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Acute actions of marine toxin latrunculin A on the electrophysiological properties of cultured dorsal root ganglion neurones

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Abstract

The effects of latrunculin A, isolated from the nudibranch *Chromodoris* sp., on the excitability of neonatal rat cultured dorsal root ganglion neurones were investigated using patch-clamp recording and Ca^{2+} imaging techniques. Under current-clamp conditions, acute application of latrunculin A (100 μM) reversibly induced multiple action potential firing and significantly increased action potential duration. No significant effects on action potential peak amplitude, threshold of action potential firing, resting membrane potential and input resistance were observed. Under voltage-clamp conditions, significant and dose-dependent suppression of K^+ current was seen with 10–100 μM latrunculin A. Additionally, a significant difference between inhibition of the current measured at the peak and the end of a 100 ms voltage step was seen with 100 μM latrunculin A. Fura-2 fluorescence Ca^{2+} imaging revealed that latrunculin A (100 μM) significantly inhibited Ca^{2+} transients evoked by KCl-induced depolarisation in all neurones. In 36% of DRG neurones, latrunculin A alone had no effect on intracellular Ca^{2+}. In 64% of neurones, latrunculin A alone evoked a transient rise in intracellular Ca^{2+}. Moreover, latrunculin A (10–100 μM) significantly inhibited the mean high voltage-activated Ca^{2+} current. The effects of latrunculin A on action potential firing and K^+ currents were attenuated by intracellular phalloidin, an indication that these effects are mediated through actin disruption.

Keywords: Action potential; Potassium current; Calcium current; *Chromodoris* sp.; Dorsal root ganglion; Latrunculin A; Phalloidin

1. Introduction

Dorid nudibranchs, or sea slugs, of the genus *Chromodoris* are brightly coloured and have few predators (Faulkner et al., 1990). They are known to sequester noxious metabolites from the sponges on which they feed and to concentrate these chemicals in their mantle tissue (Pika and Faulkner, 1995). These chemicals are then exuded from glands in the skin when nudibranchs are molested (McPhail and Davies-Coleman, 1997). Therefore, extracts from dorid nudibranchs are potentially rich sources of bioactive secondary metabolites (Faulkner, 2002). In the present study, latrunculin A (Fig. 1) was purified from a specimen of *Chromodoris* sp. collected from Indonesian waters. Separations and purifications were carried out using different column chromatographic techniques, including HPLC, and were guided by TLC and ^{1}H NMR to yield the known macrolide latrunculin A.

Latrunculin A is well known for its ability to rapidly (within 1 h), reversibly and specifically disrupt the actin cytoskeleton in mammalian cells in culture by promoting the formation of 1 : 1 molar complexes of latrunculin A with G-actin (Spector et al., 1983; Coué et al., 1987). Many ion channels and transporters are anchored in cell membranes by either direct or indirect association with the cytoskeleton (Harvey et al., 2000). Thus, altering the integrity of cytoskeletal elements, in particular actin filaments, can modulate the activity of a variety of ion channels (Janmey, 1998) and receptors (Wang et al., 1999). For example, disruption of actin filaments with cytochalasin results in activation of ATP-sensitive K^+ (K_{ATP}) channels in cardiac myocytes (Terzic and Kurachi, 1996).

In this study, the acute effects of latrunculin A on action potential configuration and K^+- and Ca^{2+}-conductances in neonatal rat cultured dorsal root ganglion (DRG) sensory neurones were investigated. DRG neurones in culture can share

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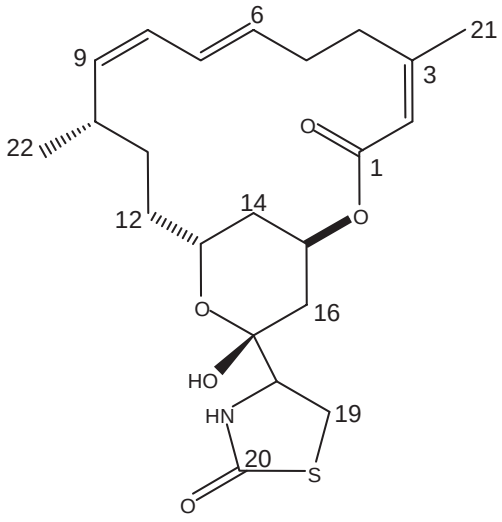

Fig. 1. Structure of latrunculin A.

characteristics with nociceptors in vivo and are frequently used to investigate the ion channels that underlie the transduction of noxious stimuli into electrical activity (Passmore, 2005) and the signal conduction and transmission during sensory processing. The wide variety of receptors and ion channels expressed on DRG neurones make them an ideal model for this study. Furthermore, the actions of latrunculin A on DRG neurones may shed light on modulation of the primary neurones in the somatosensory pathway.

2. Materials and methods

2.1. General experimental procedures

NMR experiments were recorded in DMSO-d_6 on a Varian Unity INOVA spectrophotometer operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. A low-resolution electrospray mass spectrum was obtained using a Waters ZQ4000, and high-resolution mass data were obtained on a Finnigan MAT 900 XLT. HPLC separations were carried out on a Phenomenex® column (10 × 250 mm, RP-C18, 5 μm particle size) connected to an Agilent 1100 series binary pump and monitored using an Agilent photodiode array detector. Detection was carried out at 208, 254 and 330 nm.

2.2. Isolation and structure determination

The specimen of *Chromodoris* sp. (order Nudibranchia, suborder Doridina, family Chromodorididae) was collected in November 1996 at a depth of 8 m from the reefs of North Sumatra near Bitung, Indonesia (1°33.055′ S; 125°17.507′ E). The sample was identified by Prof. Marcel Jaspars and a voucher specimen has been deposited in the Chemistry Department, University of Aberdeen under the registration no. 96304.

The sample was preserved by immersion in a mixture of EtOH/seawater (1:1) for 24 h after which the liquid was decanted and discarded and the damp specimen was transported back to Aberdeen at ambient temperature. The specimen was extracted with MeOH for 24 h (3×) and CH_2Cl_2 for 24 h (3×), and the concentrated extracts were combined and stored at −4 °C.

Desalting of the extract was carried out using a modification of the method described by West et al. (2000). The extract (1.6 g) was dissolved in the least amount (300 mL) of MeOH/H_2O (1:1) and passed through a column of DIAION HP20ss (25 × 1.5 cm) pre-equilibrated with MeOH/H_2O (1:1). The eluent was diluted with H_2O (500 mL) and passed back through the column. Finally, the eluent was diluted with H_2O (2 L) and passed back through the same column. The column was then washed with H_2O (1 L) and eluted with MeOH 100% (0.5 L). The methanolic eluent was concentrated under reduced pressure and loaded on a Sephadex LH-20 column equilibrated with CH_2Cl_2/MeOH (1:1). Two fractions were collected. Fraction 2 gave interesting ^{1}H and ^{13}C NMR spectra. This fraction was purified by reversed-phase HPLC using a mixture of MeOH and water (80:20) as eluant to yield 14 mg of latrunculin A.

2.3. Culture conditions

Primary cultures of DRG neurones were prepared following enzymatic dissociation (0.125% collagenase for 13 min and 0.25% trypsin for 6 min) and mechanical trituration of dorsal root ganglia from decapitated 2-day old Sprague–Dawley rats. The sensory neurones were plated on to laminin–polyornithine-coated coverslips and bathed in F14 culture medium (Imperial Laboratories, Andover, England) supplemented with 10% horse serum (Gibco, Paisley, Scotland), penicillin (5000 IU mL^{-1}), streptomycin (5000 μg mL^{-1}), $NaHCO_3$ (14 mM) and nerve growth factor (NGF; 20 ng mL^{-1}; Sigma, Poole, England). The cultures were incubated for up to 5 days at 37 °C in a humidified air containing 5% CO_2 and all electrophysiological and Ca^{2+} imaging recordings were made within this period.

2.4. Electrophysiology

The whole cell patch-clamp recording method was used to investigate the actions of latrunculin A (0.1, 1, 5, 10, 50 and 100 μM) on action potential firing and voltage-activated K^+ and Ca^{2+} currents. Basic electrophysiological properties and K^+ currents from DRG neurone cultures were studied using borosilicate glass patch pipettes (resistances 3–10 MΩ) filled with a solution containing in mM: KCl, 140; EGTA, 5; $CaCl_2$, 0.1; $MgCl_2$, 2; ATP, 2; HEPES, 10 and Tris to adjust the pH to 7.2. The osmolarity of the patch pipette solution was brought to 310–320 mOsm L^{-1} with sucrose. The extracellular NaCl-based solution used contained in mM: NaCl, 130; KCl, 3; $CaCl_2$, 2; $MgCl_2$, 0.6; $NaHCO_3$, 1; glucose, 5; HEPES, 10 and NaOH to adjust the pH to 7.4. This solution also contained 0.1% DMSO which was used as a solvent carrier for the natural product. The osmolarity of this extracellular bathing solution was brought to 320 mOsm L^{-1} with sucrose. Ca^{2+} currents are activated over the same voltage range as K^+ and Na^+ voltage-activated currents, so a variety of strategies are required to unmask Ca^{2+} currents (Dolphin et al., 1986). To isolate Ca^{2+} currents from other contaminating conductances, CsCl-based