kg i.v.). The extent of this antagonistic effect of AIII was comparable to that of AII and also took place site-specifically (fig. 3) in the NRGC (see fig. 6). Coadministration of PD-123319 (1.6 nmol) with AIII (40 pmol) again appreciably reversed the blunting effect of AIII on guanabenz-induced hypotension and bradycardia. Blockade of AT_1 receptors in the NRGC with losartan (1.6 nmol), on the other hand, did not discernibly affect the inhibitory action of AIII (fig. 3).

Differential effects of bilateral microinjection of AII or AIII into the NRGC on guanabenz-induced antinociception. Concomitant evaluation of the tail-flick latency

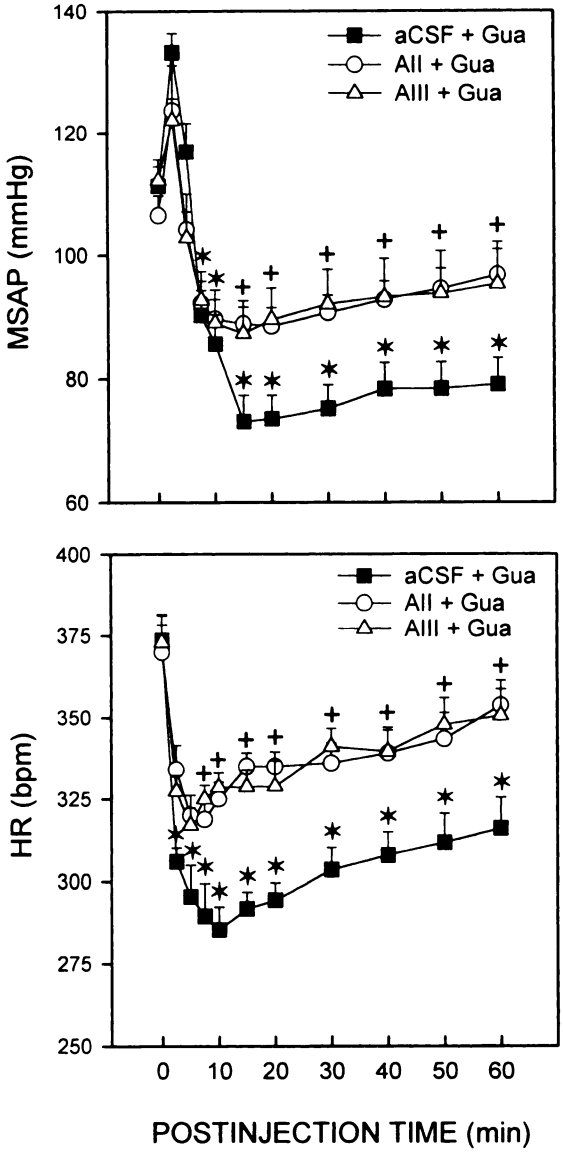

Fig. 1. Effect of bilateral microinjection of aCSF or equimolar doses (40 pmol) of AII or AIII into the NRGC on the temporal changes in MSAP and HR in response to guanabenz (Gua) (100 μg/kg i.v.). Values are mean ± S.E. (n = 6 animals/group). Significant differences ($P < .001$) exist between groups in two-way analysis of variance with repeated measures [$F(20,150) = 2.61$ for MSAP; $F(20,150) = 5.69$ for HR]. *$P < .05$ *vs.* vehicle control group; $^+P < .05$ *vs.* aCSF plus guanabenz group at comparable time points in the Student-Newman-Keuls multiple-range analysis.

revealed differential effects of bilateral microinjection of AII or AIII into the NRGC on guanabenz-induced antinociception (fig. 4, A and B). At the dose (40 pmol) used in these experiments, AII or AIII, similarly to aCSF, did not affect base-line tail-flick latency when directly applied to the bilateral NRGC.

Intravenous administration of guanabenz (100 μg/kg) appreciably increased the tail-flick latency, which endured for 30 min (fig. 4). This implied activation of *alpha*-2 adrenoceptors in the NRGC that are involved in antinociception, when coupled with bilateral microinjection of AII (40 pmol) into the same reticular nucleus, was completely depressed during the 60-min observation period (fig. 4A). Intriguingly, pretreatment with AIII (40 pmol) significantly potentiated the an-

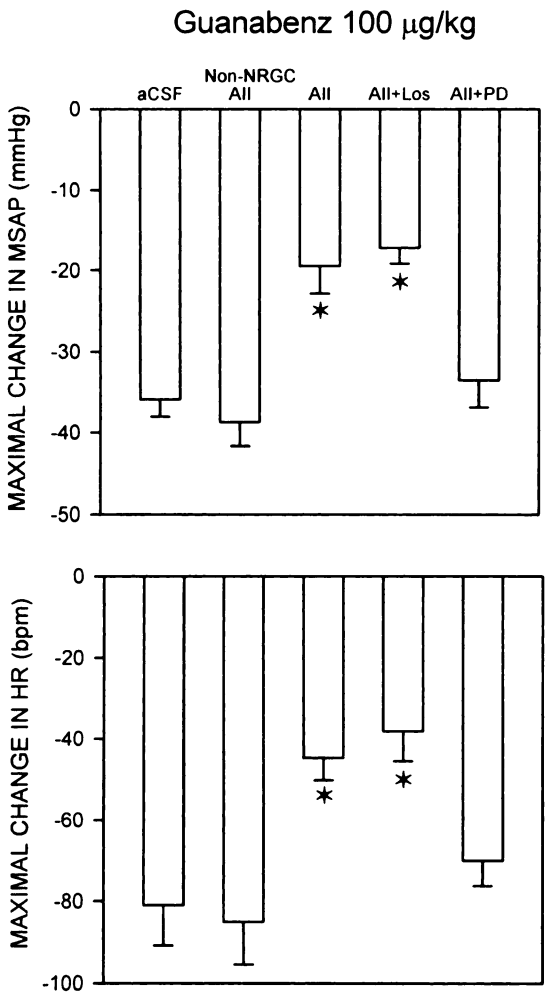

Fig. 2. Effect of microinjection of aCSF, AII (40 pmol), AII (40 pmol) plus losartan (Los) (1.6 nmol) or AII (40 pmol) plus PD-123319 (PD) (1.6 nmol) into bilateral NRGC or AII (40 pmol) into loci outside the NRGC (Non-NRGC AII) on the maximal suppression of MSAP and HR elicited by guanabenz (100 μg/kg i.v.). Values are mean ± S.E. (n = 6 animals/group). Significant differences ($P < .0001$) exist between groups in one-way analysis of variance [$F(4,25) = 24.34$ for MSAP; $F(4,25) = 18.85$ for HR]. *$P < .05$ *vs.* aCSF group in the Student-Newman-Keuls multiple-range analysis.

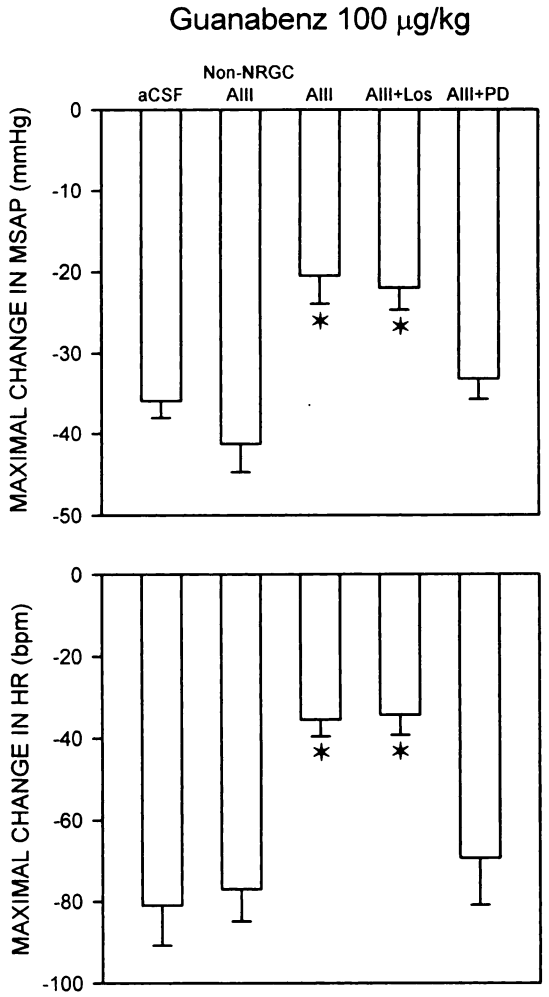

Fig. 3. Effects of microinjection of aCSF, AIII (40 pmol), AIII (40 pmol) plus losartan (Los) (1.6 nmol) or AIII (40 pmol) plus PD-123319 (PD) (1.6 nmol) into bilateral NRGC or AIII (40 pmol) into loci outside the NRGC (Non-NRGC AIII) on the maximal suppression of MSAP and HR elicited by guanabenz (100 μg/kg i.v.). Values are mean ± S.E. (n = 6 animals/group). Significant differences ($P < .0001$) exist between groups in one-way analysis of variance [$F(4,25) = 16.42$ for MSAP; $F(4,25) = 14.02$ for HR]. *$P < .05$ *vs.* aCSF group in the Student-Newman-Keuls multiple-range analysis.

tinociceptive efficacy of guanabenz by doubling the duration of the prolonged tail-flick latency (fig. 4B). Again, these differential effects of the octapeptide and heptapeptide were site-specific, because microinjection of AII or AIII into loci outside the NRGC (see fig. 6) elicited no discernible actions on the increase in tail-flick latency induced by guanabenz (fig. 4C).

Subtype of angiotensin receptors involved in the interaction between AII or AIII and guanabenz-induced antinociception. Both attenuation by AII (fig. 5A) and potentiation by AIII (fig. 5B) of guanabenz-induced prolongation of tail-flick latency were significantly antagonized upon coadministration of losartan (1.6 nmol) into the bilateral NRGC (fig. 5). In both instances, the resultant antinociceptive effect was similar to that induced by guanabenz (100 μg/kg i.v.) alone. In contrast, blockade of the AT_2 receptors in the NRGC with PD-123319 (1.6 nmol) was ineffective in influencing the respective modulatory action of AII and AIII on antinociception induced by the aminoguanidine compound (fig. 5).

Microinjection sites. Figure 6 summarizes the location of loci in the medulla oblongata upon which AII and AIII were delivered. As depicted, sites where AII or AIII elicited significant influence on guanabenz-induced cardiovascular suppression or antinociception were distributed randomly within the anatomic confines of the NRGC.

Discussion

The present study demonstrated that the activity of *alpha*-2 adrenoceptors in the NRGC that are involved in cardiovascular regulation and antinociception may be differentially affected by AII and AIII, in a process that engages specifically the AT_1 or AT_2 receptors. We observed that bilateral microinjection of AII or AIII into the NRGC equipotently reduced, *via* AT_2 receptors, the efficacy of guanabenz to elicit hypotension and bradycardia. However, antinociception induced by activation of *alpha*-2 adrenoceptors in the NRGC with guanabenz was suppressed by AII and potentiated by AIII, although both actions were subserved by the

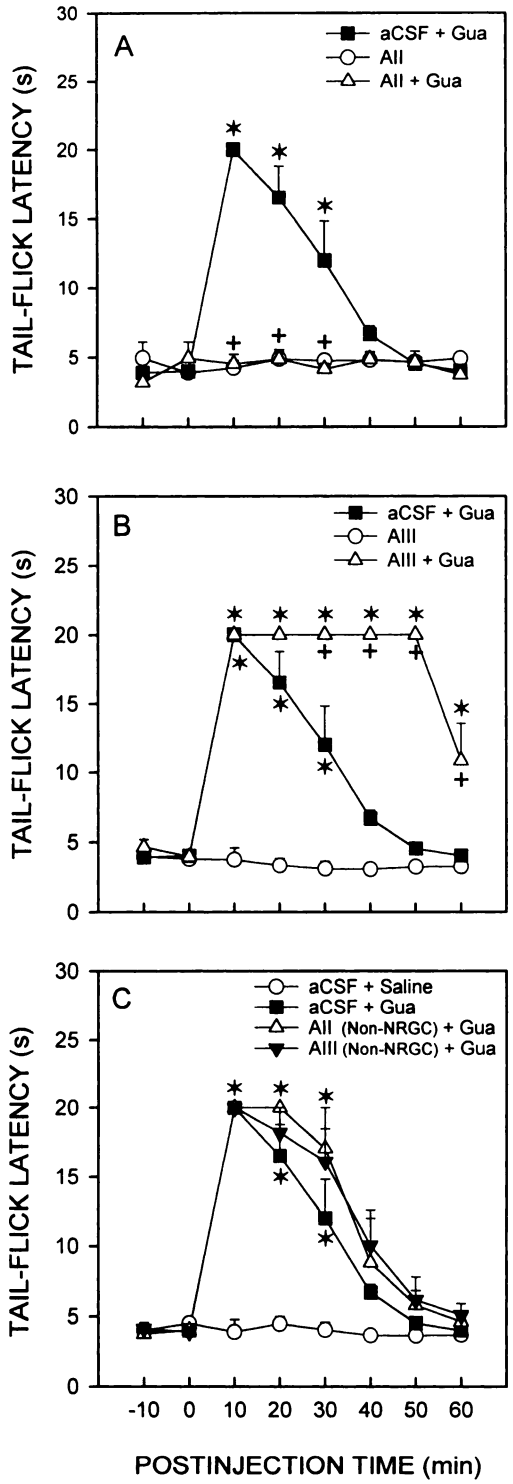

Fig. 4. Temporal changes in tail-flick latency after i.v. administration of guanabenz (Gua) (100 μg/kg) or saline and/or bilateral microinjection of AII, AIII or aCSF into the NRGC. A and B, differential effects of AII (40 pmol) (A) and AIII (40 pmol) (B) on guanabenz-induced tail-flick latency. C, temporal effects of microinjection of aCSF into the NRGC or equimolar doses (40 pmol) of AII or AIII into loci outside the NRGC (Non-NRGC) on guanabenz-induced prolongation of tail-flick latency. Values are mean ± S.E. (n = 6 animals/group). Significant differences (P < .0001) exist between groups in two-way analysis of variance with repeated measurements [F(14,105) = 21.55 in A; F(14,105) = 23.22 in B; F(21,140) = 10.02 in C]. *P < .05 *vs.* vehicle control group; $^{+}$P < .05 *vs.* aCSF plus guanabenz group at comparable time points in the Student-Newman-Keuls multiple-range analysis.

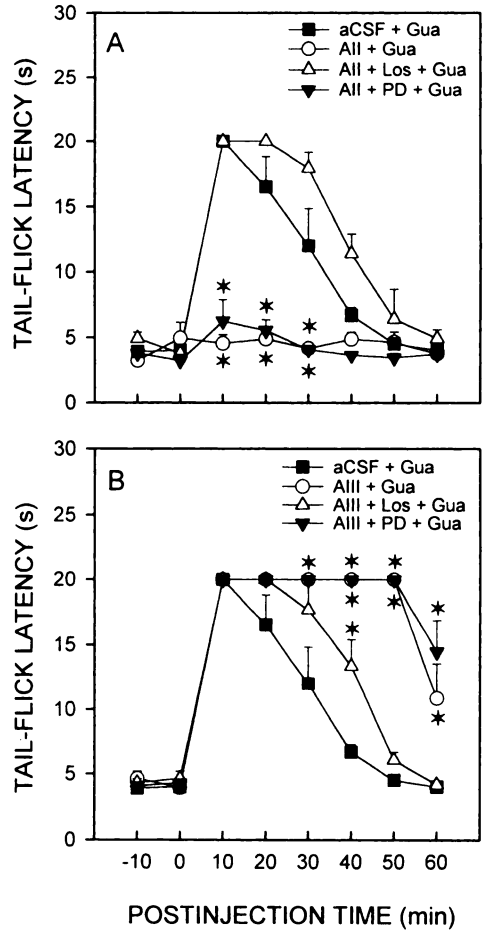

Fig. 5. Temporal changes in tail-flick latency after i.v. administration of guanabenz (Gua) (100 μg/kg i.v.) after pretreatment of the animals with bilateral microinjection into the NRGC of AII (40 pmol) (A) or AIII (40 pmol) (B), alone or combined with losartan (Los) (1.6 nmol) or PD-123319 (PD) (1.6 nmol). Values are mean ± S.E. (n = 6 animals/group). Significant differences (P < .0001) exist between groups in two-way analysis of variance with repeated measures [F(21,140) = 16.92 in A; F(21,140) = 8.35 in B]. *P < .05 *vs.* aCSF plus guanabenz group at comparable time points in the Student-Newman-Keuls multiple-range analysis.

AT_1 receptors. We further confirmed the modulatory nature of these differential actions of AII and AIII by showing that they were elicited at a dose that did not by itself induce significant alteration in base-line SAP, HR or tail-flick latency.

One novel finding in the present study is the participation of AT_2 receptors in central cardiovascular regulation. Emerging information is presently available that suggests the participation of AT_2 receptor in functions previously ascribed to the AT_1 subtype, including pressor response (Hogarty *et al.*, 1992; Tony and Porter, 1993; Nahmias and Strosberg, 1995) and autoregulation of cerebral blood flow (Stromberg *et al.*, 1993). The engagement of AT_2 receptors in the cardiovascular actions of the renin-angiotensin system has recently been ascertained in mutant mice targeted for disruption of the gene encoding this angiotensin receptor subtype (Hein *et al.*, 1995; Ichiki *et al.*, 1995). Our assignment of AT_2 receptors to the mediation of the modulatory action of AII and AIII on the activity of *alpha*-2 adrenoceptors in the NRGC involved in central cardiovascular regulation therefore lends credence to these notions. In addition, it offers a new mechanism by

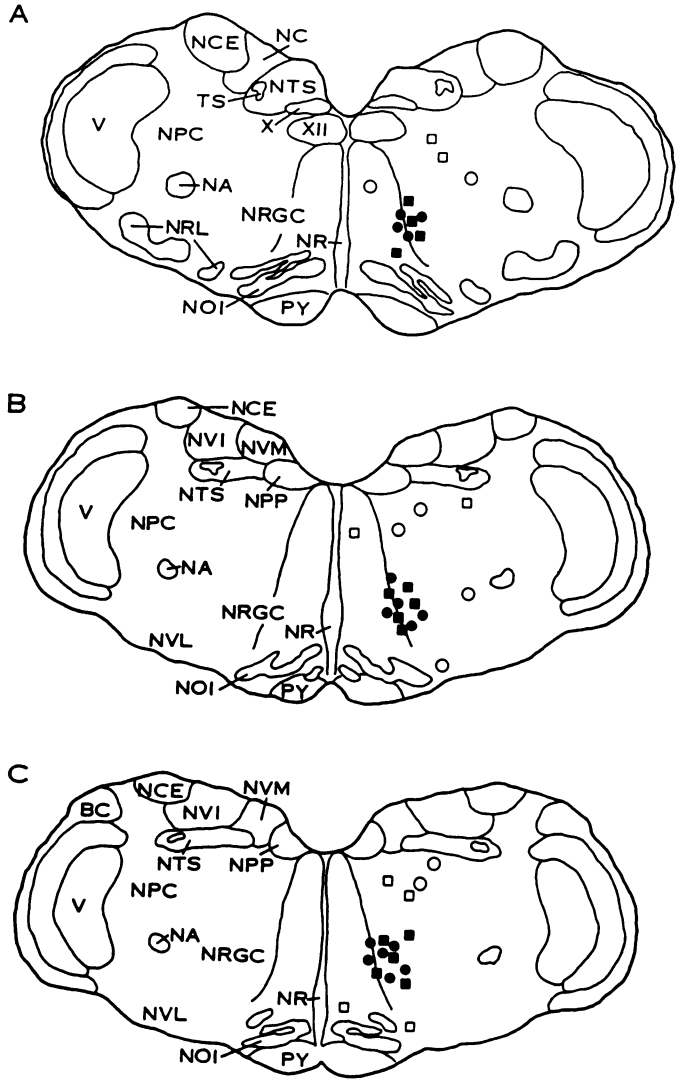

Fig. 6. Diagrammatic representation of the medulla oblongata at three rostral-caudal levels, showing the location of sites where microinjection of AII (circles) or AIII (squares) produced significant (closed symbols) or insignificant (open symbols) action on the cardiovascular suppressive or antinociceptive effects of guanabenz. BC, brachium conjunctivum; NA, nucleus ambiguus; NC, nucleus cuneatus; NCE, nucleus cuneatus externus; NOI, nucleus olivaris inferior; NCP, nucleus reticularis parvocellularis; NPP, nucleus prepositus hypoglossi; NR, nucleus raphé; NRL, nucleus reticularis lateralis; NTS, nucleus tractus solitarii; NVI, nucleus vestibularis inferior; NVL, nucleus reticularis ventrolateralis; NVM, nucleus vestibularis medialis; PY, tractus pyramidalis; TS, tractus solitarii; V, nucleus and tractus trigemini spinalis; X, nucleus dorsalis nervi vagi; XII, nucleus hypoglossi.

which the brain renin-angiotensin system may participate in the central machinery for circulatory control.

The present study demonstrated that, similarly to AIII (Lee *et al.*, 1990; Yin *et al.*, 1990), AII exerted an inhibitory action on *alpha*-2 adrenoceptors in the NRGC that are involved in central cardiovascular regulation. Furthermore, AII and AIII were shown to be equipotent in this modulatory action. These results are in line with findings from binding studies using membrane preparations from the medulla oblongata, which show a reduction in the affinity of *para*-[^{3}H]aminoclonidine for the *alpha*-2 adrenoceptor binding sites in the presence of AII or AIII (Fuxe *et al.*, 1988; Fior *et al.*, 1994). It follows that, by reducing the affinity of the *alpha*-2 adrenoceptors in the NRGC for guanabenz, activation of AT_2 receptors by AII or AIII resulted in the attenuation of the efficacy of guanabenz in its cardiovascular suppressant actions.

Another intriguing and novel finding in the present study was the antagonism by AII and potentiation by AIII of antinociception elicited by activation of the *alpha*-2 adrenoceptors in the NRGC, although both actions were reversed by losartan. Because these differential modulatory actions were elicited by equimolar doses of the octapeptide and heptapeptide, an explanation based on a difference in potency is not applicable. Likewise, because the loci upon which microinjection of AII or AIII elicited the modulatory effect were randomly distributed within the NRGC, an explanation based on heterogeneous subpopulations of responsive NRGC neurons is not forthcoming.

One possible clue may arise from recent identification of AT_{1A} and AT_{1B} receptor subclasses in rats (Iwai and Inagami, 1992). Although the functional significance of this additional delineation of AT_1 receptors is presently unclear, it is possible that AT_{1A} and AT_{1B} receptor subtypes may exhibit subtle differences in ligand-binding properties with AII and AIII and/or downstream signal transduction mechanisms. This is, in turn, reflected in the differential interactions of AII and AIII with *alpha*-2 adrenoceptors in the NRGC in antinociceptive action. It is also recognized that at least three subclasses of *alpha*-2 adrenoceptors exist, with distinct pharmacologic profiles (Bylund *et al.*, 1994) and differential effects on nociception (Millan, 1992; Khasar *et al.*, 1995). Therefore, different subclasses of *alpha*-2 adrenoceptors within the NRGC may have interacted with AII or AIII, *via* the AT_1 receptors, resulting in the differential modulatory action on guanabenz-induced antinociception.

A basic premise of the present study is that the stipulated modulatory actions of AII or AIII on *alpha*-2 adrenoceptors in the NRGC involve the presence of angiotensin receptors and *alpha*-2 adrenoceptors in this reticular nucleus. In this regard, catecholaminergic fibers are found throughout the reticular formation, including the NRGC (Fuxe, 1965). Nerve terminals that show positive immunoreactivity for dopamine-β-hydroxylase are located in the NRGC (Swanson and Hartman, 1975; Chan *et al.*, 1991). Microinjection of yohimbine into the NRGC significantly blunted the cardiovascular suppressive actions of guanabenz (Lim *et al.*, 1988b), and SAP-related neurons in the NRGC respond to this *alpha*-2 adrenoceptor agonist (Chan and Lin, 1988). Binding sites of the AT_1 receptor subtype predominate in areas of the brain thought to mediate most of the hemodynamic effects of AII, and AT_2 receptors are primarily located in sensory integrative areas of the thalamus, the septum and the medulla oblongata (Tsutsumi and Saavedra, 1991; Tony and Porter, 1993). With particular relevance to the present study, some regions such as the brainstem, periventricular nucleus and midbrain contain both receptor subtypes (Song *et al.*, 1992; Stecklings *et al.*, 1992).

The present results were obtained from animals that were maintained under stable pentobarbital anesthesia (Yang *et al.*, 1996), and we acknowledge that the findings may be quantitatively and/or qualitatively different from those seen with alert nonrestrained animals. Microinfusion into the NRGC of angiotensin ligands unaccompanied by peptidase inhibitors may result in rapid conversion of the ligand

(Wright and Harding, 1992). Thus, the contribution of metabolic products of AII and AIII to our results cannot be completely ruled out. Repeated testing of the tail-flick response as a measure of nociception may have resulted in some adaption. Although data from our control experiments significantly minimized this possibility, we are cognizant of the potential presence of this confounding factor.

An inter-relationship between central neural mechanisms involved in the regulation of nociception and arterial blood pressure has been suggested (Zamir and Segal, 1979; Randich and Maixner, 1984). Previous studies from our laboratory (Tsai *et al.*, 1994) also showed that NRGC neurons may reduce their responsiveness to nociception in the presence of elevations in SAP. We further found (Chan *et al.*, 1994) that the predominant action of AIII during hypertension was one that sensitizes the responsiveness of NRGC neurons to noxious stimuli. Because the design of the present study called for the simultaneous evaluation of cardiovascular and nociceptive responses, our observed results may have been influenced by their interactive actions. This possibility was deemed unlikely, for three reasons. First, bilateral microinjection of AII or AIII did not by itself significantly alter the base-line SAP, HR or tail-flick latency. Second, hypotension and bradycardia induced by guanabenz outlasted the duration of the simultaneously elicited antinociception. Third, whereas both AII and AIII antagonized the circulatory depressant effects of guanabenz, the octapeptide and heptapeptide exerted opposite actions on the antinociceptive action of the aminoguanidine compound.

In conclusion, the present study demonstrated, in rats maintained under pentobarbital anesthesia, that AII and AIII equipotently, but differentially, modulate the activity of *alpha*-2 adrenoceptors in the NRGC that are involved in central regulation of cardiovascular functions and processing of nociceptive information. We further showed the specific and novel engagement of AT_2 and AT_1 receptors in the respective modulatory actions.

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