Mechanism of Enhancement by Angiotensin II of Sympathetic Adrenergic Transmission in the Guinea Pig

By Christopher Bell

ABSTRACT
An electrophysiological analysis was made of the mechanism underlying the potentiation by angiotensin II of responses to adrenergic nervous stimulation of the isolated vas deferens and the uterine artery of the guinea pig. Concentrations of angiotensin II up to $5 \times 10^{-7}$ g/ml did not affect resting muscle cell membrane potential, cause spontaneous transmitter release, or increase transmitter release induced by single nerve stimuli. The time courses of excitatory junction potentials were not increased, indicating that the neuronal mechanism for transmitter inactivation was not inhibited. Angiotensin II did, however, increase the degree of facilitation of successive excitatory junction potentials during repetitive low-frequency nerve stimulation, leading to greater depolarization of the muscle cells than under control conditions. That this enhancement of facilitation was the primary mechanism underlying potentiation of transmission by angiotensin II was supported by the finding that the potentiation of mechanical responses to nerve stimulation decreased markedly as the frequency of stimulation was raised over the range where facilitation is replaced by summation as the major factor in depolarizing the muscle cells to firing threshold (2–6 pulses/sec). This mechanism of action of angiotensin II means that its potentiating effect on transmission is relatively specific for the low frequencies of nervous activity which are thought to be important in tonic cardiovascular control.

KEY WORDS
vasomotor regulation
excitatory junction potentials
arterial smooth muscle
electrophysiology
facilitation
vas deferens

Since the findings that the systemic pressor effect of angiotensin II (All) was reduced following sympathectomy or reserpine-induced depletion of catecholamines (1,2) and that All potentiated cardiovascular responses to indirectly acting sympathomimetic amines (3), numerous reports have appeared to confirm the enhancement by All of adrenergic excitation. On the basis of these findings it has been suggested that endogenous All may have a pathophysiological role in the modulation of sympathetic vasomotor activity. However, there has been considerable disagreement as to the mechanism underlying the induced enhancement (see Discussion for references).

The present investigation is concerned with elucidation of the mechanism by which All affects adrenergic transmission to single smooth muscle cells. Two preparations taken from the guinea pig have been employed: the vas deferens, in which the effects of compounds which potentiate transmission by a variety of actions have already been documented (4–6), and the uterine artery, which has physiological and morphological characteristics suggesting that it can be used as a model system for studying transmission in small muscular arteries (7,8).

Methods
Vasa deferentia from young male (200–500 g) and uterine arteries from virgin female (300–400 g) guinea pigs were prepared for recording from...
single smooth muscle cells as described previously (4, 5, 7). Electrical stimulation of the intramural adrenergic nerves in these preparations was elicited with Pt ring electrodes placed around the tissues proximal to the recording site, and trains of 0.2-1.0-msec square pulses were delivered at 0.2-1 pulses/sec and supramaximal voltage. Intracellular recordings from single muscle cells were made using glass microelectrodes having tip resistances of 20-100 megohms. The criteria used for true intracellular location of the electrode have been discussed in previous publications (4, 5, 7).

Paired vasa deferentia were suspended in modified Krebs solution (9) in a 40-ml bath maintained at 37°C, and contractile responses to electrical stimulation of intramural adrenergic nerves were recorded isometrically with Grass FT03 transducers. Stimulation was elicited using Pt ring electrodes placed around the proximal end of the tissue, and 10-second trains of 0.1-msec square-wave pulses were delivered at frequencies of 2-12 pulses/sec and supramaximal voltage every 2 minutes. For experiments in which responses to norepinephrine were obtained, the serosal coat of the vas deferens was removed (10). Norepinephrine was applied for 30 seconds at intervals of not less than 4 minutes.

Isolated uterine arteries perfused with McEwan's solution were prepared for stimulation of the periarterial vasomotor nerves as described previously (11), and vasoconstrictor responses were obtained to stimulation with 10-second trains of 1-msec square-wave pulses delivered at frequencies of 2-12 pulses/sec and supramaximal voltage every 3.5 minutes, and to intraluminal injections of norepinephrine.

Drugs used were angiotensin II (Hypertensin, Ciba); choline chloride; and norepinephrine tartrate (Levophed, Winthrop). The angiotensin was dissolved in sterile saline (0.9% w/v) and stored in plastic vessels. Norepinephrine solutions contained ascorbic acid (10⁻⁴ g/ml).

The significance of the difference of means was calculated with Student's t-test (12). Successive exposures of the tissues to AII were separated by periods of at least 60 minutes to preclude tachyphylaxis.

Results

Electrophysiology

Under control conditions the membrane properties of the smooth muscle cells in the vas deferens and the uterine artery were as documented previously (4-7, 13). In both tissues, the resting membrane potential was around 60 mv (Table 1). Spontaneous excitatory junction potentials (EJPs) were seen frequently in the vas deferens (Fig. la), but only occasionally in the uterine artery. Electrical stimulation of the intramural adrenergic nerves evoked EJPs with a time course of about 800 msec (Table 1, Figs. la and 2a). Repetitive stimulation at frequencies of 0.2 pulses/sec or more caused a progressive increase in amplitude of the first few EJPs of a

| Table 1 |

| Lack of Effect of AII (10⁻³ g/ml) on Transmission Characteristics of Smooth Muscle Cells in Guinea Pig Vas Deferens and Uterine Artery |
|-----------------|-----------------|
| Vas deferens | Uterine artery |
| | Control | AII | Control | AII |
| Membrane potential (mv) | 59.0 ± 1.2 (35) | 62.4 ± 0.9 (26) | 64.0 ± 1.9 (28) | 60.4 ± 0.9 (27) |
| Latency EJP (msec) | 19.2 ± 0.8 (17) | 18.6 ± 0.7 (18) | * | * |
| Rise time EJP (msec) | 9.6 ± 4.5 (17) | 99 ± 9.9 (15) | 55 ± 0.9 (10) | 52 ± 1.0 (12) |
| Decay constant EJP (msec) | 290 ± 10.2 (17) | 292 ± 2.7 (14) | 203 ± 3.3 (10) | 200 ± 4.0 (12) |
| Amplitude EJP (mv) | 5.6 ± 0.32 (50) | 5.2 ± 0.5 (55) | 2.0 ± 0.16 (44) | 2.0 ± 0.07 (34) |
| Spontaneous EJPs (per min) | 34 ± 13 (6)† | 39 ± 15 (6)† |  |

Each figure represents a mean ± se. The number of cells from which data were obtained is in parentheses. None of the parameters measured changed in the presence of AII (P > 0.1).

*Background noise precluded accurate measurement.
†Figures derived from the same six cells in the absence and presence of AII.
FIGURE 1
Recordings of adrenergic transmission to smooth muscle cells in the vas deferens (a) under control conditions, (b) in the presence of AII, 10^{-7} g/ml, and (c) 20 minutes after washing the bath free of AII. The upper traces in each show EJPs evoked by repetitive adrenergic nerve stimulation at 1 pulse/sec (black dots) recorded from a single cell. The lower traces show the rate of spontaneous EJP discharge recorded from another single cell. Note that AII increased facilitation of amplitude of successive EJPs without altering the amplitude of the first EJP of the train or the rate of spontaneous transmitter release and that this effect was reversed by washing. Membrane potentials: 61 mv (upper traces), 60 mv (lower traces). Calibrations: horizontal, 1 second (upper traces), 2 seconds (lower traces); vertical, 10 mv.

series. At a stimulation frequency of 1 pulse/sec, this facilitation was complete after the first 5-7 impulses, at which time further impulses evoked EJPs of stable amplitude (Figs. 1a and 2a). Repetitive stimulation at frequencies greater than 1.2 pulses/sec caused summation of successive EJPs as well as facilitation because the muscle membrane was not fully repolarized at the time of onset of the next EJP.

Exposure of the tissues to AII in concentrations as high as 5 \times 10^{-7} g/ml caused no reduction of resting membrane potential, and did not alter the amplitudes of EJPs evoked by single stimulating pulses (Table 1, Figs. 1 and 2). The time courses of evoked EJPs were also unaltered (Table 1). In the vas deferens, the frequency of discharge of spontaneous EJPs under resting conditions was not affected (Table 1, Fig. 1), and in artery the rarely occurring spontaneous EJPs were not seen more frequently in the presence of AII.

Only one aspect of the transmission process was affected in the presence of AII. This was the facilitation seen during repetitive stimulation, which was increased significantly in both tissues (Table 2), leading to greater maximum depolarization of the muscle cells when facilitation was complete than under control conditions (Figs. 1 and 2). This enhancement of facilitation was as pronounced in the presence of AII, 10^{-7} g/ml, as it was in the presence of higher concentrations (up to 5 \times 10^{-7} g/ml), and a lesser, but qualitatively similar, effect was seen during the first few minutes of AII infusion, when the bath concentration was considerably less than 10^{-7} g/ml. The enhancement of facilitation persisted up to 60 minutes in the continued presence of AII, but was rapidly reversed after washing the bath free of AII (Table 2, Fig. 1).

**CONTRACTILE RESPONSES**
Angiotensin II was added to the solution bathing the vas deferens or to the arterial perfusion fluid to produce final concentrations of 10^{-9}-5 \times 10^{-7} g/ml. These concentrations of AII had no direct contracting effect on either preparation, but did enhance the mechanical responses to electrical stimulation of intramural adrenergic nerves. This potentiation was maximal with a concentration of 10^{-7} g/ml of AII, but was also seen to a lesser degree with concentrations lower than this (10^{-8}-10^{-8} g/ml). The effect of AII persisted up to 20 minutes in the continued presence of the drug, and was reversed rapidly after washing the bath. There did appear to be some degree of tachyphylaxis to AII, as the drug had little effect if applied to the tissue a few minutes
FIGURE 2

EJPs evoked by repetitive adrenergic nerve stimulation at 1 pulse/sec (at black dots) recorded from the same muscle cell in the uterine artery (a) under control conditions and (b) in the presence of All, 10^-7 g/ml. Note the increased facilitation of amplitude of successive EJPs induced by All, and the absence of effect on the amplitude of the first EJP of the train. The electrode was dislodged from this cell before the effect of washing the bath free of All could be studied. Membrane potential 86 mV. Calibrations: horizontal, 1 second; vertical, 5 mV.

after an initial exposure. If, however, successive exposures were separated by a 60-minute interval, no tachyphylaxis was apparent. To determine the specificity of the potentiating effect of All for nervous stimulation, its effect on contractile responses of the tissues to exogenous norepinephrine was also examined. The doses of norepinephrine used were those producing 50% maximum responses for each tissue, namely 5 x 10^-6 g/ml for the vas deferens (14) and 5 x 10^-7 g by intraluminal injection for the uterine artery (11). In three experiments on each tissue, the magnitude of responses to these doses of norepinephrine were unaffected by All, 10^-7 g/ml (Fig. 3).

It is known that facilitation is the major factor in depolarizing the smooth muscle cell membrane during repetitive stimulation at frequencies below about 1.2 pulses/sec. As the stimulation frequency is increased above this value, summation becomes progressively important as successive EJPs are evoked while the membrane is still partially depolarized. At stimulation frequencies above about 5 pulses/sec, facilitation is only a minor factor in depolarization (7, 13). Thus examination of the degree of potentiating of mechanical nerve-mediated responses by All over this frequency range afforded a means of checking the electrophysiological evidence, which indicated that All potentiated transmission primarily by increasing the facilitation process.

Reproducible mechanical responses of neither vas deferens nor uterine artery could be obtained with nervous stimulation at frequencies below 2 pulses/sec. At this frequency, All, 10^-7 g/ml, caused an 11.2-fold potentiation of the response of the vas

| TABLE 2 |

<table>
<thead>
<tr>
<th>Effect of All (10^-7 g/ml) on Facilitation of EJPs during Nerve Stimulation at 1 Pulse/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Vas deferens (V/V&lt;sub&gt;0&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Uterine artery (V/V&lt;sub&gt;0&lt;/sub&gt;)</td>
</tr>
</tbody>
</table>

The degree of facilitation of EJPs is expressed as the ratio between the amplitudes of the fully facilitated EJP (V) and the first EJP of the train (V<sub>i</sub>). Each figure represents a mean ± s.e. The number of cells from which the data were obtained is in parentheses.

*P < 0.001.

Circulation Research, Vol. XXXI, September 1972
Mechanical responses of (a) vas deferens and (b) uterine artery to exogenous norepinephrine (NA, arrows) and to intramural adrenergic nervous stimulation at 8 and 2 pulses/sec (black dots) under control conditions and in the presence of AII, 10^{-7} g/ml. The perfusion pressure recording in b is discontinuous because of changes in recorder sensitivity necessary for the different responses. Note the lack of effect of AII on responses to norepinephrine and the greater potentiation in the presence of AII of responses to nervous stimulation at 2 pulses/sec than at 8 pulses/sec. Calibrations: horizontal, 1 minute; vertical, (a) 200 mg tension, (b) 40 mm Hg (norepinephrine responses), 20 mm Hg (8 pulses s^{-1} responses), 10 mm Hg (2 pulses/sec responses).

defersens and a 2.8-fold potentiation of the response of the uterine artery. As the stimulation frequency was raised over the range 2–6 pulses/sec, the degree of potentiation produced by AII decreased markedly, at 6 pulses/sec being 3.8-fold and 1.6-fold control values for vas deferens and uterine artery, respectively. Above 6 pulses/sec there was little further decrease in potentiation with higher frequencies of stimulation (Figs. 3, 4, 5).

In contrast with the pronounced frequency dependence of potentiation induced by AII, the enhancement of mechanical responses of the vas deferens in the presence of choline, 5 \times 10^{-4} g/ml, which potentiates nerve-mediated contractions of this tissue by persistent partial depolarization of the muscle cell membranes (5), was similar in degree over the entire range of stimulation frequencies tested (Fig. 3). Comparable experiments could not be performed with the uterine artery because in this tissue there is as yet no documentation of any substances which enhance transmission by muscle membrane depolarization. Cholinergic stimulants are themselves inhibitory (11).

Discussion

The enhancement by AII of adrenergic nervous excitation has been attributed by...
some workers to inhibition of the neuronal reuptake process (Uptake 1) (15) thought to be important in termination of action of norepinephrine (16-19). Other investigators have favored enhancement of transmitter release, rather than depression of its inactivation, as the underlying mechanism (20-27, and others).

In the guinea pig vas deferens, blockade of Uptake 1 causes potentiation of contractile responses both to exogenous norepinephrine and to nervous stimulation and prolongs the falling phase of the EJP (4). The EJP in the guinea pig uterine artery is similarly prolonged after Uptake 1 is blocked (unpublished observation). In contrast, the present study has demonstrated that AII does not alter the responses of the vas deferens or of the uterine artery to exogenous norepinephrine and has demonstrated that the time courses of EJPs in both tissues are unaffected by AII. Thus in these tissues AII, at least in the concentrations used, does not appear to affect Uptake 1.

The only effect of AII on transmission seen in this study was an increase in the degree of facilitation during repetitive low-frequency nervous stimulation. Facilitation of successive EJPs is characteristic of many smooth muscle tissues, and evidence has been presented in the past to support the view that in the vas deferens, as at the skeletal neuromuscular junction (28), it is a purely presynaptic phenomenon related to release of increasing amounts of transmitter with successive stimulating pulses (13). The effect of AII on facilitative recruitment did not appear to be associated with an increased capacity of the nerves to release transmitter per se, as the amplitudes of EJPs in response to single stimulating pulses were not increased. This apparent specificity of action was further supported by the observation that the degree of potentiation of contractile responses to nerve stimulation in both vas deferens and uterine artery decreased rapidly as the stimulation frequency was raised over the range of 2 to 6 pulses/sec. Over this order of frequency increase, facilitation is replaced by summation as the major factor in depolarizing the muscle cell membrane to firing threshold (7, 13). In contrast, the potentiation induced by choline in the vas deferens, which is due to partial muscle membrane depolarization (5), showed no dramatic frequency dependence over the range studied.

The smaller degree of potentiation by angiotensin II of contractile responses in the uterine artery is in accord with the less pronounced facilitation in this tissue than in the vas deferens (Table 2).

The relative specificity of the enhancing effect of AII on transmission for low frequencies of stimulation, where facilitation is pronounced, is of interest in view of the evidence that this frequency range is also that normally involved in tonic cardiovascular control (29, 30). Such a relation could be interpreted as supportive evidence for the suggestions that endogenous AII may have a pathophysiological role in the modulation of adrenergic vasomotor tone (3, 16, 19, 31-33).

In addition to the numerous reports of enhancement of transmitter release following nervous stimulation, AII has also been reported to increase spontaneous release of norepinephrine in isolated aortas (34). In the rabbit heart, however, no increase in spontaneous release has been observed (35). No evidence...
was obtained in the present study to suggest that All stimulated spontaneous transmitter release, manifested either as an increase in spontaneous EJP frequency, like that with some adrenergic neurone-blocking agents (36) and with ethyl alcohol (6), or as a persistent reduction of muscle membrane potential, like that after inhibition of monoamine oxidase activity (4) in the vas deferens.

Although only one action of All was seen in these experiments, it is likely that alternative or additional effects may contribute to the enhancement of transmission in other systems. Besides the published evidence suggesting blockade of Uptake 1, a recent report has indicated that in the rat, All may enhance the contractility of vascular smooth muscle (37), although both the present study and that by Benelli et al. (20) have demonstrated no enhancement of responses to exogenous noradrenaline by All in guinea pig tissues. The degree to which these differences reflect the species of animal used and variations in experimental conditions remains to be determined.

Acknowledgment

I should like to thank Professor G. Burnstock for his generous provision of laboratory facilities.

References

ANGIOTENSIN AND ADRENERGIC TRANSMISSION


Mechanism of Enhancement by Angiotensin II of Sympathetic Adrenergic Transmission in the Guinea Pig
Christopher Bell

Circ Res. 1972;31:348-355
doi: 10.1161/01.RES.31.3.348

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1972 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/31/3/348

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/