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Nonylphenol, an environmental estrogen, affects voltage-gated K^+ currents and L-type Ca^{2+} currents in a non-monotonic manner in GH_3 pituitary cells

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HIGHLIGHTS

- The effect of nonylphenol (NP) was studied in GH_3 cells with patch-clamp technique.
- Electrophysiological effects of NP on I_{Kv} and $I_{Ca\text{-}L}$ were obtained for the first time.
- NP could affect on I_{Kv} and $I_{Ca\text{-}L}$ at very low concentrations (10^{-15}–10^{-9} M).
- The dose-dependent effect of NP on I_{Kv} and $I_{Ca\text{-}L}$ is in a nonmonotonic manner.

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ABSTRACT

We have investigated the characteristics of voltage-gated K^+ channels and L-type Ca^{2+} channels in GH_3 rat pituitary cells and the effects of the xenoestrogen (XEs) nonylphenol (NP) on these ion channel currents. Our results have shown that the lower concentrations (10^{-15}–10^{-14} M) of NP decreased the amplitudes of voltage-gated K^+ currents (I_{Kv}) and activated L-type Ca^{2+} currents ($I_{Ca\text{-}L}$) by reducing half-activation membrane potentials of activation kinetics curves. However, the higher concentrations (10^{-10}–10^{-9} M) of NP increased the amplitudes of I_{Kv} and inhibited $I_{Ca\text{-}L}$ by reducing the peak values of $I_{Ca\text{-}L}$. Thus, NP affects I_{Kv} and $I_{Ca\text{-}L}$ in an opposite and non-monotonic manner.

1. Introduction

The GH3 cell line is derived from pituitary tumor tissue and contains mammosomatotroph cells that secret both prolactin and growth hormone (Porter et al., 1990). Because pituitary cells are excitable cells (Douglas and Taraskevich, 1978), a variety of ion channels exist in the cell membrane, leading to the depolarization and repolarization of cell membrane (Charles et al., 1999) and the generation of action potentials. Thus, the GH_3 cell is widely used for the study of ion channels' properties and their hormone-related regulations.

Environmental endocrine disrupting chemicals (EDC_S), otherwise referred to as environmental estrogens or xenoestrogens (XEs) are compounds that can inappropriately mimic many estrogenic processes and then disrupt normal endocrine function (Singleton and Khan, 2003). Examples of such disruptions include reproductive failure, feminization of male animal populations, reproductive tract malformations, endometriosis, and breast and ovarian cancers (Gotz et al., 2001; Kloas et al., 1999; Mathur et al., 2002). EDCs can be found in pesticides, flexible plastics, flame retardants, various cosmetics and numerous other products (Aksglaede et al., 2006; Singleton and Khan, 2003), and many of them are already very prevalent in the environment. Nonylphenol (NP) is one of the widely found XEs. Although it is not a very close structural mimic of estradiol, NP is considered to be an endocrine disruptor due to its ability to mimic estrogens functionally. NP is used in industrial and consumer surfactants as a detergent, in plastics as a plasticizer and in polystyrene as an antioxidant. It is reported that NP can be detected in various foods; a major route of exposure to NP is considered to be diet (Guenther et al., 2002). In Germany, New Zealand, and Taiwan, the daily adult intake of NP was estimated to be 7.5 μg/d (Guenther et al., 2002), 3.3 μg/d (Thomson et al., 2003), and 28 μg/d (Lu et al., 2007), respectively. Calafat et al. (2005) reported that NP could be detected in 51% of the urine samples examined, with a median concentration of approximately

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0.1 μg/L (about 10^{-9} M) estimated. Thus, NP is present at high enough concentrations that it may threaten the normal physiology of development and adult life.

Most previous studies have investigated the possible actions of estrogens through nuclear estrogen receptors (ERs) modifying gene activity (Bukovsky et al., 2003; Markey et al., 2005). In addition to the classical genomic pathway, it has been found that estrogens can produce rapid non-genomic signaling effects through many types of second messenger systems, such as Ca^{2+}, K^+, cAMP, and nitric oxide level changes, activation of G protein-mediated events, and activation of extracellular-regulated kinases (ERKs), phosphoinositide-3 kinase (PI3K), and Jun kinase (JNK) (Doolan and Harvey, 2003). Furthermore, it is reported that 17-β estradiol rapidly reduced I_{Kv} in preoptic neurons (Druzin et al., 2011), and that acute estradiol administration enhanced inward Na^+ and attenuated outward K^+ currents of neurons in rat hypothalamic ventromedial nucleus, leading to an increase in neuronal excitability (Kow et al., 2006). It is also reported that in the pituitary tumor cell subline GH3/B6/F10, XEs induced intracellular Ca^{2+} changes via voltage-gated L-type Ca^{2+} channels on the plasma membrane because none of the Ca^{2+} fluxes came from intracellular Ca^{2+} stores (Wozniak et al., 2005). Thus, L-type Ca^{2+} channels, or other ion channels, are likely to be involved in non-genomic mechanisms of XEs' actions.

In present study, by using the patch clamp technique, we recorded I_{Kv} and $I_{Ca\text{-}L}$ in GH3 cells to determine the characteristics of currents, and then we investigated the effects of NP on them. It has been shown that picomolar to nanomolar concentrations of both E_2 and XEs caused intracellular Ca^{2+} changes within 30 s of administration in the pituitary tumor cell subline GH3/B6/F10 (Wozniak et al., 2005). So we investigated the effects of NP on ion channel currents in low concentrations (10^{-15}–10^{-9} M) with relevance to many environmental exposures.

2. Materials and methods

2.1. Drugs and solutions

Phenol red-free Dulbecco modified eagle medium (DMEM), fetal calf serum, horse serum, and insulin–transferrin–selenium solution (ITS) were obtained from Gibco (Grand Island, NY); NP was obtained from Tokyo Chemical Industry (Japan); tetrodotoxin (TTX) was obtained from the Research Institute of the Aquatic Products of Hebei (China); Bay K8644, 4-aminopyridine (4-AP) and tetraethylammonium chloride (TEA-Cl) were obtained from Sigma (USA). NP, TTX, 4-AP and TEA were made up in DMSO, and then diluted to appropriate concentrations with experimental solutions. The final containing of DMSO is less than 0.1% in volume.

2.2. Cell culture

GH_3 cells were cultured in Phenol red-free DMEM medium supplemented with 2% sodium bicarbonate, 0.3% glutamine, 10% fetal calf serum, and 5% horse serum in a humidified environment of 5% CO_2 and 95% O_2 at 37 °C. After reaching 80% confluence, cells were plated onto cover slips in 3.5 cm culture dishes at a density of 30,000 cells/dish substituting DMEM medium containing 5 μg/ml insulin and transferrin, 5 ng/ml selenium, 0.1% BSA, 20 nM sodium pyruvate, and 25 mM HEPES (DMEM/ITS). The cover slips were then placed in culture dishes containing serum-free DMEM/ITS medium for 24–48 h prior to electrophysiological experiments. Experiments were performed on single cells.

2.3. Electrophysiological recording

The extracellular solutions used for whole-cell recordings of I_{Kv} contained (in mM): NaCl 140, KCl 5, HEPES 10, glucose 10, $CdCl_2$ 5, TTX 0.0005, buffered to pH 7.4 with NaOH. The pipette solution contained (in mM): KCl 130, NaCl 10, HEPES 10, buffered to pH 7.2 with KOH. The extracellular solutions used for whole-cell recordings of $I_{Ca\text{-}L}$ contained (in mM): NaCl 130, HEPES 5, glucose 5, CsCl 5.4, TTX 0.0005, $BaCl_2$ 20, Bay K8644 0.003, buffered to pH 7.4 with NaOH. The pipette solution contained (in mM): CsCl 130, NaCl 10, HEPES 10, buffered to pH 7.2 with CsOH.

The cover slip piece covered with the cells was placed in the chamber above the inverted microscope and perfused with corresponding extracellular solution at room temperature (20–22 °C). The patch pipettes were prepared from capillary tube using a two-step electrode puller (P-97, Sutter Instruments, USA). The electrode resistance is 2–5 MΩ after filling with the pipette solution. Data were recorded with a patch clamp amplifier (200B, Axon Instruments, USA) controlled by pClamp10.0 software (Axon Instruments, USA). Data filtered at 1 kHz were fed to a computer at a sampling rate of 5 kHz. Cells were not accepted for recording if the initial seal resistance was <2 GΩ. Solutions were applied by a gravity pressure perfusion system at a rate of approximately 2 ml/min. Steady-state activation kinetics was evaluated by fitting the peak conductance–voltage relationship to a Boltzmann equation:

$$G_{max}(a) = \frac{1}{1 + \exp(V_{1/2} - V_m)/s}$$

where V_m was the test potential, $V_{1/2}$ was the membrane potential at which half of the channels were activated, and s is the slope factor of the curve.

2.4. Statistical analysis

Electrophysiological data were analyzed using pClamp 10.0 and Origin 8.0 software. Results were expressed as means ± S.E. and n indicated the number of cells. Student's t-test was used to compare the experimental groups treated with NP to the control group. $P < 0.05$ was considered statistically significant.

3. Results

3.1. The effects of NP on I_{Kv} in GH_3 cells

3.1.1. The characteristics of I_{Kv} in GH_3 cells

The voltage-gated outward K^+ currents (I_{Kv}) were evoked by voltage steps from a holding potential of −80 mV to command potentials indicated in increments of 10 mV. As shown in Fig. 1A, the I_{Kv} consisted of a rapidly activating and inactivating transient outward K^+ current (I_{to}) and a delayed rectifier K^+ current (I_{KD}) (Fig. 1A). The initial part of the current was mainly I_{to}, and the end of the current was mainly I_{KD}. After the I_{Kv} was recorded, the cell was perfused with an extracellular solution supplemented with 4-AP, both the initial part and the later part of the currents decreased (Fig. 1B), indicating that that 4-AP-sensitive currents included both I_{to} and I_{KD}. Subsequently, the cell was then perfused with an extracellular solution supplemented with both 4-AP and TEA, the currents almost disappeared (Fig. 1C). The currents measured as the initial peak (open symbols) and at the end (filled symbols) of the stimulus pulse in the three different conditions are illustrated in Fig. 1D. The 4-AP and TEA sensitive components at the end of the currents were then subtracted from the total currents respectively and illustrated in Fig. 4E. These data showed that all outward K^+ currents were 4-AP and TEA-sensitive. In the following experiment, we observed the I_{Kv} measured at the end of the currents.

3.1.2. NP affected I_{Kv} in a non-monotonic manner

We tested a wide range of NP concentrations (10^{-15}–10^{-9} M) on the I_{Kv}. The lower NP concentrations (10^{-15}–10^{-14} M) decreased the amplitudes of I_{Kv} from 11.35 ± 1.72 pA/pF to 8.63 ± 1.21 pA/pF and 10.34 ± 2.58 pA/pF at 70 mV, respectively (Fig. 2A and C). However, the higher NP concentrations (10^{-13}–10^{-9} M) increased the amplitudes of I_{Kv} from 11.35 ± 1.72 pA/pF to 15.40 ± 2.75 pA/pF, 19.90 ± 4.14 pA/pF, 22.44 ± 4.34 pA/pF, 15.05 ± 2.91 pA/pF, 12.91 ± 2.23 pA/pF at 70 mV, respectively (Fig. 2B and C). Therefore the effect of NP on I_{Kv} was oppositely dependent upon the concentration tested. The biggest increase of the currents was 197%, which is achieved at 10^{-11} M; while the biggest decrease was about 24%, at 10^{-15} M. These results suggested that NP affected I_{Kv} in a non-monotonic dose-dependent manner.

3.2. The effects of NP on $I_{Ca\text{-}L}$ in GH_3 cells

3.2.1. The characteristics of $I_{Ca\text{-}L}$ in GH_3 cells

We recorded $I_{Ca\text{-}L}$ with the pipette solution containing CsCl to block the K^+ currents, and the extracellular solution containing TTX to block the Na^+ currents. Initially $I_{Ca\text{-}L}$ was very small (less than 50 pA) (Fig. 3A). Therefore, we then added Bay K8644 and $BaCl_2$