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Short Communication

ENDOTHELIN-1-INDUCED DEPOLARIZATION AND HYPERPOLARIZATION IN SUBMANDIBULAR GANGLION NEURONS

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Abstract

The effects of endothelin-1 were studied *in vitro* on neurons in the hamster submandibular ganglion, using the intracellular microelectrode technique. Endothelin (1μM) caused a depolarization (5.5 ± 1.2 mV) followed by a hyperpolarization (8.5 ± 2.8 mV) of the membrane potential. Membrane conductance was increased during the endothelin-induced depolarization and was decreased during the endothelin-induced hyperpolarization. The endothelin-induced depolarization was depressed (mean 43.6%) in a Krebs solution containing zero calcium and high magnesium. The results suggested that the predominant component of the depolarization was mediated by calcium ions. The calcium-insensitive component of depolarization was carried by chloride ions. Endothelin-induced slow rhythmic hyperpolarizations were probably induced by a decrease in chloride ion conductance.

Keywords: Endothelin-1 — Parasympathetic ganglia — Depolarization — Calcium ion — Current clamp

INTRODUCTION

Endothelial cells produce endothelin-1, one of the most vasoconstricting agents known. Endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3) are all members of a family of similar 21-amino-acid polypeptides. Each endothelin is encoded by a different gene[1]. Endothelin-1 is not stored in secretory granules, and most regulatory factors alter the transcription of its gene, with changes in secretion occurring promptly thereafter[1].

Submandibular responses to stimulation of the chorda tympani nerve have been investigated in anesthetized sheep during and after an intracarotid infusion of endothelin, which reduced the blood flow through the gland[2,10]. Endothelin-1 induced *in vitro* biphasic responses on neurons in colonic parasympathetic ganglia of felines and vesical parasympathetic ganglia of rabbits[4,5]. It caused a membrane depolarization followed by a membrane hyperpolarization. Membrane conductance was increased during the endothelin-induced depolarization and

decreased during the endothelin-induced hyperpolarization[4,5].

We studied *in vitro* ET-1 responses of submandibular ganglion neurons, using the intracellular microelectrode recording technique.

MATERIALS AND METHODS

Experiments were conducted according to the guidelines for the treatment of experimental animals at Tokyo Dental College. An isolated submandibular ganglion was superfused continuously with oxygenated Krebs solution (30°C) at 2 ml/min. Intracellular recordings were conducted using glass microelectrodes filled with 3 M KCl (input resistance; 40–60 MΩ). Penetration of the glass microelectrode was achieved under visual control with Zeiss Nomarski optics (10× ocular; 25.8× objective, UD40). Endothelin-1 (ET-1) was applied at a concentration of 1 μM by pressure ejection from a pipette with a tip 3–5 μm in inner diameter. The micropipette was positioned 20–30 μm away from a target cell. Thirty pulses of air pressure (2 kg/cm^2, 50 msec) were delivered to the pipette at 1 Hz through a pressure ejection system. Cellular responses were recorded using a strip chart recorder. For measurement of the membrane input resistance of each ganglion neuron, hyperpolarizing currents (0.2–0.3 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 neuron was similarly changed by injection of direct currents (0.1–0.5 nA) using the balanced bridge circuit. The preganglionic nerve was stimulated through a suction electrode.

The ionic composition of Krebs solution was as follows (mM): 136 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 1.2 NaH_2PO_4 and 11 Glucose. A chloride channel blocker, 4-acetamide-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS; Sigma) was applied to neurons through perfusate. ET-1 was purchased from Peptide Institute Inc.

RESULTS

Nine neurons responded to ET-1. The parameters of these ET-1-induced membrane responses were as follows: resting membrane potential -61.5 ± 4.6 mV, amplitude of depolarization 5.5 ± 1.2 mV, duration of depolarization 3.2 ± 0.4 min, membrane input resistance (R_m) decrease in depolarization $19.4 \pm 3.3\%$, amplitude of hyperpolarization 8.5 ± 2.8 mV, duration of hyperpolarization 8.2 ± 0.9 min and R_m increase in hyperpolarization $32.8 \pm 6.1\%$ (mean ± S.E.). After endothelin-induced hyperpolarization, spontaneous rhythmic hyperpolarizations induced by activation of Ca^{2+}-activated K^+ channels were evoked[7].

In one neuron, ET-1 caused a membrane depolarization followed by slow rhythmic hyperpolarizations, about 6–8 mV in amplitude at intervals of about 9.6 min. R_m of slow rhythmic hyperpolarization increased by 85.7% at maximum (Fig. 1). The rhythmic hyperpolarization continued for 38 min. Subsequently, the membrane potential was stabilized at -80 mV.

In Ca^{2+}-free Krebs solution, the parameters of the ET-1-induced membrane responses were as follows: resting membrane potential -63.8 ± 1.5 mV, amplitude of depolarization 2.4 ± 0.2 mV (mean 43.6% of the total potential), duration of depolarization 2.5 ± 0.2 min (mean 78.1% of the total potential), R_m decrease in depolarization $17.8 \pm 5.4\%$, amplitude of hyperpolarization 21.8 ± 11.2 mV, duration of hyperpolarization 10.8 ± 1.7 min, R_m increase in hyperpolarization $76.3 \pm 30.2\%$ (mean ± S.E., $n = 4$). Addition of SITS (0.5 mM) to the Ca^{2+}-free solution completely inhibited the calcium-insensitive depolarization ($n = 4$). It is presumed that the endothelin-1-induced depolarization also involves activation of chloride channels.

DISCUSSION

Endothelin has been reported to have many functions in different tissues, including

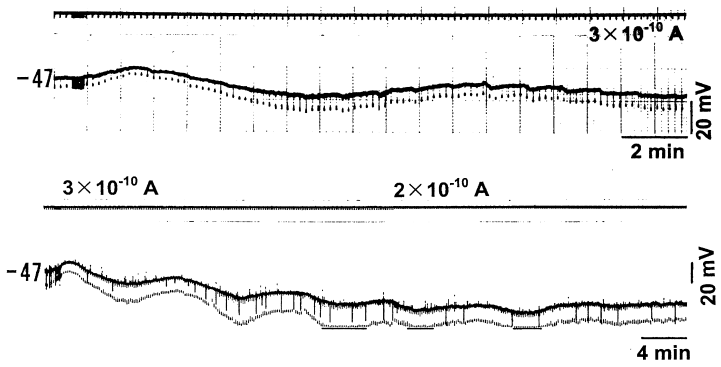

Fig. 1 Effects of endothelin-1 on submandibular ganglion neurons

Upper trace: ET-1 (1 μM) caused a depolarization followed by a hyperpolarization of the membrane potential. Membrane conductance was increased during ET-1-induced depolarization and decreased during ET-1-induced hyperpolarization. Resting $E_m = -47$ mV. Hyperpolarizing current pulses (0.3 nA) were applied for 50 msec at 0.1 Hz through the recoding electrode by means of a balanced bridge circuit. Thirty pulses of air pressure (2 kg/cm^2, 50 msec) were delivered to a pipette at 1 Hz through a pressure ejection system. After ET-1-induced hyperpolarization, spontaneous rhythmic hyperpolarizations induced by activation of Ca^{2+}-activated K^+ channels were evoked[7].

Lower trace: In one neuron, ET-1 caused a membrane depolarization followed by slow rhythmic hyperpolarizations, about 6–8 mV in amplitude at intervals of about 9.6 min. R_ms during them increased by 85.7% at a maximum. The rhythmic hyperpolarization continued for 38 min. Subsequently, the membrane potential was stabilized at -80 mV. Spontaneous rhythmic hyperpolarizations were recorded as vertical bars[7]. Underlines show current pulses for R_m measurement off the scale.

the central and peripheral nervous system[3,6]. Endothelin-1 is present at the dorsal root ganglion, the autonomic ganglion and the ventral column in the peripheral nervous system[1,4,5]. In submandibular ganglia *in vivo*, the origin of endothelin-1 is the trigeminal ganglion and afferent branches of the submandibular gland innervate the submandibular ganglia. This study investigated *in vitro* effects of endothelin-1 on submandibular ganglion neurons.

Our results suggest that endothelin-1-induced depolarization is primarily carried out by calcium ions. In Ca^{2+}-free Krebs solution, the amplitude of depolarization was 2.4 ± 0.2 mV and its duration was 2.5 ± 0.2 min. These small depolarizations were probably caused by chloride ions. A chloride channel blocker, SITS, completely eliminated the calcium-insensitive depolarization produced by endothelin-1. The equilibrium potential of the chloride ion is at -16 ± 3.3 mV (mean $\pm$ S.E.)[9]. However, slow rhythmic hyperpolarizations are probably induced by inactivation of calcium-sensitive chloride channels[8].

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