Facilitation of Adrenergic Transmission by Locally Generated Angiotensin II in Rat Mesenteric Arteries

K. U. Malik, D.Sc., Ph.D., and A. Nasjletti, M.D.

ABSTRACT When studied on isolated rat mesenteric arteries perfused with Tyrode’s solution, angiotensin I and angiotensin II (1 ng/ml), a synthetic tetradecapeptide renin substrate, and a purified hog renin substrate (50–100 ng/ml) potentiated vasoconstrictor responses to sympathetic nerve stimulation and to injected norepinephrine without altering basal pressure. These agents produced a greater augmentation of the vasoconstrictor responses to nerve stimulation than to injected norepinephrine. The potentiation of vasoconstrictor responses to sympathetic nerve stimulation and injected norepinephrine which was elicited by renin substrate and angiotensin I was abolished by an inhibitor of angiotensin I-converting enzyme, SQ 20,881, and by an angiotensin II receptor antagonist, [Sar$^1$-Ile$^8$]-angiotensin II. In contrast, the potentiating effect of angiotensin II was blocked only by the latter compound. We conclude that utilization of renin substrate within the vascular wall by renin or renin-like enzymes results in the formation of angiotensin I, which is converted to angiotensin II. Angiotensin in turn potentiates the vasoconstrictor responses to adrenergic stimuli presumably by augmenting release of the adrenergic transmitter and inhibiting its neuronal reuptake as well as by increasing vascular reactivity to norepinephrine.

ANGIOTENSIN II (A II), in addition to exerting a potent vasoconstrictor effect, enhances sympathetic activity in several tissues innervated by adrenergic nerves where it augments the response to sympathetic nerve stimulation as well as to injected norepinephrine. Facilitation of adrenergic nervous effects by A II appears to be due to enhanced synthesis and release, as well as to inhibition of neuronal reuptake, of norepinephrine. The demonstration of the presence of angiotensin-forming enzymes (renin, angiotensin I-converting enzyme) in extrarenal tissues including blood vessels raises the possibility that A II formed locally within vascular tissues modulates adrenergic nervous activity. To test this hypothesis we have examined the effect, in rat mesenteric arteries perfused with Tyrode’s solution, of natural and synthetic (tetradecapeptide) renin substrates and of both A I and A II on vasoconstrictor responses to periarterial nerve stimulation and to injected norepinephrine. To distinguish direct effects of the precursors of A II from those produced by local generation of A II within the vascular tissue, we have investigated the action of these agents on the vasoconstrictor responses to adrenergic stimuli after blockade of the activity of the renin-angiotensin system with either (1) an inhibitor of A I-converting enzyme, SQ 20,881 or (2) a competitive antagonist of A II, [Sar$^1$-Ile$^8$]-angiotensin II. The effect of A II and other agents on the vasoconstrictor responses to periarterial nerve stimulation described in this study is presumed to be due to their action on the adrenergic neuromuscular junction, since the work of McGregor and our previous observations indicate that the periarterial nerves to rat mesenteric arteries are postganglionic adrenergic fibers.

Methods

Experiments were performed on female albino rats weighing 300–350 g. The superior mesenteric artery was cannulated with the animal under ether anesthesia and isolated with its small resistance vessels according to the method of McGregor (15). The isolated vessels were perfused with Tyrode’s solution at a constant flow of 20 ml/min with a Harvard peristaltic pump (model 1210). Details of the method have been described earlier. Tyrode’s solution of the following composition (in mmol/liter) was used: NaCl, 137; KCl, 2.7; CaCl$_2$, 1.8; MgCl$_2$, 1.1; NaHCO$_3$, 12; NaH$_2$PO$_4$, 0.42; and D(+)-glucose, 5.6. The temperature of the perfusion fluid was maintained at 35°C. The solution was gassed with a mixture of 95% oxygen and 5% carbon dioxide. Changes in perfusion pressure were recorded with a manometer by a frontal lever writing on a kymograph. Before cannulation of the superior mesenteric artery, the pressure in the cannula was 60 mm Hg at a flow rate of 20 ml/min. During perfusion of the mesenteric blood vessels the pressure was 80 mm Hg.

The periarterial nerves were stimulated for 22 seconds at 4-minute intervals with a Grass stimulator (model S44) using supramaximal biphasic rectangular pulses 1 msec in duration at 6 Hz.

For each preparation at 4-minute intervals either the periarterial nerves were stimulated or norepinephrine was infused directly into the arterial cannula for 20 seconds with an infusion pump (Braun-Melsungen) using an automatic timer. The dose of norepinephrine in a preparation was varied (range, 200–500 ng) to produce control vasoconstrictor responses whose magnitude was not significantly different ($P < 0.01$) from the control responses produced by stimulation of periarterial nerves for 22 seconds. For each
preparation only a single dose of a drug was infused; drugs were selected randomly. The degree of augmentation produced by an agent in various preparations was independent of the height of control responses.

The concentration of norepinephrine bitartrate (Levophed, Winthrop Laboratories) is expressed as the free base. The following drugs were used: A II (1-Asp-5-Val-angiotensin II) (Hypertensin, CIBA); A I (1-Asp-5-Ile-angiotensin I) (Schwartz/Mann); tetradecapeptide renin substrate (Asp-Arg-Val-Tyr-Ile-His-Pro-Orn-Leu-Leu-Leu-Tyr-Ser); [Sar'-Ile*]angiotensin II; SQ 20,881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) (Squibb); natural hog renin substrate purified by (NH₄)₂SO₄ fractionation, acid denaturation, and batchwise DEAE fractionation; bovine albumin (Metrix, Armour Pharmaceutical Co.); and vasopressin (Sigma). All drugs were dissolved in small volumes of Tyrode's solution and added to the perfusion fluid to obtain the desired final concentration. The percent change in the vasoconstrictor responses to nerve stimulation and to injected norepinephrine produced by the various agents was calculated by comparing the height of maximal vasoconstrictor responses during the infusion of an agent for 16–24 minutes with the mean height of control responses recorded in the absence of the drug over the same period of time.

For statistical analysis paired and unpaired t-tests were performed according to the methods described by Steel and Torrie.19

Results

Effect of Angiotensin II, Angiotensin I, and Renin Substrate on Vasoconstrictor Responses

Effect of angiotensin II and modification of its action by [Sar'-Ile*]angiotensin II and an inhibitor of angiotensin I-converting enzyme, SQ 20,881. A II, 1 ng/ml, infused for 16–24 minutes, did not alter the basal pressure but it significantly (P < 0.001) potentiated the vasoconstrictor responses of mesenteric vessels to nerve stimulation and to injected norepinephrine (Fig. 1); the degree of augmentation was greater for the former than the latter (Table I). Potentiation of the vasoconstrictor responses to injected norepinephrine by A II gradually diminished over 15–20 minutes despite continued infusion of the latter agent. Higher concentrations of A II, e.g., 10 ng/ml, constricted mesenteric vessels as shown by an increase in perfusion pressure of 7 ± 1.0 mm Hg (SEM) in 10 preparations. However, the increase was not maintained and the basal pressure returned to control levels within 12 minutes during infusion of A II. Infusion of the A II receptor antagonist, [Sar'-Ile*]angiotensin II, 100 ng/ml, did not alter the basal pressure nor the vasoconstrictor responses to nerve stimulation or to injected norepinephrine, but in four preparations abolished the potentiating effect of A II on the vasoconstrictor responses to both of these adrenergic stimuli (Fig. 1). [Sar'-Ile*]angiotensin II, 100 ng/ml, did not alter the potentiating effect of another peptide, vasopressin, 100 nU/ml, on the vasoconstrictor responses of two preparations to both types of adrenergic stimuli.

Angiotensin I. A I (1 and 10 ng/ml) also potentiated the vasoconstrictor responses to nerve stimulation and to injected norepinephrine without altering the basal pressure. As for A II, the degree of augmentation of vasoconstrictor response to nerve stimulation was greater than that to injected norepinephrine (Fig. 1 and Table I). The potenti-

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dose (ng/ml)</th>
<th>No. of preparations</th>
<th>Responses to nerve stimulation (% increase ± SEM)</th>
<th>No. of preparations</th>
<th>Responses to injected norepinephrine (% increase ± SEM)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II</td>
<td>1</td>
<td>11</td>
<td>68 ± 8*</td>
<td>11</td>
<td>36 ± 6*</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>10†</td>
<td>7</td>
<td>188 ± 31†</td>
<td>7</td>
<td>117 ± 16†</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Angiotensin I</td>
<td>1</td>
<td>5</td>
<td>47 ± 8*</td>
<td>7</td>
<td>25 ± 4*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>10†</td>
<td>10</td>
<td>136 ± 16†</td>
<td>18</td>
<td>76 ± 9*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tetradecapeptide (renin substrate)</td>
<td>100</td>
<td>12</td>
<td>99 ± 12*</td>
<td>16</td>
<td>76 ± 11*</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* P < 0.001.
† Concentration which raised the basal perfusion pressure by 7 mm Hg.
tion by A I of the vasoconstrictor responses to nerve stimulation and to injected norepinephrine was abolished by the simultaneous infusion of [Sar'-Ile']angiotensin II, 10 ng/ml, in four preparations (Fig. 2). To determine whether the potentiating effect of A I on the vasoconstrictor responses to adrenergic stimuli was mediated either by its direct action on the adrenergic neuromuscular junction or by its conversion to A II within the vascular tissue, we examined the effect of an inhibitor of A I-converting enzyme, SQ 20,881. This inhibitor, at 10 µg/ml, did not alter the basal pressure or the vasoconstrictor responses to adrenergic stimuli. However, the simultaneous infusion of this agent abolished the potentiating effect of A I (10 ng/ml) on the vasoconstrictor responses to nerve stimulation in three preparations and to injected norepinephrine in three preparations (Fig. 2). In contrast, SQ 20,881 failed to block the potentiating effect of A II (1 ng/ml) on the vasoconstrictor responses to nerve stimulation in three preparations and to injected norepinephrine in two (Fig. 1).

Renin substrate. To determine whether angiotensins generated locally in the vascular tissue also facilitate adrenergic nervous activity we examined the effect of tetradecapeptide renin substrate and purified natural hog renin substrate on the vasoconstrictor responses to adrenergic stimuli. Infusion of tetradecapeptide renin substrate, 50–100 ng/ml, did not alter the basal pressure but significantly \( (P < 0.001) \) potentiated the vasoconstrictor responses to nerve stimulation and to injected norepinephrine (Fig. 3). The degree of augmentation of the former response was greater than the latter (Table 1). In contrast, infusion of albumin in similar concentrations, 50–100 ng/ml, failed to alter vasoconstrictor responses to either adrenergic stimulus in four prepara-

Augmentation of the vasoconstrictor responses to sympathetic nerve stimulation and to injected norepinephrine produced by tetradecapeptide renin substrate, 100 ng/ml, was abolished by the simultaneous infusion of SQ 20,881, 10 µg/ml (Fig. 3) in four preparations. In two preparations the latter agent also abolished the potentiating effect of purified natural renin substrate on the vasoconstrictor responses to nerve stimulation (Fig. 4). The augmentation of the vasoconstrictor responses to sympathetic nerve stimulation in four preparations and to injected norepinephrine in four preparations produced by tetradecapeptide renin...
substrate, 100 ng/ml, also was blocked by the simultaneous infusion of [Sar1-Ile8]angiotensin II (Fig. 3).

Discussion

Our demonstration that A II potentiates the vasoconstrictor responses of isolated, perfused rat mesenteric arteries to sympathetic nerve stimulation and to injected norepinephrine confirms the observations of McGregor.14 Similar findings have been reported for cut mesenteric arteries28 and other vascular beds.1 Augmentation of the vasoconstrictor responses to nerve stimulation and injected norepinephrine in our experiments could be due to enhanced vascular reactivity to norepinephrine or to inhibition of neuronal uptake of this amine,6 or to both. Since augmentation of vasoconstrictor responses to nerve stimulation was greater in degree and duration than to injected norepinephrine, a presynaptic effect of the octapeptide may contribute to the potentiation of vasoconstrictor responses to nerve stimulation. This view is supported by the demonstrated ability of A II to enhance the synthesis of A I as well as the release of the transmitter in tissues innervated by adrenergic nerves.2-4 The facilitatory effect of A II on vasoconstrictor responses to adrenergic stimuli presumably is mediated by stimulation of A II receptors at the adrenergic neuroeffector junction, since the competitive antagonist of A II, [Sar1-Ile8]angiotensin II, abolished the potentiating effect of A II. The specificity of [Sar1-Ile8]angiotensin II is shown by the failure of this agent to alter the potentiating effect of vasopressin on vasoconstrictor responses to adrenergic stimuli.

A I, the peptide precursor of A II, shared with the latter its ability to potentiate vasoconstrictor responses to sympathetic nerve stimulation to a greater degree than vasoconstrictor responses to injected norepinephrine. The demonstration that the converting enzyme inhibitor, SQ 20,881, blocked the potentiating effect of the decapeptide, but not that of A II, on the vasoconstrictor responses to adrenergic stimuli strongly suggests that the facilitatory effect of A I on adrenergic transmission is preceded by its conversion to A II. Blockade by [Sar1-Ile8]angiotensin II of the potentiating effects of A I and A II on the vasoconstrictor responses to adrenergic stimuli supports this view. These observations and the demonstration of A I-converting enzyme in vascular tissues15-18 are consistent with the hypothesis that conversion of A I to A II within the vascular tissue29 affects adrenergic transmission. Further, our demonstration that tetradecapeptide, as well as purified renin substrate,14 augmented the vasoconstrictor responses of rat mesenteric arteries to adrenergic stimuli raises the possibility that renin or renin-like enzymes occurring in vascular tissues30 may release A I which, after being converted to A II, potentiates vascular responses to adrenergic stimuli. The likelihood that tetradecapeptide renin substrate exerted a direct effect on either vascular reactivity or adrenergic mechanisms is remote since the facilitatory effect of this agent was completely prevented by A II receptor blockade as well as by inhibition of A I-converting enzyme activity. Similarly, a direct effect of the natural substrate on adrenergic mechanisms is improbable for the same reasons. In addition, albumin did not alter the vasoconstrictor responses to adrenergic stimuli. The presence in extrarenal tissues of enzymes other than renin, i.e., tonin23 and pseudorenin,24 which are able to release angiotensins from renin substrate has been established. They may be involved in the generation of angiotensin in the rat mesenteric arteries. However, the possibility that tonin might have generated angiotensin under these experimental conditions is unlikely since this enzyme is not blocked by converting enzyme inhibitors and does not release angiotensin from the natural substrate.25 Similarly, generation of A I by pseudorenin is improbable since this enzyme is almost devoid of activity at pH 7.4.25 This was the pH of the fluid we used to perfuse the mesenteric arteries. Our contention that renin substrate is utilized by renin within blood vessels to generate A I is supported by the demonstration of renin-like enzymes in vascular tissues.7-8 Such renin-like activity has been demonstrated in arteries and veins of hog, rat, and dog.5-6 The enzyme appears to be similar to kidney renin and it has been suggested that it is transported to blood vessels from plasma.7 More recently, however, the demonstrations that renin activity is present in mesenteric vessels of dogs 24 hours after nephrectomy, and that this activity increases after severe hemorrhage,27 appear to indicate that the enzyme is synthesized locally in blood vessels. These observations and the demonstration that renin cannot release A II directly from tetradecapeptide renin substrate28 suggest that the facilitatory effect of renin substrate on the adrenergic neuroeffector unit is mediated by the generation of A I, which subsequently was converted to A II within the vascular tissue. Thus, local generation of A II in blood vessels may play an important role in the regulation of vascular tone by exerting a direct effect on the vascular smooth muscle and, perhaps more importantly, by augmenting sympathetic activity.

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References

Renal Cortical Blood Flow in Glycerol-Induced Acute Renal Failure in the Rat

THEODORE W. KURTZ,* ROY M. MALETZ, M.D.,† AND CHEN H. HSU, M.D.

ABSTRACT Renal hemodynamics and renal function were evaluated in rats at 3, 24, and 48 hours and at 7 days after the induction of acute renal failure (ARF) by glycerol injection. Three hours after induction of ARF, creatinine clearance was 0.04 ml/min/100 g; renal blood flow (RBF), 1.99 ml/min/100 g; and filtration fraction, 3.7%. All were abnormally low. Although the administration of isotonic saline (total dose, 3% of body weight) to rats 3 hours after glycerol injection significantly improved creatinine clearance (0.17 ml/min/100 g), RBF (2.54 ml/min/100 g), and filtration fraction (12.9%), these values still were significantly lower than those of controls (creatinine clearance = 0.50 ml/min/100 g, RBF = 4.92 ml/min/100 g, filtration fraction = 20.0%, all P values <0.001). Serum creatinine concentrations were significantly lower than those of controls (creatinine clearance = 0.50 ml/min/100 g, RBF = 4.92 ml/min/100 g, filtration fraction = 20.0%, all P values <0.001). Serum creatinine concentrations were significantly elevated at 24 hours (3.72 gm/100 ml), 48 hours (4.69 mg/100 ml), and 7 days (0.66 mg/100 ml) after glycerol injection compared to control (0.46 mg/100 ml, all P <0.01). RBF during these phases was not different from normal (4.41 ml/min/100 g). RBF 3 hours after bilateral ureteral obstruction was measured to determine the effects of tubular obstruction on renal hemodynamics. The RBF of rats with ureteral obstruction (4.12 ml/min/100 g) was not significantly different from controls (4.41 ml/min/100 g), suggesting that tubular obstruction in this model of ARF is probably not the cause of decreased RBF. The depressed glomerular filtration, as reflected by the decreased creatinine clearance that occurs during glycerol-induced ARF, is probably related to altered intrarenal vascular resistance or to changes in glomerular capillary permeability, or both.

THE PATHOGENESIS of the oliguria in myoglobinuric acute renal failure has been the subject of investigation ever since the association between myoglobin and renal injury was first noted. 1 The preponderance of recent evidence has indicated that depression of glomerular filtration due to sustained cortical ischemia is responsible for the acute renal failure (ARF). 2-6 These studies, however, have involved the use of gas washout techniques to evaluate renal blood flow (RBF) under circumstances in which tissue necrosis, cell swelling, and edema occur. That such conditions may impinge the reliability and accuracy of hemodynamic data obtained by washout methods was explained by Aukland et al., 7 who thoroughly examined the methodology of hydrogen washout. Development of the radioactive microsphere technique for evaluating renal hemodynamics has obviated the technical problems associated with the gas washout methods. The recent adaptation of the microsphere technique for use in the
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