Polycyclic Aromatic Hydrocarbons Phenanthrene and Retene Modify the Action Potential via Multiple Ion Currents in Rainbow Trout *Oncorhynchus mykiss* Cardiac Myocytes

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Abstract: Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in aqueous environments. They affect cardiovascular development and function in fishes. The 3-ring PAH phenanthrene has recently been shown to impair cardiac excitation–contraction coupling by inhibiting Ca$^{2+}$ and K$^{+}$ currents in marine warm-water scombrid fishes. To see if similar events take place in a boreal freshwater fish, we studied whether the PAHs phenanthrene and retene (an alkylated phenanthrene) modify the action potential (AP) via effects on Na$^{+}$ ($I_{\text{Na}}$), Ca$^{2+}$ ($I_{\text{CaL}}$), or K$^{+}$ ($I_{\text{K}}$, $I_{\text{K1}}$) currents in the ventricular myocytes of the rainbow trout (*Oncorhynchus mykiss*) heart. Electrophysiological characteristics of myocytes were measured using whole-cell patch clamp. Micromolar concentrations of phenanthrene and retene modiﬁed the shape of the ventricular AP, and retene profoundly shortened the AP at low micromolar concentrations. Both PAHs increased $I_{\text{Na}}$ and reduced $I_{\text{CaL}}$ and $I_{\text{K}}$, but retene was more potent. Neither of the PAHs had an effect on $I_{\text{K1}}$. Our results show that phenanthrene and retene affect cardiac function in rainbow trout by a mechanism that involves multiple cardiac ion channels, and the final outcome of these changes (shortening of AP) is opposite to that observed in scombrid fishes (prolongation of AP). The results also show that retene and aryl hydrocarbon receptor (AhR) agonist have an additional mechanism of toxicity besides the previously known AhR-mediated, transcription-dependent one. Environ Toxicol Chem 2019;38:2145–2153. © 2019 SETAC

Keywords: Aquatic toxicology; Cardiotoxicity; Mode of action; Polycyclic aromatic hydrocarbons

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants that occur as complex mixtures in aquatic environments. They originate from petrogenic or pyrogenic sources, and they enter the waters via atmospheric deposition, oil accidents, municipal and industrial efﬂuents, and urban runoff. Individual PAHs as well as PAH mixtures (such as oil) affect the development and function of the heart in several fish species (Billiard et al. 1999; Incardona et al. 2004, 2006, 2009, 2011, 2014; Dubansky et al. 2013; Brette et al. 2017; Raine et al. 2017).

Phenanthrene and retene (1-methyl-7-isopropyl phenanthrene) are 3-ring PAHs. Phenanthrene is common in both petrogenic and pyrogenic mixtures of PAHs, and it causes reversible bradycardia and atrioventricular conduction block in zebrafish (*Danio rerio*) and a slight increase in heart rate and reduction of circulation in marine medaka (*Oryzias melastigma*; Incardona et al. 2004; Mu et al. 2014; Sun et al. 2015; Cypher et al. 2017). In Pacific bluefin tuna (*Thunnis orientalis*), phenanthrene affects the cardiac action potential (AP) and ion currents (Brette et al. 2017). Retene is an alkylated phenanthrene, and it has been found in sediments downstream from pulp and paper mills, in landﬁlls, and in oil sand–produced water (Leppanen and Oikari 1999a, 1999b; Legler et al. 2011; Cheng et al. 2018). Retene is an aryl hydrocarbon receptor (AhR) agonist, and it activates the AhR and causes changes in the transcription of several genes, leading to developmental defects in the cardiovascular system (Billiard et al. 1999; Scott et al. 2011; Vehniäinen et al. 2016).

Contraction of the vertebrate heart is triggered by cardiac AP, which originates from the primary pacemaker center at the border zone between the sinus venosus and the atrium (Yamauchi and Burnstock 1968; Haverinen and Vornanen 2007). From there AP spreads throughout the atrium and via the atrioventricular canal further to the ventricular wall, thereby triggering sequential contractions of atrium and ventricle.
Aquaria at a rate of 150 to 200 L/04.10.07.

...ion with compressed air. Groundwater (average pH 8.0,\textsuperscript{(Sedmera et al. 2003). Cardiac AP is generated by the complex interaction between several voltage-gated ion currents in the sarcolemma of cardiac myocytes. In fish ventricular myocytes, there are 2 major inward currents, the fast Na\textsuperscript{+} current (I\textsubscript{Na}) and L-type Ca\textsuperscript{2+} current (IC\textsubscript{aL}; long-lasting), and 2 major outward K\textsuperscript{+} currents, the fast component of the delayed rectifier K\textsuperscript{+} current (IK\textsubscript{r}) and the background inward rectifier K\textsuperscript{+} current (IK\textsubscript{1}; Vornanen 2016). Besides these major ion currents, fish ventricular myocytes may have T-type Ca\textsuperscript{2+} current (IC\textsubscript{aT}; transient) and the slow component of the delayed rectifier K\textsuperscript{+} current (IK\textsubscript{s}; Nemtsas et al. 2010; Hassanen et al. 2011; Abramochkin et al. 2018; Haverinen et al. 2018a). The shape of the cardiac AP is dependent on the antagonistic effects of INa and IK1 on membrane potential and an important factor in uninterrupted propagation of cardiac AP (Varghese 2016; Vornanen 2016). The aim of the present study was to investigate if phenanthrene and retene modulate the 4 major ion currents of the rainbow trout (Oncorhynchus mykiss) ventricle, which could reveal novel toxic effects of these PAHs on the fish heart.

MATERIAL AND METHODS

Animals

Hatchery-reared rainbow trout (Oncorhynchus mykiss; 73.43 ± 11.69 g, n = 18) were obtained from the local fish farm (Kontiolahti, Finland). In the animal facilities of the University of Eastern Finland, the trout were maintained in 500-L metal aquaria for a minimum of 3 wk before use in the experiments and fed aquarium fish food (Ewos) at least 5 times a week. Water temperature was regulated at 14 ± 0.5 °C (Computec Technologies), and oxygen saturation was maintained by aeration with compressed air. Groundwater (average pH 8.0, conductivity 13 µS/cm) was constantly flowing through the aquaria at a rate of 150 to 200 L/d (permission E5AV12832/04.10.07/2015).

Myocyte isolation

All experiments were conducted in vitro on enzymatically isolated ventricular myocytes. Fish were killed by a cranial concussion and pithing, and the heart was rapidly excised. Ventricular myocytes were isolated using retrograde perfusion of the heart and the standard concentrations of hydrolytic enzymes as reported for the method developed in our laboratory (Vornanen 1997). Cell isolation was conducted at room temperature (20–22 °C). Isolated myocytes were used in the experiments within 10 h from isolation.

Whole-cell patch clamp

Whole-cell current-clamp recordings were made by using an Axopatch 1D amplifier (Axon Instruments). Clampex 9.2 software was used for data acquisition, and off-line analysis of the recordings was done using the Clampfit 10.4 software package. During the experiments, myocytes were continuously superfused with external saline solution at a rate of 1.5 to 2 mL min\textsuperscript{−1}. The temperature of the external solution in the recording chamber was regulated at 14 °C by using a Peltier device (CL-100 from Warner Instruments or HCC-100A from Dagan) and continuously recorded on the same file with electrophysiological data. Patch pipettes were pulled (PP-83; Narishige) from borosilicate glass (King Precision) and had a resistance of 2.7 ± 0.06 MΩ when filled with the internal saline solution. After gaining a gigahm seal, the membrane under the pipette tip was ruptured by a short-voltage pulse (zap) to gain access to the cell, transients attributable to series resistance (7.3 ± 0.26 MΩ) and pipette capacitance were canceled, and the capacitive size of ventricular myocytes was determined.

For recording of APs and K\textsuperscript{+} currents, the external saline solution contained (mmol/L\textsuperscript{−1}) 150 NaCl, 5.4 KCl, 1.2 MgCl\textsubscript{2}, 1.8 CaCl\textsubscript{2}, 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 10 glucose, with pH adjusted with NaOH to 7.6 at 20 °C (giving a pH of 7.68 at the experimental temperature). The composition of the pipette (internal) solution was as follows (mmol/L\textsuperscript{−1}): 140 KCl, 4 MgATP, 1 MgCl\textsubscript{2}, 0.03 Tris-GTP, and 10 HEPES (pH adjusted with KOH to 7.2 at 20 °C). To elicit APs, ventricular myocytes were stimulated with current pulses of constant duration (4 ms) and with increasing amplitude. The initial stimulus strength was 200 pA, and it was raised with 20-pA increments until an all-or-none AP was elicited (Badr et al. 2018). The stimulation frequency was 1 Hz. The following AP parameters were analyzed off-line: resting membrane potential (V\textsubscript{rest}), threshold potential of AP (V\textsubscript{th}), critical depolarization (V\textsubscript{th} – V\textsubscript{rest}), AP overshoot (mV), AP amplitude (AMP, mV), AP duration at 50% repolarization level (APD50, ms), maximum rate of AP upstroke (+dV/dt, mV ms\textsuperscript{−1}), and the maximum rate of AP repolarization (–dV/dt, mV ms\textsuperscript{−1}; Figure 1). Measures V\textsubscript{in}, I\textsubscript{th}, and critical depolarization are for electrical excitability of ventricular myocytes, that is, the ease with which AP can be triggered by a depolarizing current.

Voltage dependency of the rapid component of the delayed rectifier K\textsuperscript{+} current (IK\textsubscript{r}) and the inward rectifier K\textsuperscript{+} current (IK\textsubscript{1}) were measured using standard stimulation protocols (Vornanen et al. 2002a) from the holding potential of −80 mV. When recording IK\textsubscript{1}, the external saline included 2 µM E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonyl-aminobenzoyl)piperidine), 0.5 µM tetrodotoxin (TTX; Tocris Cookson), and 10 µM nifedipine, to block IK\textsubscript{r}, INa, and IC\textsubscript{aL} respectively. Also, IK\textsubscript{1} was recorded in the presence of TTX (0.5 µM), nifedipine (10 µM), and 0.2 mM BaCl\textsubscript{2} (to block IK\textsubscript{1}).

The fast Na\textsuperscript{+} current (INa) was measured under a reduced Na\textsuperscript{+} gradient (20 mM [Na\textsuperscript{+}]\textsubscript{e}, 5 mM [Na\textsuperscript{+}]\textsubscript{i}) across the sarcolemma to obtain good control of the membrane voltage. The composition of the external saline was (mmol/L\textsuperscript{−1}): 20 NaCl, 120 CsCl, 1 MgCl\textsubscript{2}, 0.5 CaCl\textsubscript{2}, 10 glucose, and 10 HEPES at pH 7.7 (adjusted with CsOH at 20 °C; Haverinen and Vornanen 2004). Nifedipine (10 µmol L\textsuperscript{−1}) was included in the external solution to block IC\textsubscript{aL}. The pipette solution consisted of (in mmol L\textsuperscript{−1}) 5 NaCl, 130 CsCl, 1 MgCl\textsubscript{2}, 5 etgazic acid (EGTA), 5 Mg\textsubscript{2}ATP, and 5 HEPES (pH adjusted to 7.2 with CsOH at 20 °C). Using
established stimulus protocols, $I_{\text{Na}}$ was elicited from a holding potential of $-120 \text{ mV}$ (Haverinen and Vornanen 2006; Haverinen et al. 2018b).

The composition of the external saline solution for recording $I_{\text{CaL}}$ was as follows (mmol/L$^{-1}$): 150 NaCl, 5.4 CsCl, 1.8 CaCl$ _2$, 1.2 MgCl$ _2$, 10 HEPES, and 10 glucose (pH adjusted to 7.6 at 20 °C with CsOH). We included TTX (0.5 μM) in this saline to block Na$^+$ current ($I_{\text{Na}}$, Vornanen 1998). Because Cs$^+$ may flow through the Erg K$^+$ channels, 2 μM E-4031 was included in the external solution to prevent contamination by $I_{\text{Kr}}$. The pipette solution contained (mmol L$^{-1}$) 130 CsCl, 15 tetraethylammonium chloride, 5 MgATP, 1 MgCl$ _2$, 5 oxaloacetate, 10 HEPES, and 5 EGTA (pH adjusted to 7.2 at 20 °C with CsOH; all chemicals from Sigma). We elicited $I_{\text{CaL}}$ from a holding potential of $-80$ to $+10 \text{ mV}$ at a frequency of 0.2 Hz.Recording of $I_{\text{CaL}}$ is complicated by time-dependent rundown (decline) of the current. To minimize the effect of rundown on results, time-dependent changes in $I_{\text{CaL}}$ were monitored after getting access to the whole configuration. For the same reason, the analysis of PAH effects was limited to the 2 highest concentrations. Only those cells where $I_{\text{CaL}}$ stabilized within approximately 5 min from the start of recording were accepted for analysis.

**PAHs**

The stocks of phenanthrene (Sigma-Aldrich) and retene (MP Biomedicals) were made in dimethyl sulfoxide (DMSO) at 20 mM. Test solutions at concentrations of 0.3, 1.0, 10, and 30 μM for phenanthrene and 0.1, 1.0, and 10 μM for retene were made daily in external saline solutions. Effects of the highest DMSO concentration in the experimental solutions on AP parameters and ion currents were tested in separate experiments. No statistically significant effects were noticed.

**Statistical analyses**

After checking the normality of distribution and equality of variances, one-way analysis of variance (with Tukey’s or Dunnett’s T3 post hoc test) or nonparametric test (with Friedman’s test) were used for evaluating the effect of different PAH concentrations on AP parameters and maximum ion currents. All statistical tests were performed using SPSS (IBM; Ver 21.0) software. Data are presented as mean ± standard error of the mean (SEM), and $p < 0.05$ was considered statistically different.

**RESULTS**

**Phenanthrene and retene differentially modify the AP in rainbow trout ventricular myocytes**

Phenanthrene had no effect on the duration of AP at the level of 50% repolarization (APD50; Figure 2A and E) but shortened it at the zero voltage level (APD0) at 30 μM (Figure 2A). Phenanthrene increased the maximum upstroke velocity (+dV/dt) at 1 and 10 μM and accelerated the maximum rate of AP repolarization (−dV/dt) at 10 and 30 μM (Figure 2C). Retene had more pronounced effects on APs than phenanthrene. The duration of the AP (APD50 and APD0) was strongly shortened at 1 and 10 μM concentrations (Figure 3A and E). Retene augmented the AP amplitude at 10 μM and increased the overshoot at 1 and 10 μM (Figure 3B). The maximum rate of AP upstroke became faster at all concentrations of retene, but the effect on −dV/dt was significant only at 10 μM (Figure 3C). Excitability of ventricular myocytes was decreased at the highest (10 μM) retene concentration as the critical depolarization needed to elicit AP was approximately 18% higher than in the control (Figure 3B).

**Phenanthrene and retene modulate cardiac $I_{\text{Na}}$, $I_{\text{CaL}}$, and $I_{\text{Kr}}$ currents but have no effect on $I_{\text{K1}}$**

Phenanthrene and retene affected all studied ventricular ion currents except $I_{\text{K1}}$, but retene caused the effects at lower concentrations than phenanthrene. Under exposure of 10 μM phenanthrene or 1 μM retene, the peak density of $I_{\text{Na}}$ was
increased by 12 and 17%, respectively (Figure 4A and B). The effects of the highest concentrations (30 µM phenanthrene, 10 µM retene) were slightly less and statistically nonsignificant (Figure 4B).

After getting electrical access to the cell, there was a clear increase in the amplitude of \( I_{Ca,L} \) attributable to the buffering of intracellular free Ca\(^{2+} \) by EGTA of the pipette solution (removal of Ca\(^{2+} \)-dependent inactivation of \( I_{Ca,L} \)). Then, the current stabilized and enabled the recording of drug effects on \( I_{Ca,L} \) (Figure 5A and B). Phenanthrene reduced \( I_{Ca,L} \), but the effect was statistically significant only at the highest concentration tested, 30 µM (Figure 5C). Retene diminished \( I_{Ca,L} \) at 1 and 10 µM (Figure 5C).

Whereas phenanthrene attenuated \( I_{Kr} \) at 10 and 30 µM, retene was effective even at the lowest test concentration (0.1 µM; Figure 6). Both phenanthrene and retene decreased the \( I_{Kr} \) tail currents at all voltages, where the tail current was activated (Figure 6C and D). The maximum inhibition of \( I_{Kr} \) tail at +40 mV was 79.3 and 59.2% for phenanthrene and retene, respectively. During the depolarizing prepulse, phenanthrene and retene inhibited \( I_{Kr} \) (\( I_{Kr,activ} \)) in the voltage range between 0 and +20 mV but did not have any effect at +40 and +60 mV (Figure 6E and F). This suggests that there is a phenanthrene- and retene-resistant current underlying \( I_{Kr} \), probably the slow component of the delayed rectifier K\(^+\) current, \( I_{Ks} \). Neither of the PAHs had an effect on the background inward rectifier, \( I_{K1} \) (Supplemental Data, Figure S1A and B).

**DISCUSSION**

**Effects on AP**

Both 3-ring PAHs affected the ventricular AP of the rainbow trout heart, but retene was a much stronger AP modifier than
FIGURE 4: Phenanthrene and retene increase the fast Na\(^+\) current (I\(_{Na}\)) in rainbow trout ventricular cardiomyocytes. (A) Current–voltage relationship of I\(_{Na}\) in the absence and presence of phenanthrene (left) and retene (right). The stimulus protocol is shown between the graphs. (B) Effects of phenanthrene (10, 30 µM) and retene (1, 10 µM) on the peak density of I\(_{Na}\). The results are means ± SEM of 12 to 14 myocytes from at least 3 animals. Groups denoted by the same letter do not differ significantly from each other.

Phenanthrene did not change V\(_{rest}\) or AMP, consistent with the findings from bluefin tuna cardiomyocytes (Brette et al. 2017); V\(_{rest}\) is maintained by the I\(_{K1}\), which remained untouched by phenanthrene. Phenanthrene had only minor effects on APD; APD was slightly reduced at the zero-voltage level but remained unchanged at the 50% repolarization level. In this respect, rainbow trout clearly differs from bluefin tuna, where phenanthrene lengthened ventricular APD (Brette et al. 2017). The APD is regulated by a delicate balance between influx of Ca\(^{2+}\) via I\(_{CaL}\) and efflux of K\(^+\) via I\(_{Kr}\), I\(_{Ks}\), and I\(_{K1}\) (Grant 2009). Because the resistance of the sarcolemma at the AP plateau is high (Ca\(^{2+}\) and K\(^+\) fluxes are small), small changes in the amplitude and activation/inactivation rate of Ca\(^{2+}\) and K\(^+\) currents will affect APD (Zaza 2010). Shortening of AP at the zero-voltage level suggests that in the early plateau I\(_{CaL}\) is reduced more than I\(_{K}\) by phenanthrene. The results of the present study show a small but clear increase in +dV/dt in rainbow trout with 10 and 30 µM phenanthrene. Phenanthrene also steepened the rate of repolarization (−dV/dt). These are novel actions of PAHs on fish cardiac I\(_{Na}\). Retene was more potent than phenanthrene at enhancing I\(_{Na}\), which is in line with its larger effect on +dV/dt and overshoot of the AP. At the level of intact tissue the larger I\(_{Na}\) means a faster propagation of AP in the ventricular wall. To our knowledge, there are no earlier data on the effects of PAHs on fish cardiac I\(_{Na}\). However, in bluefin tuna ventricular myocytes, phenanthrene did not affect the upstroke velocity of the AP, thus suggesting species-specific differences in PAH modulation of I\(_{Na}\).

Effects on cardiac ion currents

The Na\(^+\) current (I\(_{Na}\)) is active during the upstroke of the AP, causing depolarization of the sarcolemma by fast and large influx of Na\(^+\). Both phenanthrene and retene increased the peak I\(_{Na}\) density in the ventricular myocytes of rainbow trout. Retene was more potent than phenanthrene at enhancing I\(_{Na}\), which is in line with its larger effect on +dV/dt and overshoot of the AP. At the level of intact tissue the larger I\(_{Na}\) means a faster propagation of AP in the ventricular wall. To our knowledge, there are no earlier data on the effects of PAHs on fish cardiac I\(_{Na}\). However, in bluefin tuna ventricular myocytes, phenanthrene did not affect the upstroke velocity of the AP, thus suggesting species-specific differences in PAH modulation of I\(_{Na}\).

Currents I\(_{CaL}\) and I\(_{K}\) are the main determinants of the long AP plateau. They are counteracting currents because I\(_{CaL}\) is depolarizing and I\(_{K}\) repolarizing. The net outcome of the inhibition of these currents can be seen as changes in APD. Notably, both PAHs caused shortening of APD, but retene was much more powerful than phenanthrene. Strong shortening of APD by retene indicates that the net charge influx via I\(_{CaL}\) is inhibited more than the K\(^+\) efflux via I\(_{K}\). The final phase 3 repolarization is accelerated by the background inward rectifier I\(_{K1}\). The increase in the rate of −dV/dt by PAHs is probably attributable to the resistance of I\(_{K1}\) to retene and phenanthrene whereby the uninhibited I\(_{K1}\) overwhelms the reduced I\(_{CaL}\).

The lowering of Ca\(^{2+}\) influx in phenanthrene-treated rainbow trout cardiac myocytes is in line with previous research showing that phenanthrene decreased Ca\(^{2+}\) transients in
Intracellular free Ca^{2+} concentration. In trout ventricular myocytes, the activation of contraction is largely dependent on the sarcolemmal Ca^{2+} influx during the AP plateau because approximately two-thirds of the activator Ca^{2+} is estimated to come from the extracellular space (Vornanen et al. 2002b). Inhibition of I_{CaL} and shortening of the plateau means that Ca^{2+} influx is smaller and there is less time for Ca^{2+} entry. In the intact ventricle, this should appear as reduced force of contraction. Indeed, exposure to PAHs or oil reduces atrial and ventricular contraction and diminishes cardiac stroke volume in larval fish (Incardona et al. 2013; Jung et al. 2013; Edmunds et al. 2015; Esbaugh et al. 2016; Serhus et al. 2016; Khursigara et al. 2017; Perrichon et al. 2018). In rainbow trout yolk sac larvae, retene causes pericardial and yolk sac edemas (Billiard et al. 1999; Scott et al. 2011; Vehniäinen et al. 2016), phenomena often seen with PAH and oil exposures and proposed to be caused by reduced cardiac output (Incardona and Scholz 2016). Taken together, inhibition of I_{CaL} and shortening of the AP plateau would compromise contractility and cardiac output of the heart with the outcome of reduced physical performance level and fitness of the fish. These effects would be particularly strong under the intoxication by retene.

The I_{Kr} channels are notorious for their susceptibility to inhibition by low concentrations of various small-molecule compounds (Sanguinetti and Tristani-Firouzi 2006). The wide pore cavity of the channel allows access of small molecules to the pore (Vandenbregg et al. 2001). Therefore, it is no surprise that also PAHs can block these channels in fish cardiac myocytes. In rainbow trout ventricular myocytes, 10 and 30 μM phenanthrene reduced I_{Kr} by 43 and 75%, respectively. This is slightly less than the inhibition in bluefin tuna, where 5 and 25 μM phenanthrene decreased I_{Kr} by 60 and more than 85%, respectively (Brette et al. 2017). In rainbow trout, the effect of retene on I_{Kr} was more pronounced because 10 μM retene caused a 60% reduction in I_{Kr} (Supplemental Data, Figure S2).

In mammalian heart, blockade of I_{Kr} by many drugs is shown to be proarrhythmic and able to induce chaotic ventricular tachycardia, torsades de pointes (Vandenbregg et al. 2001). However, if both I_{Kr} and I_{CaL} are inhibited simultaneously and at similar drug concentrations, the effect is antiarrhythmic, even when drugs prolong, shorten, or triangulate ventricular APs (Kramer et al. 2013; Obejero-Paz et al. 2015). A typical example is verapamil, a useful human cardiovascular medicine, which inhibits human I_{Kr} and I_{CaL} at similar concentrations (Shetuan et al. 1999; Kang et al. 2012). Because PAHs inhibit both I_{Kr} and I_{CaL} at similar micromolar concentrations, they should not be proarrhythmic in fish ventricle. However, I_{Kr} and I_{CaL} are essential components of the cardiac pacemaker, which determines the rate and rhythm of the heartbeat (Schram et al. 2002). Half-maximal inhibition of I_{Kr} by E-4031 is known to reduce the beating rate of rainbow trout sinoatrial preparations, and therefore inhibition of I_{Kr} might explain the PAH-induced bradycardia of larval fish (Haverinen and Vornanen 2007). Inhibition of I_{CaL} is likely to affect impulse generation and conduction of the nodal tissues (sinoatrial pacemaker and atrioventricular canal) because I_{CaL} is the main determinant for the rate of AP upstroke and impulse conduction (I_{Na} is absent.
or small in nodal cells; Schram et al. 2002). Inhibition of ICaL might therefore appear as atrioventricular block and ventricular bradycardia, phenomena seen in larval fish exposed to PAHs or oil (Incardona et al. 2004, 2005, 2009, 2011; Zeltser et al. 2004; Perrichon et al. 2016, 2018). However, care must be taken when applying results from mature fish to embryos or larval fish.

Because retene is quite hydrophobic (logKow ≈ 6), the actual concentrations in the test chamber most probably were lower than nominal. It must also be borne in mind that retene is quickly metabolized by cytochrome P450 (CYP1A) in fish, and this may lower the concentration of parent retene that reaches cardiac myocytes in vivo (Hawkins et al. 2002). In nature, however, fish are exposed to PAH mixtures that frequently contain CYP1A inhibitors, which in turn decrease the metabolism of PAHs and thus increase the concentration of parent compounds (Hawkins et al. 2002).

Retene is an AhR agonist, and it disturbs cardiovascular development in fish via activating AhR and altering transcription (Scott et al. 2011; Vehniäinen et al. 2016). The
present study shows that in addition to this transcriptional route, retene has a direct effect on cardiac function via modulating voltage-gated ion channel activity. Because normal cardiac function is important for cardiovascular development (Glickman and Yelon 2002; Incardona et al. 2015), as well as the development of other tissues and organs (Incardona et al. 2004), retene may cause developmental defects also independently of the AhR. The ability to modulate the activity of cardiac ion channels also means that in addition to early-life stages, retene may be cardiotoxic to juvenile-niles and adults.

CONCLUSION

The 3-ring PAHs phenanthrene and retene differentially modified ventricular APs in rainbow trout cardiomyocytes. Retene was more potent and strongly reduced the duration of ventricular AP. Although phenanthrene and retene had qualitatively similar effects on ion currents, phenanthrene only slightly affected AP duration, probably because of its weaker inhibition of $I_{Ks}$ and $I_{Ca}$ in comparison to retene. Furthermore, the effects of phenanthrene on ventricular AP differed from those reported earlier for the marine warm-water scromboid fish bluefin tuna. The present results suggest that different PAHs may have different direct effects on cardiac function and that these effects may be partly species-specific. This further complicates the environmental risk assessment of PAHs.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4530.

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Data Accessibility—Data are available from the authors (eeva-riikka.vehniainen@jyu.fi).

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