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Suppression and recovery of voltage-gated currents after cocaine treatments of olfactory receptor cells

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ABSTRACT

Objective: Cocaine (1–5% concentrations) is commonly used as a local anesthetic for the otorhinolaryngeal surgery of the nasal cavity. Recent reports indicate that some patients complain of olfactory deficits after surgery, and decreased olfaction is found in cocaine abusers. In spite of these reports, the effects of cocaine on the olfactory receptor cells are unknown.
Methods: Effect of cocaine was examined in olfactory receptor cells isolated from the newt. Under the voltage clamp with the whole-cell recording configuration, the voltage-gated currents were recorded when the membrane potential was depolarized from a holding potential of −100 mV in a step wise between −90 mV and +40 mV.
Results: When cocaine was applied by a puff pressure (5%) and the extracellular solution, the voltage-gated currents, including inward and outward components, were significantly reduced. The dose–suppression curves of cocaine for sodium and potassium currents could be fitted by the Hill equation. Half-blocking concentration of sodium and potassium currents were 43 μM and 557 μM; Hill coefficient was 1.1 and 0.9, respectively.
Conclusion: This rapid and complete recovery from the suppression was confirmed even after the treatments with the high concentration cocaine. This fact implies that cocaine does not affect olfactory ability after locally high dose treatments of nasal cavity in surgical operation.

1. Introduction

Cocaine produces anesthesia by inhibiting excitation of nerve endings or by blocking conduction in peripheral nerves. This is achieved by reversible binding to and inactivating sodium channels, thus inhibiting the action potential [1,2]. Local anesthetic for the otorhinolaryngeal surgery of the nasal cavity is commonly established with cocaine. To use cocaine in the nasal cavity, cotton pledgets are soaked in 1–5% cocaine solution in distilled water. It has been reported that some patients complain of olfactory deficits after the surgery [3], and decreased olfaction is found in cocaine abusers [4]. In spite of these reports, the effects of cocaine on olfactory receptor cells are unknown. Olfactory receptor cells, the primary sensory neuron performing the signal transduction in the olfactory system, are located on the olfactory epithelium in the nasal cavity. These cells are neurons with a dendrite facing the interior space of the nasal cavity and characterized by the possession of cilia playing a role in the transduction of odorous stimulation. Odorant binding to receptor proteins at the ciliary surface of the receptor cell activates enzymatic cascades [5,6] causing the opening of two types of ionic channels; cAMP-gated cationic channel and Ca^{2+}-gated Cl^- channel [7–9]. They cause a slow and graded voltage change, which is called receptor potentials [10]. A graded receptor potential initiates voltage-gated ionic currents. These currents are then encoded into spike trains that transmit olfactory information to the brain. It has been reported that newt olfactory receptor cells express various kinds of ionic currents: Na^+ current (I_{Na}), a T-type Ca^{2+} current ($I_{Ca,\ T}$), an L-type Ca^{2+}current ($I_{Ca,\ L}$), a delayed rectifier K^+ current (I_K) and a Ca^{2+}-activated K^+ current ($I_{K\ (Ca)}$) [11]. Similar currents have been observed in olfactory receptor cells from several species such as a catfish [12], coho salmon [13], xenopus [14], tiger salamander [15], mouse [16], and rat [17].

In the present study, we focused on the effects of cocaine on I_{Na} and I_K because the transient inward current such as I_{Na} is essential to initiate an action potential in olfactory receptor cells [12–17], and the late sustained outward current such as I_K contributed to a repolarization of an action potential [18]. In short, I_{Na} and I_K are not

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only the major components of inward and outward currents, respectively, but in the course of encoding olfactory information on the olfactory receptor cell; they also generate an action potential in the end. We also compared the feature of inhibition on cocaine with TTX [19], other local anesthetic [20] and high concentration of odorants [21].

Our experiments were performed under the whole-cell voltage clamp using isolated newt olfactory receptor cells. The present findings revealed the safety of cocaine usage in the nasal surgery by establishing whether the effects of cocaine are reversible or irreversible.

2. Materials and methods

2.1. Preparation

Olfactory receptor cells were dissociated enzymatically from the olfactory epithelium of the newt, *Cynops pyrrhogaster*. Dissociation protocols were similar to those reported previously [22]. In short, the animal was anaesthetized by cooling on ice, decapitated, and then pithed. The mucosae excised from the olfactory cavity were cut into small pieces and incubated for 5 min at 36 °C in a Ringer solution (in mM): 110 NaCl, 3.4 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 2 HEPES, 10 glucose, 1 pyruvic acid, and 0.03 phenol red (pH adjusted to 7.4 with NaOH) containing 0.1% collagenase (Sigma Chem. Co., St. Louis, MO) with no added Ca^{2+} and Mg^{2+}. The tissue was then rinsed and triturated with a normal Ringer solution. Isolated cells were placed on concanavalin A-coated dishes. Cells were maintained for 5 min at 4 °C before use. We conformed to the ethical treatment of animal study in Mie University.

2.2. Recording procedures

Membrane currents were recorded in a whole-cell recording configuration [23]. Pyrex tubing (1.2 mm o.d.) was pulled in three steps on a pipette puller (Sutter Instruments, USA, P-97). Residual capacitance was compensated electrically. The recording pipette was filled with pseudo-intracellular (K^+) solution (in mM): 119 KCl, 1 $CaCl_2$, 5 EGTA, 10 HEPES, and 0.03 phenol red (pH adjusted to 7.4 with KOH), or Cs^+ solution (in mM): 119 CsCl, 1 $CaCl_2$, 5 EGTA, 10 HEPES, and 0.03 phenol red (pH adjusted to 7.4 with CsOH). The resistance of the pipette was around 15 MΩ. For recording, the Petri dish was mounted on a stage of an inverted microscope with phase contrast optics (Axiovert 135; Zeiss, Germany). An indifferent electrode was an Ag–AgCl wire connected to the culture dish via an agarose bridge. A patch–clamp amplifier (Axopatch-200A; Axon Instruments Inc., Burlingame, CA) linked to an IBM-compatible PC, was used to measure membrane current. The membrane voltages were controlled by using the pCLAMP software (Molecular Devices). Current signals from the amplifier were monitored on PC display. Data were low-pass filtered (4-pole Bessel type) with a cut-off frequency of 5 kHz by an analog-to-digital interface (Digi data 1200; Molecular Devices). All experiments were performed at room temperature (22–25 °C).

2.3. Application of drugs

Cocaine that dissolved in Ringer's solutions for the membrane current analysis just before that application, was applied to the cell from grass pipette by pressure ejection (10 psi) or perfused continuously at a rate of 3–4 ml/min using a peristaltic pump. The recording chamber (Narishige, Japan) containing olfactory receptor cells had a solution reservoir of around 3 ml. To block I_K, $I_{K\,(Ca)}$ and I_{Ca}, we used K^+–Ca^{2+} block solution (in mM): 100 NaCl, 3 KCl, 3 HEPES, 10 glucose, 1 pyruvic acid, 20 tetraethylammonium (TEA), 3 $CoCl_2$, and 0.03 phenol red (pH adjusted to 7.4 with NaOH) and block I_{Na}, $I_{K\,(Ca)}$ and I_{Ca}, we used Na^+–Ca^{2+} block solution (in mM): 100 Choline chloride, 3 KCl, 3 HEPES, 10 glucose, 1 pyruvic acid, 1 $MgCl_2$, and 0.03 phenol red (pH adjusted to 7.4 with KOH). Applied cocaine by pressure ejection was 5% concentration in distilled water. One of these solutions was added to the extracellular perfusate to result in final, diluted concentrations of 0.001–10 mM, respectively. Cocaine solutions were protected from the light exposure to avoid degeneration. Choline chloride, NaCl, KCl, $CoCl_2$, $MgCl_2$, HEPES, glucose, pyruvic acid, phenol red and TEA were purchased from Nacalai Tesque Inc. (Kyoto, Japan), Wako Pure Chemical Industries Inc. (Osaka, Japan) and Sigma Chem. Co. (St. Louis, USA).

3. Results

3.1. Suppression of membrane currents by cocaine

Under the voltage clamp (holding potential, $V_h = -100$ mV), depolarizing step pulses induced time- and voltage-dependent currents (Fig. 1A). At step voltages between −40 mV and +40 mV, current responses consisted of a transient (~15 ms) inward current and a delayed outward current. With 5% cocaine, the initial transient inward current was almost completely blocked, and the late sustained outward current was reduced (Fig. 1B). Membrane currents measured after the washout of 5% cocaine were almost perfectly reversible (Fig. 1C). Similar phenomena were observed in all the cells recorded ($n = 6$). It is remarkable that the inward currents were almost abolished and outward one reduced. Fig. 2 illustrates current–voltage relationships of the inward (Fig. 2A) and outward (Fig. 2B) currents in the absence and the presence of 5% cocaine. In control, the transient inward current was activated at membrane potentials more positive than −40 mV, showing a peak value at −30 mV (Fig. 2A). These values are similar to those of I_{Na}. In the presence of 5% cocaine, the transient inward current was abolished in the entire voltage regions tested. After the washout of cocaine, the *I–V* relation for the inward component recovered almost completely.

The outward current started to activate at membrane voltages more positive than −40 mV (Fig. 2B). As the membrane potential was shifted to positive, the current amplitude increased monotonically. Application of 5% cocaine reduced the outward current, but the *I–V* curve still showed a slight outward rectification. It is interpreted that 5% cocaine blocks I_K that mediates the outward current.

3.2. Dose–suppression relation of cocaine on I_{Na} and I_K, and its recovery

It is highly likely that majority of the transient inward and the delayed outward currents observed in Figs. 1 and 2 were carried through the voltage-gated Na^+ and K^+ channels, respectively. In the present study, we specifically studied the effects of cocaine on I_{Na} and I_K.

To determine the effective concentration of cocaine on I_{Na} and I_K, we isolated I_{Na} and I_K by pharmacological agents, and the recorded cells were exposed to several concentrations of cocaine. As the concentration of cocaine was increased with time, the peak amplitude of I_{Na} and I_K was reduced (Fig. 3A-a and B-a). Fig. 3A-b ($n = 5$) and B-b ($n = 6$) illustrate the dose–suppression curve of cocaine. The data could be fitted by the Hill equation,

$$\frac{R}{R_0} = 1 - \frac{C^n}{(C^n + IC_{50}^n)} \quad (1)$$

where R_0 is the peak amplitude of membrane current without the cocaine, R is that in the presence of the cocaine, C is concentration