Central Opiate System Modulation of the Area Postrema Pressor Pathway

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SUMMARY Angiotensin II, when given into the vertebral arteries, acts at the area postrema to augment central sympathetic vasomotor activity. The mechanism of action is unknown but recent evidence implicates an interaction with the opiate system. In dogs anesthetized with chloralose either alone or in combination with morphine, naloxone blunted the pressor response to vertebrally administered angiotensin II by 50%. Addition of morphine to dogs anesthetized with chloralose only doubled the pressor response to identical doses of angiotensin II. On the other hand, the magnitude of the pressor responses to intravenously infused angiotensin II were unaltered by either naloxone or morphine. Likewise, responses to norepinephrine given vertebrally and intravenously were not similarly affected. Therefore, naloxone-induced changes in vascular responsiveness were not responsible for the altered sensitivity of the area postrema to angiotensin II following blockade of endogenous opiates. The data suggest that there exists a previously unrecognized interaction of the endogenous opiate system in the medulla in mediating the pressor effects of angiotensin II at the level of the area postrema.

KEY WORDS • angiotensin II • area postrema • opiates • morphine • naloxone

AMONG the effects of angiotensin II is its ability to augment central sympathetic vasomotor activity by an action at the area postrema, a blood-brain barrier deficient structure along the walls of the dog's fourth ventricle. The intrinsic mechanism by which angiotensin II acts at the area postrema has not been clarified. For example, it is not known whether it acts directly on specific angiotensin II receptors or through inhibition and/or release of other neurotransmitters. Recent studies of the vertebrate central nervous system (CNS) have demonstrated the presence of opiate receptors throughout the brain and, of special interest here, the area postrema. Additionally, a recent study has provided evidence that enkephalin-containing neurons are located in the area postrema. This finding intrigued us because earlier studies in this laboratory had stressed the need of using morphine to unmask the centrally mediated effects of angiotensin II.

Therefore, we reexamined the contribution of morphine to the centrally mediated effects of angiotensin II with the assumption that it signified the possibility of a specific interaction between angiotensin II and opiate receptors at the level of the area postrema.

Methods

The centrally mediated cardiovascular effects of angiotensin II produced by the infusion of the peptide in the vertebral artery circulation were compared in dogs anesthetized with either chloralose alone (66 mg/kg i.v.) (n = 15) or a combination of morphine-chloralose (2 mg/kg i.m.) (n = 8). In all experiments, both vertebral arteries were cannulated using fine 20 gauge catheters (Angiocath, Deseret Co., Sandy, Utah) so as not to interrupt blood flow. Additional catheters were placed into a femoral artery to record arterial blood pressure (BP) and into the jugular vein for the purpose of infusions.

The octapeptide (1-Aspartic acid, 5-Isoleucine) angiotensin II (synthetized by Dr. M. Khosla of the Research Division of the Cleveland Clinic) or 1-norepinephrine were dissolved in 0.9% sodium chloride and infused either intravertebrally or intravenously at doses of 10, 20, 100, and 200 ng/kg/min respectively at a rate of 1 ml/min for 3 minutes. Infusions were randomized and compared to equivalent amounts of 0.9% saline.

Eight dogs were anesthetized with the combination of morphine-chloralose, and pressor responses were obtained before and 30 minutes after administration of naloxone (0.04 mg/kg, i.v.). This group served to illustrate the effect of removing the influence of morphine on the mechanisms of the central action of angiotensin II.

In 15 other dogs anesthetized with a-chloralose, pressor responses due to infusion of angiotensin II and
norepinephrine, given via either the intravenous or vertebral artery route of administration, were recorded both before and after the addition of a specific opiate agonist or antagonist. In eight of the 15 dogs, facilitation of the central action of angiotensin II by exogenous activation of the opiate system was evaluated by comparing the effect of intravertebral infusions of angiotensin II before and 1 hour after intramuscular administration of 2 mg/kg of morphine. In a remaining seven dogs, the direct contribution of the endogenous opiate system was determined by recording the effect of blockade of the opiate system 30 minutes after the dogs were given naloxone (0.04 ng/kg, i.v.).

The mean and standard error for the peak of the pressor responses from all dogs for each dose and condition were calculated. These data were compared using the Student's *t* test for paired data, and were considered significant when *p* < 0.05.

### Results

#### Alteration of Pressor Responses Due to Morphine Blockade

In eight dogs anesthetized with the combination of morphine and chloralose, baseline mean arterial pressure (MAP) averaged 125 ± 4 mm Hg, and baseline heart rate was 39 ± 4 beats/min. The average peak increase in BP produced by a 3-minute intravertebral infusion of either 10 or 20 ng/kg/min of angiotensin II averaged 23 ± 2 and 26 ± 2 mm Hg (*p* < 0.01) above control values respectively (fig. 1). The BP increases were rapid in onset (< 1 min) and not accompanied by any significant change in heart rate. During the intravenous infusion of angiotensin II (10 and 20 ng/kg/min), the MAP rose by an average of 10 ± 2 and 18 ± 2.3 mm Hg respectively. Heart rate did not change. Pressor responses obtained during the intravenous infusion of identical amounts of angiotensin II were thus significantly (*p* < 0.05) less than those obtained via the vertebral artery route.

Addition of the competitive morphine antagonist naloxone (0.8 mg i.v.) caused an increase in baseline MAP (148 ± 3 mm Hg, *p* < 0.05) and heart rate (94 ± 13 beats/min, *p* < 0.05). Pressor responses to intravertebral angiotensin II were markedly reduced to about one-half of those obtained before administration of naloxone (fig. 1). The reduced responsiveness was specific for the vertebral artery route since the peak of the pressor responses to intravenous administration of angiotensin II remained unchanged.

Three-minute intravertebral infusion of 1-norepinephrine at doses of 100 and 200 ng/kg/min increased MAP by 12 ± 6 and 13 ± 4 mm Hg respectively. Increases above baseline MAP values were statistically significant (*p* < 0.05) for the highest but not the lowest dose infused. Heart rate decreased by 6 ± 2 and 7 ± 1 beats/min. The responses obtained from intravenous infusions of identical doses of 1-norepinephrine were significantly (*p* < 0.05) less than those obtained via the vertebral artery route.

### Figures

**Figure 1. Alteration in the vertebral (top) and intravenous (bottom) angiotensin II elicited pressor response due to (left) naloxone in morphine-chloralose anesthetized dogs, (middle) morphine in chloralose anesthetized dogs, and (right) naloxone in chloralose anesthetized dogs, p values refer to difference from control pressor response.**

**Figure 2. Alteration in the vertebral (top) and intravenous (bottom) 1-norepinephrine elicited pressor response due to (left) naloxone in morphine-chloralose anesthetized dogs, (middle) morphine in chloralose anesthetized dogs, and (right) naloxone in chloralose anesthetized dogs, p values refer to difference from control pressor responses.**
norepinephrine produced effects not different from those obtained via the vertebral artery route (fig. 2). The increases in MAP due to either intravenous or intravertebral infusions of these doses of norepinephrine were in most cases not significantly affected by administration of naloxone. The height of the pressor response appeared to be potentiated but reached significance (p < 0.05) only at the highest dose tested intravenously (200 ng/kg/min of norepinephrine). In the other case, this difference was not statistically significant (p > 0.05), probably due to increased variability as indicated by the large standard error. The reflex bradycardia associated with the infusion of norepinephrine via both routes of administration was significantly potentiated after treatment with naloxone.

Heart rate decreased 25 ± 2 (p < 0.05, vertebral) and 30 ± 9 (p < 0.05, intravenous) with 100 ng/kg/min norepinephrine and 26 ± 7 (p < 0.05) and 31 ± 6 (p < 0.05) respectively with 200 ng/kg/min.

Alteration of Pressor Responses Due to the Addition of Morphine

In a subgroup of dogs anesthetized with chloralose alone (8 of the 15), baseline MAP was 145 ± 6 mm Hg and heart rate was 105 ± 9 beats/min. Angiotensin II given vertebrally (10, 20 ng/kg/min) produced a much smaller increase in MAP than in dogs anesthetized with morphine-chloralose; on the average, the MAP increase amounted to 9 ± 1 and 12 ± 2 mm Hg respectively (fig. 1). The increases in pressure were associated, however, with tachycardia (25 ± 8 and 28 ± 11 beats/min respectively), a feature not present in morphine-chloralose dogs. Pressor responses to identical doses of Angiotensin II given intravenously were not different from those due to vertebral administration; thus, a differential responsiveness between the two routes of administration could not be documented. Intravenous infusions of 10 and 20 ng/kg/min Angiotensin II resulted in slight decreases in heart rate (3 ± 3 and 8 ± 4 beats/min).

Following the addition of morphine (2 mg/kg i.m.) baseline MAP averaged 112 ± 6 mm Hg and heart rate was 72 ± 6 beats/min. The height of the pressor responses to intravertebral infusions of Angiotensin II were significantly augmented to about twice those recorded prior to administration of morphine (18 ± 2 and 22 ± 2 mm Hg) (fig. 1). The addition of morphine again prevented the heart rate component of the pressor response seen in dogs anesthetized with chloralose alone; the heights of the Angiotensin II pressor response via the intravenous route were not modified.

Addition of morphine inhibited the pressor responses to intravenous vasoconstrictor doses of norepinephrine, which was significant only at the lowest dose (12 ± 2 before; 6 ± 2 mm Hg after) (fig. 2). Vertebrally elicited pressor responses were not significantly altered. The magnitude of the reflex bradycardia was also reduced.

Alteration of Pressor Responses After Blocking Endogenous Opiates

In another subgroup of dogs anesthetized with chloralose alone (7 of the 15), baseline MAP and heart rate were 147 ± 7 mm Hg and 108 ± 11 beats/min respectively. Pressor responses to vertebrally administered Angiotensin II (10, 20 ng/kg/min) were again of small magnitude (12 ± 2 and 15 ± 3 mm Hg) and identical to those obtained intravenously (10 ± 1 and 14 ± 1 mm Hg) (fig. 1). During the pressor response, heart rate increased 16 ± 6 and 17 ± 6 beats/min. Intravenous administration of the same doses did not cause significant changes in heart rate.

Naloxone (0.04 mg/kg i.v.) did not alter baseline hemodynamics (151 ± 7 mm Hg and 114 ± 8 beats/min) of dogs anesthetized with chloralose, but reduced the magnitude of the pressor responses to vertebrally infused Angiotensin II to even smaller values (7 ± 1 and 11 ± 2, both p < 0.05); the intravenous responses remained unchanged (12 ± 1 and 16 ± 1 mm Hg).

Pressor responses and heart rate changes due to the infusion of 100 and 200 ng/kg/min of norepinephrine given by either route were not significantly altered by the addition of naloxone.

Pressor responses to 100 and 200 ng/kg/min of norepinephrine given intravertebrally were 9 ± 1 and 12 ± 2 before and 6 ± 2 and 9 ± 2 mm Hg after naloxone. Intravenous responses were 10 ± 2 and 15 ± 3 mm Hg before naloxone and 9 ± 2 and 11 ± 2 mm Hg after the morphine antagonist.

Discussion

These present findings support the possibility of a previously unrecognized important interaction between the central actions of Angiotensin II at the level of the dog's area postrema and the brain opiate receptor system. Both Scoop and Lowes and Ferrario et al. had previously documented the need of employing morphine in conjunction with chloralose to unmask the central action of Angiotensin II infused into the vertebral arteries of anesthetized dogs. Previous studies had indicated that a-chloralose anesthesia preserved the activity of the sympathetic nervous system while barbiturates caused the opposite effect. The addition of morphine was believed to reduce the total dose of anesthesia required to carry out the experiment, preserving a preparation sensitive to subtle changes in sympathetic vasomotor tone. While this concept was supported by various studies, no one until now considered that the central effects of morphine, rather than the use of either a-chloralose or pentobarbital, influenced the magnitude and characteristics of the cardiovascular pressor response mediated by the neural action of Angiotensin II in the area postrema.

In dogs anesthetized with chloralose after premedication with morphine, the magnitude and characteristics of the pressor responses to two doses of
angiotensin II, within range of the previously described summit of the vertebral artery dose-response curve, were essentially the same as reported elsewhere. Intravenous responses to identical doses of the peptide were always of lesser magnitude than those obtained via the vertebral artery route. The differential sensitivity of the cardiovascular pressor response obtained by comparing the vertebral artery versus the intravenous routes was entirely abolished by the administration of the competitive antagonist of morphine (naloxone). Similarly, the differential effects could not be reproduced in animals anesthetized with α-chloralose alone and no pretreatment with morphine. The effects of naloxone appear to be due to blockade of a central opioid mechanism, since vascular responsiveness to peripheral infusions of angiotensin II were unaffected while that to norepinephrine was not affected in the same way.

That the interaction between angiotensin and the brain opiate system occurs at the level of the area postrema is only inferential. First, the area postrema contains the receptors mediating the cardiovascular action of blood-borne angiotensin II infused via the vertebral arteries. Second, presynaptic morphine receptors have been demonstrated in the brain stem, particularly in the area postrema and nucleus tractus solitarii (NTS) of a number of species. Third, it has been shown that the area postrema is the chemo-receptor trigger zone for the emetic reflex. Recent studies have indicated that the participation of an endogenous opiate at the area postrema may facilitate the emetic actions of drugs. On the other hand, further studies will be required to ascertain the specific site of interaction.

The importance of this interaction and its possible mediation by the area postrema becomes clear in light of recent results from our laboratory. Electrical stimulation of the area postrema evokes pressor responses due to increased central sympathetic outflow; this mechanism resembles the actions of angiotensin II at the area postrema. Moreover, ablation of the area postrema is accompanied by hypotension and a significant decrease in hemodynamic variability. Those studies have provided evidence that the area postrema participates in the tonic regulation of cardiovascular function by the CNS. Furthermore, the participation of the opiate system in the effects of angiotensin II at the area postrema may provide insight into the neurochemical substrate of cardiovascular regulation at this level of the brain stem.

The mechanism by which morphine potentiates the central action of angiotensin II at the level of the area postrema has not been determined yet. That this effect is due to a change in peripheral vascular responsiveness can be excluded by the absence of similar changes in the pressor responses to either intravenously administered angiotensin II or norepinephrine, given by either route following injection of naloxone in dogs with or without morphine pretreatment. Thus, it seems possible that morphine may act on a specific receptor system modulating the action of angiotensin II on central vasomotor cardiovascular neurons. Terminans of noradrenergic neurons have been shown to be endowed with specific opiate receptors. Activation of these presynaptic receptors by morphine and congener leads to depression of the amount of transmitter released per impulse. Furthermore, the depression is antagonized by naloxone. Considering that the area postrema and NTS are heavily innervated with noradrenergic terminals, which presumably inhibit vasomotor sympathetic discharge, we could speculate that the action of morphine is related to suppression of noradrenergic inhibitory inputs to bulbar cardiovascular neurons.

Both barbiturate and chloralose anesthesia could bring about a decrease in central vasomotor activity by acting either at an enkephalinergic or noradrenergic brainstem site. Addition of morphine may prevent their inhibiting effects, rendering the reflex sensitivity of the preparation approaching the “awake” condition. Dickinson and Yu, Fukiyama et al., and Sweet et al. have shown the occurrence of a centrally mediated hypertensive response by the prolonged administration of angiotensin II into the vertebral arteries of the conscious rabbit or dog. It is not known, however, whether a further potentiation of the centrally mediated cardiovascular response could occur by the addition of morphine to awake animals.

Injection of enkephalin into a lateral cerebral ventricle of awake rats reduces both angiotensin II elicited drinking behavior and the accompanying pressor response. This is another indication of the different mechanism and pathways involved in the action of angiotensin II above (OVLT) and below (area postrema) the tentorium.

The response to the infusion of pressor doses of norepinephrine via either route of administration appeared to be potentiated after blockade of morphine with naloxone. The data are compatible with findings that suggest that morphine acts to diminish activation of central postsynaptic α-receptors. Additionally, morphine blocks the uptake of norepinephrine across the cell membrane.

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Central opiate system modulation of the area postrema pressor pathway.
J E Szilagyi and C M Ferrario

doi: 10.1161/01.HYP.3.3.313

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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