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Effects of PFOS and PFOA on L-type Ca^{2+} currents in guinea-pig ventricular myocytes

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Abstract

Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are amphiphiles found ubiquitously in the environment, including wildlife and humans, and are known to have toxic effects on physiological functions of various tissues. We investigated the effects of PFOS and PFOA on action potentials and L-type Ca^{2+} currents, I_{CaL}, in isolated guinea-pig ventricular myocytes using whole-cell patch-clamp recording. In current-clamp experiments, PFOS significantly decreased the rate of spike, action potential duration, and peak potential at doses over 10 μM. In voltage-clamp experiments, PFOS increased the voltage-activated peak amplitude of I_{CaL}, and shifted the half-activation and inactivation voltages of I_{CaL} to hyperpolarization. PFOA had similar effects PFOS, but showed significantly lower potency. These findings are consistent with previous observations for anionic *n*-alkyl surfactants, suggesting that PFOS and PFOA may change membrane surface potential, thereby eliciting general effects on calcium channels. These findings provide further insights into the mechanisms of PFOA and PFOS toxicities.

Keywords: Perfluorooctane sulfonate; Perfluorooctanoate; L-type calcium channel; Guinea pig; Ventricular myocyte; Negatively charged amphiphile

Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are a class of specialty chemicals that are used in a variety of products, such as lubricants, paints, cosmetics, and fire-fighting foams. Recently, these chemicals were found ubiquitously in the environment, including humans [1–8]. The worldwide distribution of these chemicals has been attributed to their resistance to degradation by ecological systems and their bioconcentration [9,10].

PFOS and PFOA have been reported to have toxic in vivo effects on peroxisomal proliferation, endocrine function, carcinogenicity, development, and reproduction [11–14]. Regarding their carcinogenicity, PFOS has been reported to inhibit gap junctional intercellular communication both in vitro and in vivo [15,16].

The amphiphilic natures of PFOS and PFOA suggest that their effects could be primarily associated with cell membrane functions. In addition to its inhibition of gap junctional intercellular communication, PFOS has been reported to increase the permeability of cell membranes to hydrophobic ligands [17–19]. For example, it increases membrane fluidity in fish leukocytes and slightly increases the intrinsic proton leakage of the mitochondrial inner membrane, which resemble surfactant-like changes in membrane fluidity. Furthermore, it is able to increase the non-selective permeability of mitochondrial membranes. However, the physiological alterations to the cellular membranes have not yet been implicated in specific effects on cellular functions.

Previously, alkyl anionic surfactants, *n*-octyl sulfate and *n*-dodecyl sulfate, were reported to change the surface charge and modulate voltage-dependent ion channels in a variety of preparations, including sodium

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channels from rat dorsal root ganglion cells [20], mammalian cardiac myocytes [21], and squid giant axon [22], as well as calcium channels from rabbit cardiac myocytes [23] and cardiac myocyte delayed rectifier potassium channels [21]. It is therefore possible that perfluorinated amphiphiles have similar pharmacological actions on ion channels. If so, the various effects of PFOA and PFOS may be explainable by their effects on ion channels. Here, we investigated the effects of PFOS and PFOA on L-type calcium currents (I_{CaL}) in isolated guinea-pig ventricular myocytes using a whole-cell patch-clamp recording technique.

Materials and methods

Drugs. Heptadecafluorooctane sulfonic acid potassium salt (FW.538.22), used as a standard for PFOS, and pentadecafluorooctanoic acid ammonium salt (FW.431.10), used as a standard for PFOA, were purchased from Fluka (Milwaukee, WI). The purities of these standards were greater than 98%. The test chemicals were analyzed for, and shown not to contain, fluoride, sodium, and aluminum (Shimadzu Analytical & Measuring Center, Japan). The test chemicals were dissolved in dimethyl sulfoxide as 10 mM stock solutions. Prior to experiments, the stock solutions were diluted in an external solution to obtain the concentrations described in the text.

Cell isolation. Ventricular cells were isolated from the hearts of guinea pigs (300–400 g body weights) using an enzymatic dissociation technique [24,25]. Briefly, guinea pigs were anesthetized with pentobarbital (50 mg kg^{-1}) and the chest was opened under artificial respiration. The heart was dissected out and the ascending aorta was cannulated to start coronary perfusion with normal Tyrode solution. After perfusion of 100 ml Ca^{2+}-free Tyrode solution, the perfusate was switched to a Ca^{2+}-free Tyrode solution containing 0.02% collagenase (Wako Pure Chemicals, Osaka, Japan), and the heart was digested for about 30 min. Next, the heart was rinsed with a high K^+/low Cl^- storage solution. The left ventricle was then dissected from the digested heart and stored in the storage solution at 4 °C for later use.

The protocols for isolating ventricular cells were approved by the Animal Committee of the Akita University School of Medicine, and the 'Guidelines for Animal Experimentation' of the University were completely adhered to in all subsequent experiments.

Solutions. Normal Tyrode solution contained (in mM): NaCl, 136.9; KCl, 5.4; $MgCl_2$, 0.53; $CaCl_2$, 1.8; NaH_2PO_4, 0.33; glucose, 5.5; and Hepes, 5.0 (adjusted to pH 7.4 with NaOH). Na^+/K^+-free Tyrode solution contained (in mM): Tris–HCl, 140; $MgCl_2$, 1.0; $CaCl_2$, 2.5; glucose, 10; and Hepes, 5 (adjusted to pH 7.4 with Tris-base).

High K^+/low Cl^- storage solution contained (in mM): potassium glutamate, 70; KCl, 20; KH_2PO_4, 10; $MgCl_2$, 1.0; taurine, 20; glucose, 10; and EGTA, 0.3 (adjusted to pH 7.2 with KOH).

The pipette solution for the conventional whole-cell clamp experiments contained (in mM): KOH, 150; HCl, 30; NaCl, 10; $CaCl_2$, 2; EGTA, 5; Na_2ATP, 5; Na_2GTP, 0.1; $MgCl_2$, 5; and Hepes, 5.0 (adjusted to pH 7.2 with aspartic acid). The free concentrations of Ca^{2+} and Mg^{2+} were calculated to be 0.1 μM and 0.55 mM, respectively [26]. The pipette solution used for recording I_{CaL} was a Cs^+-rich solution that contained (in mM): CsOH, 110; TEA-Cl, 30; EGTA, 10; MgATP, 5; Na_2GTP, 0.1; and Hepes, 5.0 (adjusted to pH 7.2 with aspartic acid). The pipette solution for perforated patch-clamp experiments contained (in mM): K-aspartate, 110; KCl, 30; NaCl, 10; $CaCl_2$, 1.0; and Hepes, 5.0 (pH 7.2 with KOH). Amphotericin B was dissolved in dimethyl sulfoxide as a stock solution (400 mg/ml; Sigma) and added to the pipette solution to give a final concentration of 200 μg/ml just before use.

Electrophysiological experiments. A drop of cell suspension was added to the recording chamber (0.5 ml in volume) filled with normal Tyrode solution. After the cells had settled on the floor of the chamber, they were perfused with normal Tyrode solution at 2–3 ml/min. Experiments were performed at 36–37 °C on quiescent single cells that had clear sarcomere striations.

The whole-cell patch-clamp technique was used for electrical recording from single cells with amphotericin-permeabilized or ruptured patches [27,28]. The currents were recorded with a patch-clamp amplifier (List EPC-7; Darmstadt, Germany). An agar–Tyrode–Ag–AgCl bridge was used as an indifferent electrode. Patch pipettes were pulled from glass capillaries (Corning #7052;Warner Instrument, CT, USA) using a micropipette puller (Model P-97; Sutter Instrument, Novato, CA, USA), and the electrode resistance ranged 3–5 MΩ when filled with the internal solution. For voltage-clamp recording, the series resistance was electronically compensated (>80%) and the current signal was filtered by a low-pass Bessel filter with a cut-off frequency of 2 kHz. Data were digitized at 5 kHz using a Digidata 1200A A/D converter (Axon Instruments) and stored on a computer for later analysis using the pCLAMP version 6.0 software (Axon Instruments) [28].

The results are expressed as means ± SEM (n, number of cells). Comparisons between two groups were performed using Student's paired or unpaired t test and values of $P < 0.05$ were considered statistically significant.

Results

Effects of PFOS on action potentials

Membrane potentials were measured using an amphotericin-perforated whole-cell current-clamp. Fig. 1A shows a typical recording of the membrane potential of a ventricular myocyte before and during exposure to PFOS. The baseline indicates the resting membrane potential. PFOS decreased the rate of rise of spike and the peak potential, and shortened the action potential in a dose-dependent manner. The action potential parameters are summarized in Figs. 1B–E. At the highest concentration of 100 μM, the action potential was completely inhibited. All these effects were reversible when the PFOS was washed out. The resting potential remained unchanged at all the concentrations evaluated.

Effects of PFOS on whole-cell currents of guinea-pig ventricular cells

Under conventional whole-cell voltage-clamp conditions using the standard internal solution and normal Tyrode solution, square pulses of 500 ms duration were applied from a holding potential of −40 mV to various test potentials in control cells and cells exposed to 10 μM PFOS (Fig. 2). The current–voltage (I–V) relationships measured at the initial peak (circle) and at the end (full circle) of the pulses are shown in Fig. 2B. Exposure of cells to PFOS markedly decreased the inward peak of the current at test potentials more positive than −20 mV. In addition, the outward current at the end of 300 ms test pulses appeared to be increased, albeit