Original Article

ANGIOTENSIN II-INDUCED DEPOLARIZATIONS IN HAMSTER SUBMANDIBULAR GANGLION NEURONS

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Abstract

Angiotensin II acts as an excitatory neurotransmitter of the sympathetic ganglia. In the parasympathetic neurons, however, angiotensin II (angio II)-induced responses have not been recorded. In this study, we investigated the effects of angio II in the hamster submandibular ganglion (SMG) neurons using a intracellular recording technique. Approximately 70% of these ganglion cells responded with persistent depolarization (3–6 mV, 5–9 min) at a concentration of 10 μ M. The angio II-induced depolarizations were caused by the combination of an increase in Na⁺ conductance and a decrease in K⁺ conductance. An involvement of K⁺ channels such as M channels and SK channels in the electrogenesis mechanism was suggested by an inhibitory effect of Ba²⁺ ion on the component of the increased membrane input resistance during the angio II-induced depolarization. In addition, the angio II-induced depolarizations were mediated through angio II type I (AT₁) receptors. Thus angio II is probably a neurotransmitter that increases their excitability in most of the neurons in parasympathetic ganglia.

Key words: Angiotensin II—AT₁ receptor—g_{Na,k}—Depolarization—
Submandibular ganglion neuron

INTRODUCTION

Angiotensin II (angio II) is an octapeptide most familiar for its role in the renin-angiotensin-aldosterone system that helps to maintain blood volume and pressure²⁾. In addition, angiotensin is also involved in local functions through the activity of tissue renin-angiotensin systems that occur in many organs, including the brain, adrenal gland, vascular wall, reproductive organs, and heart²⁾. In both the central and peripheral nervous systems, angio II acts as a neurotransmitter, often *via*

G-protein²⁾. In the peripheral nervous system, angio II potentiates sympathetic transmission through the sympathetic ganglia²⁾. Angio II inhibits N-type calcium currents and M-type potassium currents in acutely dissociated superior cervical ganglion cells, thus increasing the excitability of these neurons¹¹⁾. Up to the present, angio II-induced depolarizations have not been recorded in the parasympathetic neurons^{2,6)}. In cat ciliary ganglion neurons, angio II caused no depolarization in concentrations up to 10 μ M⁶⁾. In cardiac parasympathetic ganglia, angio II inhibits release

of acetylcholine from the preganglionic neurons *via* a presynaptic mechanism^{2,9}. *In vitro* autoradiographic techniques have demonstrated a high density of angio II-type I (AT₁) receptors over cells in intracardiac ganglia, although the resolution of this technique did not allow differentiation between presynaptic and postsynaptic elements^{3,10}.

In this study, we investigated angio II-induced responses in the submandibular ganglion neurons.

MATERIALS AND METHODS

Experiments were conducted according to the guidelines for the treatment of experimental animals at Tokyo Dental College. The intracellular recording technique in SMG neurons was used as previously described⁸. Briefly, an isolated hamster SMG was superfused continuously with oxygenated Krebs solution (30°C) at 2 ml/min. Intracellular recordings were conducted using glass microelectrodes filled with 3M KCl (input resistance of 30–60 MΩ). Penetration of the glass microelectrode was achieved under visual control with Zeiss Nomarski optics (10×ocular; 25.8×objective, UD40). Angio II was applied at concentration of 10 μM by pressure ejection from a pipette with a tip 5 μm in inside diameter. The micropipette was positioned 20–30 μm away from a target cell. Twenty pulses of air pressure (2 kg/cm², 50 msec) were delivered to the pipette through a pressure ejection system. Cellular responses were recorded by a strip chart recorder. For estimating the membrane input resistance of the ganglion cell, hyperpolarizing currents (0.1–0.2 nA) were applied for 50 msec at 0.1 Hz through the recording electrode by means of a balanced bridge circuit. The membrane potential of the ganglion cell was changed by applying direct currents (0.1–0.5 nA) using the balanced bridge circuit.

The ionic composition of the normal Krebs solution was as follows (mM): 136 NaCl, 5 KCl, 2.5 CaCl₂, 1 MgCl₂, 12 NaHCO₃, 1.2 NaH₂PO₄ and 11 glucose. Na⁺-free Krebs solu-

tion was prepared by adding 150 mM TrisCl instead of NaCl, NaHCO₃, and NaH₂PO₄. The pH was adjusted to 7.4 with HEPES. In Krebs solution containing 1 mM BaCl₂, the pH was adjusted with TrisCl. [Sar¹, Ala⁸]-angiotensin II, a peptide angiotensin II receptor antagonist¹⁴, losartan, a selective non-peptide AT₁ receptor antagonist¹³, and PD-123,319 ditrifluoroacetate, a potent selective non-peptide AT₂ receptor antagonist¹³, were respectively applied to cells at concentrations of 10 μM through perfusate. Losartan was a gift from Merck & Co., Inc.

RESULTS

Fourteen out of 20 tested SMG cells from 20 male hamsters, 4–6 weeks old, responded with depolarizations. The depolarizations produced by 20 angio II-ejections at a concentration of 10 μM were accompanied by a decrease in membrane input resistance (R_m) in 7 cells, an increase in R_m in 5 cells, and both a decrease and an increase in R_m in the remaining 2 cells, in which the depolarization was preceded by an increase in R_m . Three representative angio II-induced depolarizations are shown in Fig. 1. The depolarization accompanied by a decrease in R_m was 6.3 ± 1.2 mV in peak amplitude, 1.9 ± 0.3 min in time to peak, and 7.5 ± 0.8 min in duration at resting membrane potential (resting E_m) = -64.9 ± 4.5 mV (mean $\pm$ S.E., $n = 7$). The membrane input resistance was 45.9 ± 12.4 MΩ under the resting conditions and decreased by $22.0 \pm 4.2\%$ of control at a maximum during the depolarization. The depolarization accompanied by an increase in R_m was 2.6 ± 0.4 mV in peak amplitude, 1.4 ± 0.3 min in time to peak, and 5.2 ± 0.6 min in duration at resting E_m = -37.8 ± 1.8 mV (mean $\pm$ S.E., $n = 5$). The R_m was 54.7 ± 6.1 MΩ under the resting conditions and increased by $31.0 \pm 9.6\%$ of control at a maximum during the depolarization. This type of the depolarization changed to those accompanied by a decrease in R_m at a more negative E_m level to -60 mV. The depolarization accompanied by both a decrease

Fig. 1 Three representative angiotensin II-induced depolarizations of hamster submandibular ganglion cells

Three representative depolarizations produced by 20 angio II-ejections at a concentration of $10\mu\text{M}$ were accompanied by a decrease in membrane input resistance (R_m) in the upper trace, an increase in R_m in the middle trace, and both a decrease and an increase in R_m in the lower trace.

Upper trace: R_m decreased by 37.5% of control ($26.7\text{M}\Omega$) during the depolarization. Resting E_m : -66mV , Time to peak: 3.2min, Duration: 7.4min, Peak amplitude: 11mV.

Middle trace: R_m increased by 30% of control ($66.7\text{M}\Omega$) during the depolarization. Resting E_m : -31mV , Time to peak: 2.0min, Duration: 5.1min, Peak amplitude: 2mV, Hyperpolarizing currents (0.2nA) applied for 50msec at 0.1 Hz through the recording electrode generated anodal break-action potentials.

Lower trace: R_m increased by 10.5% of control ($85\text{M}\Omega$) in the rising phase of the depolarization and decreased by 47.4% at the peak of the depolarization. Hyperpolarizing currents (0.2nA) applied for 50msec at 0.1 Hz generated anodal break-action potentials. Resting E_m : -53mV , Time to peak: 4.9min, Duration: 9.1min, Peak amplitude: 16mV.

and an increase in R_m in one cell was 16 mV in peak amplitude, 4.9 min in time to peak, and 9.1 min in duration at resting $E_m = -53\text{mV}$. The R_m was $85\text{M}\Omega$ under the resting conditions, increased by 10.5% in the rising phase, and decreased by 47.4% at the peak of the depolarization. In Tris-substituted Na^+ -free solution, the depolarization accompanied by a decrease in R_m decreased the peak amplitude by 84.4% from 32 mV to 5 mV (shown in Fig. 2). In Krebs solution containing 1mM BaCl_2 , the component of an increase in R_m of angio II-induced depolarization accompanied by both a decrease and an increase in R_m was prevented (shown in Fig. 2). Effects of angio II receptor antagonists on the angio II-induced depolarization are shown in Fig. 3.

Fig. 2 Effects of Na^+ -free solution and Ba^{2+} ion on the angiotensin II-induced depolarization

Effect of Na^+ -free solution:

This angio II-induced depolarization was 32 mV in peak amplitude, 5.2 min in time to peak, and 13.2 min in duration at resting $E_m = -62\text{mV}$ in normal Krebs solution. The R_m decreased by 30% of control at a maximum during the depolarization.

In Tris-substituted Na^+ -free solution, the peak amplitude decreased from 32 mV to 5 mV (resting $E_m = -58\text{mV}$). A component accompanied by R_m decrease of the depolarization reduced markedly. The R_m instead increased by 22% of control at a maximum during the depolarization.

Effect of Ba^{2+} ion:

This angio II-induced depolarization was 4 mV in peak amplitude, 2.3 min in peak amplitude and 6.3 min in duration at resting $E_m = -43\text{mV}$ in normal Krebs solution. The R_m decreased by 23% of control near peak of the depolarization. Hyperpolarizing currents (0.2nA) applied for 50msec at 0.1 Hz through the recording electrode generated anodal break-action potentials. In 1 mM Ba^{2+} -containing Krebs solution, a component accompanied by R_m increase in the rising phase of the depolarization was partially prevented. The R_m decreased by 33% of control near peak of the depolarization.

$10\mu\text{M}$ $[\text{Sar}^1, \text{Ala}^8]$ -angio II reversibly prevented the angio II-induced depolarizations ($n=3$) completely. Additionally, $10\mu\text{M}$ losartan also reversibly prevented the angio II-depolarizations ($n=3$) completely. Furthermore, $10\mu\text{M}$ PD-123,319 partially prevented the depolarizations ($n=3$) and the peak amplitudes of depolarization decreased by 60% (mean). In one cell, rhythmic hyperpolarizations were induced at a frequency of

Fig. 3 Effects of antagonists on the angiotensin II-induced depolarization

Effect of a peptide angiotensin II receptor antagonist:

This angio II-induced depolarization was 3 mV in peak amplitude, 0.6 min in time to peak, and 3 min in duration at resting $E_m = -57$ mV in normal Krebs solution. Hyperpolarizing currents (0.2 nA) applied for 50 msec at 0.1 Hz through the recording electrode generated anodal break-action potentials during the depolarization. $10 \mu\text{M}$ $[\text{Sar}^1, \text{Ala}^8]$ -angiotensin II prevented completely the angio II-induced depolarization. Thirty minutes after washing out with normal Krebs solution, the angio II-depolarization recovered completely.

Effect of a selective non-peptide AT_1 receptor antagonist:

This angiotensin II-induced depolarization was 6 mV in peak amplitude, 5 min in time to peak, and 10 min in duration at resting $E_m = -80$ mV in normal Krebs solution. Notably, $10 \mu\text{M}$ losartan prevented completely the angio II-induced depolarization. Ninety-four minutes after washing out with normal Krebs solution, the angio II-induced depolarization recovered partially to 3 mV. Hyperpolarizing currents (0.2 nA) applied for 50 msec at 0.1 Hz through the recording electrode generated three anodal break-action potentials in these three traces. The amplitude (8 mV) of spike-afterhyperpolarizing potential during exposure to losartan was the same with control before losartan application in the 1st trace, but was reduced by angio II in the 3rd trace (see the amplitude, 3 mV). This was a sign of an inhibition of SK channels by angio II.

Effect of a selective non-peptide AT_2 receptor antagonist:

This angio II-induced depolarization was 5 mV in peak amplitude, 4.6 min in time to peak, and 13.3 min in duration at resting $E_m = -92$ mV in normal Krebs solution. Notably, $10 \mu\text{M}$ PD-123,319 partially prevented (by 60% of control) the angio II-induced depolarization to 2 mV. Fifty-two minutes after washing out with normal Krebs solution, the angio II-induced depolarization recovered partially to 4.5 mV.

2.4/min during angio II-induced depolarization; they were 24 mV in amplitude and 1,400 msec in duration at a maximum at resting $E_m = -73$ mV (not shown).

DISCUSSION

In the cat superior cervical ganglion (SCG) neurons, the effect of angiotensin II was primarily due to an increase of the permeability of Na^+ ions⁽⁶⁾. In rat SCG neurons, the effect was usually accompanied by a decreased membrane conductance, probably to K^+

ions⁽⁴⁾. Subsequently, Constanti and Brown (1981)⁽⁵⁾ observed a decrease in K^+ conductance by muscarine and angio II in SCG neurons, which they suggested was due to common suppression of the I_M . Shapiro *et al.* (1994)⁽¹¹⁾ revealed that angio II inhibits calcium and M currents channels in rat sympathetic neurons *via* G proteins. In this study, we obtained two kinds of information about the electrogenesis mechanism of angio II-induced depolarization; an increase in Na^+ conductance and a decrease in K^+ conductance. The three types of angio II-induced depolarizations in the SMG neurons were

probably induced by combinations of two different ionic conductances. In any resting E_m , either of two ionic conductances could be predominant. Ba^{2+} ion is known as a blocker of M channels and Ca^{2+} -activated K^+ channels¹⁾. In this study, 1 mM $BaCl_2$ prevented the component of an increase in R_m in angio II-induced depolarization. This suggests the involvement of K^+ channels such as M channels and SK channels in the electrogenesis mechanism of angio II-induced depolarization. Furthermore, we demonstrated inhibitory effects of angio II on M current and Ca^{2+} -activated K^+ current (probably $I_{K,Cal}$) in voltage-clamped SMG neurons using whole-cell configuration of the patch-clamp technique (unpublished observation).

In the parasympathetic neurons, angio II-induced depolarization has not been recorded²⁾. Angio II in concentrations up to 10 μ M did not cause any detectable membrane depolarization in the cat ciliary ganglion neurons⁶⁾. In the rat intracardiac ganglia, a high density of AT_1 receptors was demonstrated, although differentiation between preganglionic and postganglionic elements was not possible^{3,10)}. The results of this study revealed that the angio II-induced depolarization in SMG neurons was mediated by AT_1 receptors over the cell body. In the freshly dissociated SMG neurons, angio II inhibits N- and L-type calcium currents at concentrations of 10 nM to 1 μ M (unpublished observation).

We already reported that collaterals of the sensory neurons regulate the excitability of SMG neurons through CGRP receptors and neurokinin receptors^{7,8,12,15)}. The origin of angio II may also be the collateral terminals of the sensory neurons. AT_1 receptors were demonstrated in the caudal nucleus of the spinal trigeminal tract in the medulla, suggesting an association with the terminals of trigeminal sensory neurons²⁾; the localization of AT_1 receptors in the superior salivatory nucleus in medulla and the preganglionic axon terminals remains unknown²⁾.

The AT_1 receptor can engage many second messenger systems *via* G-proteins, predominantly G_q , with activation of phospholipase C_β

leading to increased inositol triphosphate and diacylglycerol generation and subsequent increases in intracellular Ca^{2+} concentrations and protein kinase C activity²⁾. The AT_1 receptor may also mediate a decrease in adenylate cyclase activity *via* G_i , stimulate tyrosine phosphorylation of Jak2 (activating the Jak-STAT pathway), and activate the mitogen-activated protein kinase pathway, leading to increased *c-fos* transcription²⁾. In neuronal cultures, AT_1 receptor stimulation increases phospholipase C, intracellular calcium, protein kinase C, and the immediate early gene *c-fos*—an end result being increased transcription of the tyrosine hydroxylase gene²⁾. In this study, one cell responded with rhythmic hyperpolarizations during angio II-induced depolarization. This is surely evidence of AT_1 receptor signalling in the SMG neurons.

In a further study, we will investigate the effects of angio II on high voltage-gated calcium currents, M-type potassium current, and calcium-activated potassium current in acutely dissociated SMG cells.

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