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A neuroactive steroid, allotetrahydrocorticosterone inhibits sensory nerves activation in guinea-pig airways

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Abstract

We examined the effects of a neuroactive steroid, allotetrahydrocorticosterone on the activation of capsaicin-sensitive afferent sensory nerves (C-fibers). Allotetrahydrocorticosterone (0.0001–1.0 μg/ml) dose-dependently inhibited electrical field stimulation-induced guinea-pig bronchial smooth muscle contraction, but not the substance P-induced contraction at 1.0 μg/ml. Allotetrahydrocorticosterone (0.01–1.0 μg/ml) also reduced the capsaicin-induced release of substance P-like immunoreactivity from guinea-pig airway tissues in a dose-dependent manner. The inhibitory effect of allotetrahydrocorticosterone on electrical field stimulation-induced bronchial contraction were reduced by the pretreatment of voltage-dependent K^+ channel blockers, tetraethylammonium (1 mM). This evidence suggests that allotetrahydrocorticosterone negatively modulate the activation of C-fibers and substance P release from their endings in airway tissues via the opening of voltage-dependent K^+ channels.

Keywords: Neuroactive steroid; C-fibers; Substance P; K^+ channels; Airway; Guinea-pig

1. Introduction

Several previous studies showed that neurogenic inflammation in the airway must have an important role in the pathogenesis of asthma (Barnes, 1996; Joos and Pauwels, 2001). By using tachykinin antagonists, Murai et al. (1992a,b) directly demonstrated that airway inflammation is generated by tachykinin released from capsaicin-sensitive afferent sensory nerves (C-fibers), which are stimulated by various types of irritants like cigarette smoke (Morimoto et al., 1992), cold air (Yoshihara et al., 1995) and hypertonic saline (Ono et al., 1999). These irritants activate C-fibers by opening non-selective cation channels with a high Ca^{2+} permeability (Caterina et al., 1997). Morimoto et al. (1996) have showed ω-conotoxin GVIA blocked the activation of C-fibers and the release of substance P from their endings, and suggested that the opening of N-type voltage-dependent Ca^{2+} channels might activate C-fibers in guinea-pig airway tissues.

Neuroactive steroids are synthesized de novo in the brain from cholesterol, primarily in oligodendrocytes (Baulieu and Robel, 1990). Biochemical, electrophysiological and behavioral evidences suggest a neuromodulatory role for neuroactive steroids in the central nervous systems, particularly a biomodel regulation of certain neurotransmitter receptors. The ability of certain neuroactive steroids to influence brain activity including the firing rate of neurons, induction of sedation, anesthesia, neurosecretion and behavioral changes is well known (McEwen, 1991; Majewska, 1992; Mensah-Nyagan et al., 1999). Ffrench-Mullen et al. (1994) examined the effect of neuroactive steroids on the Ca^{2+} channel currents in freshly dissociated pyramidal neurons from the adult guinea-pig hippocampal CA1 region and demonstrated that a neuroactive steroid,

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allotetrahydrocorticosterone primarily inhibits the ω-conotoxin GVIA-sensitive N-type Ca^{2+} channel current. In the peripheral nervous system, the steroid anesthetic alphaxalone rapidly and reversibly blocked sodium and potassium currents (Benoit et al., 1988). The 3α-hydroxy ring A-reduced metabolites of progesterone and deoxycorticosterone have been shown to potentiate γ-aminobutyric acid $(GABA)_A$-gated Cl^- current (Majewska et al., 1986). In contrast, dehydroepiandrosterone sulfate reversibly antagonizes the $GABA_A$-gated Cl^- current, acting as an allosteric antagonist of the $GABA_A$ receptor (Majewska et al., 1990). According to these reports, steroid hormones are known to influence nervous excitability.

In this report, we examined the effects of a neuroactive steroid, allotetrahydrocorticosterone on the activation of C-fibers in guinea-pig airway tissues and suggested it showed the inhibitory effects on airway inflammation induced by the activation of C-fibers via the opening of voltage-dependent K^+ channels.

2. Materials and methods

2.1. Animals

Twelve-week-old male Hartley guinea-pigs (270–330 g) were purchased from SLC (Hamamatsu, Japan) at least 1 week before the experiments. Animals were housed in a temperature- and humidity-controlled environment under a 12 h light/12 h dark cycle with light on at 7:00 a.m. Animals were allowed free access to food and water ad libitum.

2.2. Contractile response of isolated guinea-pig bronchi in vitro

The procedure of Fox et al. (1997) was used with certain modifications. Male Hartley guinea-pigs were killed and their bronchi were removed rapidly. A ring preparation with 4–5 mm length of the main bronchi (only one bronchi/ animal) was mounted in 5 ml organ baths filled with warmed (37 °C) and oxygenated (95% O_2, 5% CO_2) standard Tyrode's solution (pH 7.5) containing 137.0 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.05 mM $MgCl_2$, 0.4 mM $NaHPO_4$, 5.6 mM dextrose, 11.9 mM $NaHCO_3$ under a resting tension of 0.5 g. After a 60 min equilibration period, bronchi were stimulated by pre-established (data not shown) submaximal concentrations or stimuli of 10 nM substance P or electrical field stimulation in the presence of atropine, propranolol and phosphoramidon, respectively, 1 μM. Electrical field stimulation was delivered using two parallel platinum wires connected to a stimulator and square-wave pulses of supramaximal voltage (50 V) and 1 ms pulse duration were applied for 30 s every 30 min at a frequency of 10 Hz. After two reproducible responses were obtained (control response) and the tension of the preparation returned to basal levels by washing, the neuroactive steroid, allotetrahydrocorticosterone was added and, 10 min later, contraction was induced with the same stimuli of substance P or electrical field stimulation. The contractile response obtained in the presence of drug was compared with the control response. In separate experiments, a voltage-dependent K^+ channel blocker, tetraethylammonium were added 10 min before application of the agonist. Only one concentration of one agonist and/or antagonist was tested per bronchi preparation. All of the drugs employed in this study, did not affect the baseline tone.

2.3. Substance P release from guinea-pig airway tissues in vitro

The procedure of Ray et al. (1991) was used with certain modifications. Male Hartley guinea-pigs were killed and lungs were perfused (6 ml/min, 37 °C) with oxygenated (95% O_2, 5% CO_2) Krebs-Ringer HEPES buffer (pH 7.5) containing 138.0 mM NaCl, 5.6 mM KCl, 1.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM NaH_2PO_4, 11 mM $NaHCO_3$, 10 mM dextrose, 20 mM HEPES, 30 μM bacitracin, 1 μM phosphoramidon via a cannula that was inserted into the pulmonary artery through the right ventricle. The left atrium was opened to collect the outflow. Fifteen minutes after the start, perfusates from one period (15 min; i.e. 90 ml) were collected on ice in beakers containing hydrochloric acid to give a final concentration of 0.1 M. Each fraction was desalted on Sep-Pak C_{18} cartridges (Waters, Milford, MA) as described for somatostatin (Wu et al., 1983) and the peptides were concentrated to a final volume of 1 ml. The recovery from the Sep-Pak cartridge was more than 90% for radiolabeled substance P. Chemical irritation of tissues was achieved by perfusion with buffer containing 1 μM capsaicin for 5 min during the second collection period. Substance P-like immunoreactivity was measured by radioimmunoassay. The amount of substance P released by capsaicin was calculated by subtracting the level detected in the first period perfusate from that in the second period perfusate. Drugs were added in Krebs-Ringer HEPES buffer throughout the experiment. After the experiments finished, the lungs including trachea and bronchi, were dissected out and weighed. The increase of substance P-like immunoreactivity release was calculated in fmol per gram of tissues.

2.4. Materials

Substance P and phosphoramidon were purchased from Peptide Institute Inc. (Osaka, Japan). Bacitracin and allotetrahydrocorticosterone were obtained from Sigma Chemical Company (St. Louis, MO). Capsaicin and tetraethylammonium were from Nakalai Tesque Chemical Company (Kyoto, Japan). [^{125}I]Substance P (74 TBq/mmol) and anti-substance P anti-serum for the radioimmunoassay were purchased from Amersham Int. Ltd. (Buckinghamshire, U.K.). Capsaicin and allotetrahydrocorti costerone were dissolved in dimethylsulfoxide. Tetraethylammonium