Electrical Excitation of the Heart in a Basal Vertebrate, the European River Lamprey (*Lampetra fluviatilis*)

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ABSTRACT

Hagfishes and lampreys (order Cyclostomata) are living representatives of an ancient group of jawless vertebrates (class Agnatha). Studies on cyclostome hearts may provide insights into the evolution of the vertebrate heart and thereby increase our understanding of cardiac function in higher vertebrates, including mammals. To this end, electrical excitability of the heart in a basal vertebrate, the European river lamprey (*Lampetra fluviatilis*), was examined. Ion currents of cardiac myocytes, action potentials (APs) of atrial and ventricular muscle, and electrocardiogram (in vivo) were measured using the patch-clamp method, intracellular microelectrodes, and trailing wires, respectively. The characteristic features of fairly high heart rate (28.4 ± 3 beats min^{-1}) and short AP duration (550 ± 44 and 122.1 ± 28.5 for ventricle and atrium, respectively) at low ambient temperature (5°C) are shared with cold-active teleost fishes. However, the ion current basis of the ventricular AP differs from that of other fishes. For inward currents, sodium current density (I_{Na}) is lower and calcium current density (I_{Ca}) higher than in teleost ventricles, while the kinetics of I_{Na} is slow and that of I_{Ca} is fast in comparison. Among the ventricular repolarizing currents, the delayed rectifier K^+ current is smaller than in myocytes of several teleost species. Unlike mammalian hearts, ATP-sensitive K^+ channels are constitutively open under normoxic conditions, thus contributing to negative resting membrane potential and repolarization of APs. Upstroke velocity of AP (5.4 ± 0.9 and 6.3 ± 0.6 V s^{-1} for ventricular and atrial myocytes, respectively) is slower than in teleost hearts. Excitability of the lamprey heart seems to possess both primitive and advanced characteristics. Short APs are appropriate to support brief and vigorous contractions (in common with higher vertebrates), while relatively low AP upstroke velocities enable only relatively slow propagation of contraction over the heart.

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Introduction

Large differences exist in excitation and excitation-contraction (E-C) coupling of the heart and its autonomic nervous control among vertebrates. For example, isoforms and molecular assemblies of ion channels responsible for electrical activity at the sarcolemma (SL) vary between piscine and mammalian hearts (Haverinen et al. 2007; Hassinen et al. 2008*b*, 2011). Relative roles of sarcoplasmic reticulum (SR) Ca^{2+} release and SL Ca^{2+} influx in the activation of contraction in cardiac myocytes also differ between ectothermic (fishes, frogs, reptiles) and endothermic (mammals and birds) vertebrates (Fabiato 1983; Klizner and Morad 1983; Driedzic and Gesser 1994; Vornanen et al. 2002*b*; Vornanen and Haverinen 2012). To better understand the functional and adaptive roles of these differences in cardiac E-C coupling, physiological studies on extant species of ancient vertebrate groups may be used to provide insights into the evolutionary origin, adaptive value, and genetic limitations of physiological traits.

Hagfishes and lampreys (order Cyclostomata) are living representatives of an ancient group of jawless vertebrates (class Agnatha). The agnathans appeared more than 555 million years ago, but most of the species became extinct about 200 million years later. Currently, the group comprises about 120 cyclostome species (Shu et al. 1999). Although molecular data suggest a shared ancestry of lampreys and hagfishes—that is, they diverged after the split of Agnatha and Gnathostome (the jawed vertebrates) lineages (Delarbre et al. 2002; Heimberg et al. 2010)—in morphological and physiological terms lampreys share a number of characteristics with both Gnathostomes and hagfishes. Because lampreys and hagfishes are close relatives, it would be informative to know how the shared and divergent physiological traits of lampreys, with hagfishes on one hand and gnathostomes on the other, are associated with the current biology of these groups, that is, which traits are ancestral, degenerate, or adaptive for each group.

The lamprey heart displays some distinctive features whose mechanistic basis remains poorly elucidated. For example, neural regulation deviates from that of both hagfishes and gnathostomes (for review, see Farrell 2007 and Satchell 1991). Autonomic control of the lamprey heart differs from the typical vertebrate pattern in three respects: (i) it completely

lacks sympathetic adrenergic control; (ii) cholinergic control is excitatory, in contrast to the inhibitory control of other vertebrates; and (iii) cholinergic control is mediated via nicotinic rather than muscarinic receptors (Greene 1902; Carson 1906; Augustinsson et al. 1956). The hagfish heart lacks any autonomic control and therefore differs from the lamprey heart in regard to the parasympathetic system. Although the lamprey heart is devoid of adrenergic control, the endothelial surface of the myocardium has catecholamine-containing chromaffin cells, which may release adrenaline and noradrenaline to cardiomyocytes (Augustinsson et al. 1956).

Another characteristic feature of both lamprey and hagfish, evident from electrocardiograms (ECG), is a slow rate of action potential (AP) transmission over the heart (Davie et al. 1987). Electrical conduction rate depends on the density of sarcolemmal sodium current (I_{Na}). On other hand, the effects of adrenaline and noradrenaline on E-C coupling are mediated by SL β-adrenergic receptors, which in vertebrates are coupled to L-type Ca^{2+} channels. Because there are no previous cellular studies on the lamprey heart, the objective of this study was to analyze ion currents to explore the basal arrangement underlying electrical excitation of the vertebrate myocardium.

Material and Methods

Animals

Experiments were conducted on European river lamprey (*Lampetra fluviatilis*; 56 ± 12 g, $n = 32$) caught from the river Kemijoki (Finland) in October 2008–2011. In the animal house, lampreys were maintained in 500-L metal aquaria at a water temperature of 5°–6°C. No food was provided for the fish. Photoperiod was a 12L:12D cycle. The experimental protocols were approved by the Animal Experiment Board in Finland (permissions STH998A and PH472A).

Recording of Electrocardiograms (ECG)

ECG recordings were made following previously described methods (Campbell et al. 2004). Lampreys were anesthetized by MS-222 (0.85 mg L^{-1}, Sigma) in neutralized water ($NaHCO_3$, pH ~7) and placed ventral side up on an operating table. Recording electrodes were made of two seven-strand Teflon-coated wires (length = 40 cm, diameter = 0.23 mm; A-M Systems), which were obliquely inserted from the ventral side of the fish laterally close to the pericardium. The trailing wires were bound together and attached by a suture to the back of the lamprey. The operated fish was placed into a glass jar (2.3 L) that was immersed in the fish tank (500 L, O_2 concentration about 11 mg L^{-1}). The ECG wires and the reference electrode were connected to a bioamplifier (AD Instruments, Oxford, UK) for continuous recording of bipolar ECG signals on computer. Off-line analysis of the recordings was made by using LabChart 7 software (AD Instruments).

Heart rate (HR) and HR variability of resting fish were determined 3–12 d (mean ± SEM = 6 ± 1.16) after the attachment of the recording electrodes. To this end, 1-h stretches of constant ECG recordings were automatically analyzed using the HR variability subroutine of LabChart 7. HR variability was evaluated from Poincaré plots, where each RR interval (fig. 1*A*; RRn, *X*-axis; fig. 1*C*) is plotted against the next RR interval (RRn+1, *Y*-axis; fig. 1*C*). Points falling above and below the line of identity indicate interbeat intervals longer and shorter than the preceding RR interval, respectively. For each Poincaré plot, standard deviations of the longer and shorter axis (SD1 and SD2, respectively; fig. 1; Tulppo et al. 1996) were determined from the following equations, where NN refers to normalized RR intervals generated by discarding artifacts and ectopic beats from the raw RR intervals (LabChart 7):

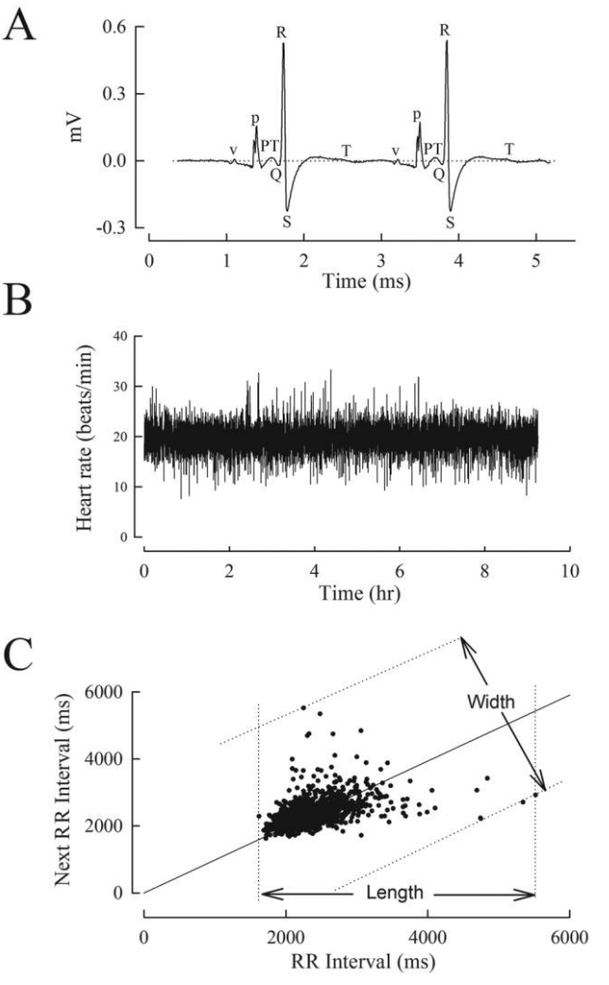

Figure 1. Electrocardiogram of *Lampetra fluviatilis* at 5°C. *A*, Representative electrocardiogram showing depolarization of sinus venosus (v), depolarization of atrium (P), repolarization of atrium (PT), depolarization of ventricle (QRS), and repolarization of ventricle (T). The dotted line indicates the zero voltage line. *B*, Tachogram from bipolar electrocardiogram recording of a nonanesthetized lamprey showing a stable long-term cardiac rhythmicity. *C*, Poincaré plot showing a larger long-term variability (length) than short-term variability (width) in heart rate. All recordings are from the same fish. RR interval refers to the interval between the peaks (R) of two consecutive QRS complexes of the electrocardiogram.

$$SD1^2 = 0.5 \times SD\Delta NN^2,$$

$$SD2^2 = 2 \times SDNN - 0.5 \times SD\Delta NN^2.$$

SDNN and SDΔNN are standard deviation of the NN intervals and standard deviation of the differences between adjacent NN intervals, respectively.

Recording of Action Potentials

The whole heart was gently fixed with insect pins on the Sylgard-coated bottom of a 10-mL recoding chamber filled with continuously oxygenated (100% O_2) physiological saline solution containing (in mmol L^{-1}) 150 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose, and 10 HEPES, with pH adjusted to 7.7 with NaOH (at 5°C). Cardiac preparations were allowed to stabilize for about 1 h before APs were recorded with sharp microelectrodes as described earlier (Haverinen and Vornanen 2009). Recordings were analyzed in Clampfit software (Axon Instruments) to determine resting membrane potential (RMP), AP overshoot, AP amplitude, and AP duration at 50% of repolarization (APD_{50}).

Patch-Clamp Recordings

Ventricular myocytes were isolated with enzymatic digestion with established methods (Vornanen 1997; Vornanen and Haverinen 2012) and were used within 8 h of isolation. A small aliquot of dissociated cells was placed in a small (150 μL) recording chamber and then superfused continuously, with the external solution precooled to 5° ± 1°C.

For measurement of K^+ currents the external solution contained (in mmol L^{-1}) 150 NaCl, 5.4 KCl, 1.5 $MgSO_4$, 0.4 NaH_2PO_4, 2.0 $CaCl_2$, 10 glucose, and 10 HEPES at pH 7.7. Tetrodotoxin (TTX; 1 μM), nifedipine (10 μM), and glibenclamide (30 μM) were added in the external saline solution to block Na^+, Ca^{2+}, and ATP-sensitive K^+ currents, respectively. Pipette solution contained (in mmol L^{-1}) 140 KCl, 4 MgATP, 1 $MgCl_2$, 5 EGTA, and 10 HEPES at pH 7.2.

Na^+ current (I_{Na}) was measured in Cs^+-based, low-Na^+ saline solution, which contained (in mmol L^{-1}) 20 NaCl, 120 CsCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 10 glucose, and 10 HEPES (pH adjusted to 7.7 with CsOH). In addition, 10 μM nifedipine (Sigma) was added to both solutions to block L-type Ca^{2+} currents. The pipette solution contained (mmol L^{-1}) 5 NaCl, 130 CsCl, 1 $MgCl_2$, 5 EGTA, 5 MgATP, and 5 HEPES (pH adjusted to 7.2 with CsOH). To ensure adequate voltage control, a minimum of 80% series resistance compensation was applied. I_{Na} was elicited from the holding potential of −120 mV.

The composition of the physiological solution used for recording Ca^{2+} current (I_{Ca}) contained (in mmol L^{-1}) 130 NaCl, 5.4 CsCl, 1.5 $MgSO_4$, 0.4 NaH_2PO_4, 1.8 $CaCl_2$, 10 glucose, and 10 HEPES (adjusted to pH 7.7 with CsOH). For recording I_{Ca}, the pipette solution contained (in mmol L^{-1}) 130 CsCl, 5 MgATP, 15 tetraethylammonium chloride, 1 $MgCl_2$, 5 succinate, 5 EGTA, 0.3 Na_2GTP, and 10 HEPES (adjusted to pH 7.2 with CsOH).

Single-channel currents of Kir2 potassium channels and ATP-sensitive K^+ channels were recorded in the cell-attached configuration using an EPC-9 amplifier and Pulse software or Axopatch 1D amplifier and PClamp 9 (Axon; Paajanen and Vornanen 2004; Hassinen et al. 2008*b*). Electrode resistance varied between 9 and 11 MΩ when they filled with K^+-based solution (in mmol L^{-1}): 134 KCl, 1.8 $CaCl_2$, 2 $MgCl_2$, 10 glucose, and 10 HEPES (adjusted to pH 7.7 with KOH; $[K^+] = 141$ mM). Physiological saline solution (see "Recording of Action Potentials") was used as the bath solution. All single-channel recordings were sampled at 4 kHz and low-pass filtered at 2 kHz. Single-channel conductance was determined by applying 30-s square pulses from −120 to −20 mV in 20-mV increments every 10 s.

Statistical Analyses

After checking normality of distribution and homogeneity of variances, a *t*-test for independent samples was used to compare the parameters of atrial and ventricular APs and effects of isoprenaline and acetylcholine on L-type Ca^{2+} current. Interspecies differences in voltage dependence and rate of inactivation of I_{Na} and I_{Ca} were tested with one-way ANOVA using Tukey's honest significant difference as the post hoc test. A P value of 0.05 was the limit of statistical significance. Data are presented as means ± SEM.

Results

Heart Rate and Electrocardiogram

A typical ECG of the lamprey heart included v, P, PT, QRS, and T waves (fig. 1*A*) corresponding to depolarization of sinus venosus, atrial depolarization, atrial repolarization, ventricular depolarization, and ventricular repolarization, respectively. The mean in vivo HR of lamprey at 5°C was 28.5 ± 3.0 beats min^{-1} ($n = 7$), with a Q-T interval (ventricular AP duration) of 831 ± 92 ms (fig. 1*B*). Figure 1*C* shows a typical ellipsoid Poincaré plot of a resting lamprey. Typical for the HR of a resting vertebrate, the short-term variability (width) of HR is less than the long-term variability of HR (length). For all animals ($n = 7$), Poincaré plots were elliptical in shape with a mean standard deviation of 196 ± 38 ms (SD1) and 311 ± 65 ms (SD2) for short- and long-term variability, respectively ($P < 0.05$). The SD1/SD2 was 0.64 ± 0.02. Duration of the QRS complex, a measure for the rate of AP propagation over the ventricle, was 218.4 ± 55.8 ms.

Cardiac Action Potentials

APs were recorded with sharp microelectrodes from intact atrial and ventricular muscle of the lamprey heart. APs of the lamprey ventricle had a typical shape of the vertebrate cardiac AP with an upstroke velocity of 5.4 ± 0.9 V s^{-1} (mean ± SEM), prominent AP overshoot (18.7 ± 7.9 mV), and long plateau duration (APD_{50} = 550 ± 44.6 ms) at 5°C (fig. 2; table 1). The RMP was −67.7 ± 9.8 mV. In comparison to ventricular APs,

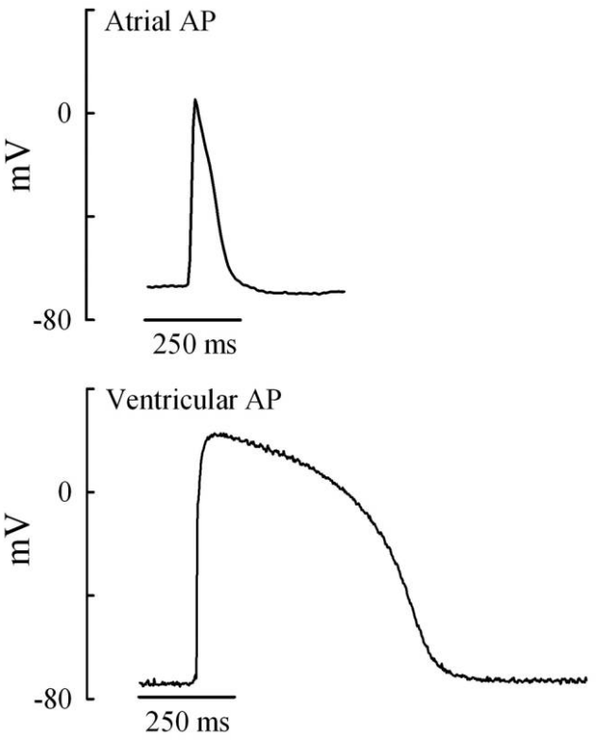

Figure 2. Representative microelectrode recordings of atrial and ventricular action potentials (AP) of the lamprey (*Lampetra fluviatilis*) heart at 5°C.

atrial APs were much shorter in duration (APD_{50} = 122.1 ± 28.5 ms, $P < 0.001$) and had a slightly smaller AP overshoot (13.0 ± 10.1 mV, $P < 0.01$) and similar upstroke velocity (6.3 ± 0.6 V s^{-1}, $P > 0.05$; fig. 2; table 1).

Sodium Current (I_{Na})

The fast upstroke of cardiac AP is produced by a rapid influx of Na^+ ions through the voltage-gated Na^+ channels generating I_{Na}. Ventricular myocytes of the lamprey heart had a robust I_{Na}, which was blocked by low concentrations of tetrodotoxin (data not shown), a specific blocker of Na^+ channels (Vornanen et al. 2011). I_{Na} had a peak amplitude of 14.9 ± 3.5 pA pF^{-1} at −30 mV, and the current reversed at +33 ± 3.3 mV, that is, close to the theoretical reversal potential of Na^+ ions (+33.2 mV; fig. 3). Typical for I_{Na}, the current inactivated relatively quickly during maintained depolarization, with a time constant of 2.2 ± 0.3 ms at −30 mV.

L-Type Ca^{2+} Current (I_{CaL})

The long duration of ventricular AP is maintained by a balance between Ca^{2+} influx through L-type Ca^{2+} channels and K^+ efflux through different K^+ channels. The peak density of I_{CaL} occurred at +10 mV, and the current inactivated much slower than I_{Na} (fig. 4). I_{CaL} was 90% blocked by 10 μM nifedipine (not shown), a blocker of L-type Ca^{2+} channels. A nonspecific β-adrenergic agonist, isoprenaline (2 μM), increased the peak density of I_{CaL} by 65% from 3.0 ± 0.42 to 4.2 ± 0.56 pA pF^{-1}. In contrast, acetylcholine (1 μM) had no effect on I_{CaL} (fig. 4).

Inward Rectifier K^+ Current (I_{K1})

When I_{Na}, I_{CaL}, and I_{Kr} were blocked with TTX, nifedipine, and E-4031, respectively, large inward and outward currents remained in lamprey ventricular myocytes (fig. 5*A*). Low concentrations of Ba^{2+} (0.2 mM) blocked a part of the inward and outward currents. The Ba^{2+}-sensitive current had a typical shape of the inward rectifier K^+ current (I_{K1}), and the current reversed at −82 mV, close to the reversal potential of K^+ ions (fig. 5*B*). The remaining current had a linear voltage dependence with a reversal potential at about −60 mV, suggesting that it was also mainly carried by K^+ ions, possibly representing the ATP-sensitive K^+ current ($I_{K,ATP}$). Similar to the $I_{K,ATP}$ of teleost fishes (Paajanen and Vornanen 2002; Abramochkin and Vornanen 2014), the lamprey current was only partially (50%–60%) blocked by 10 μM glibenclamide, a specific blocker of $I_{K,ATP}$ (data not shown).

To identify the linear Ba^{2+}-insensitive current, single-channel recordings of K^+ currents were conducted under the same experimental conditions (temperature, external saline solution) as the whole-cell recordings (fig. 5*C*, 5*D*). Single-channel recordings were overwhelmed by frequent occurrence of large-amplitude (10 ± 1.2 pA at −120 mV) currents, with frequent and rapid openings and closings. This channel had a slope conductance of 61 pS. These features are characteristic of the ATP-sensitive K^+ channels. Small-amplitude (1.2 pA at −120 mV) and kinetically slower inward rectifier currents were also present, with a slope conductance of only 9 pS. Such data indicate that under these conditions, at least two types of inward

Table 1: Characteristics of atrial and ventricular action potentials (APs) of the lamprey (*Lampetra fluviatilis*) heart at 5°C

	Ventricle	Atrium	P^a
Resting membrane potential (mV)	−67.7 ± 9.8	−70.8 ± 4.2	NS
Action potential overshoot (mV)	18.7 ± 7.9	13.0 ± 10.1	<.01
Action potential duration (APD_{50}; ms)	550 ± 44.6	122.1 ± 28.5	<.001
Maximum rate of AP upstroke (V s^{-1})	5.4 ± .9	6.3 ± .6	NS

Note. Data are means ± SEM of 10–12 stable impalements of at least four hearts.

[a]This column shows statistically significant differences between atrial and ventricular APs. NS = not significant.

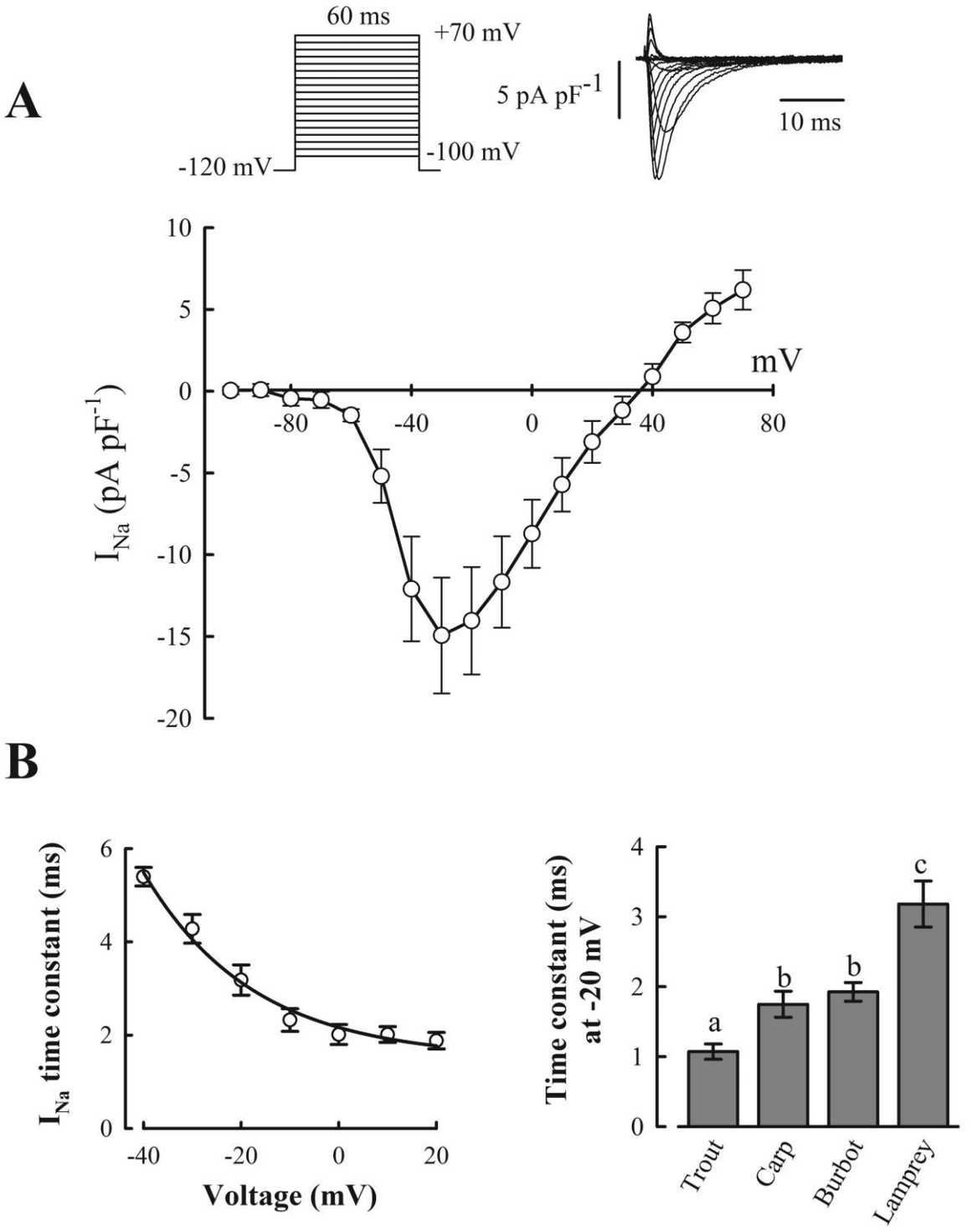

Figure 3. Sodium current (I_{Na}) of lamprey ventricular myocytes at 5°C. *A*, Mean current-voltage relation (± SEM) of I_{Na}. Voltage protocol and representative current tracings are shown at the top. *B*, Voltage dependence of the inactivation time constant (τ) of the lamprey I_{Na} (*left*) and comparison of I_{Na} inactivation time constants between lamprey and teleost fishes at 0 mV (*right*). The results are means (± SEM) of 10–12 cells. Dissimilar letters indicate a statistically significant difference ($P < 0.05$) between species. Data for trout (*Oncorhynchus mykiss*), crucian carp (*Carassius carassius*), and burbot (*Lota lota*) I_{Na} are from Haverinen and Vornanen (2004).

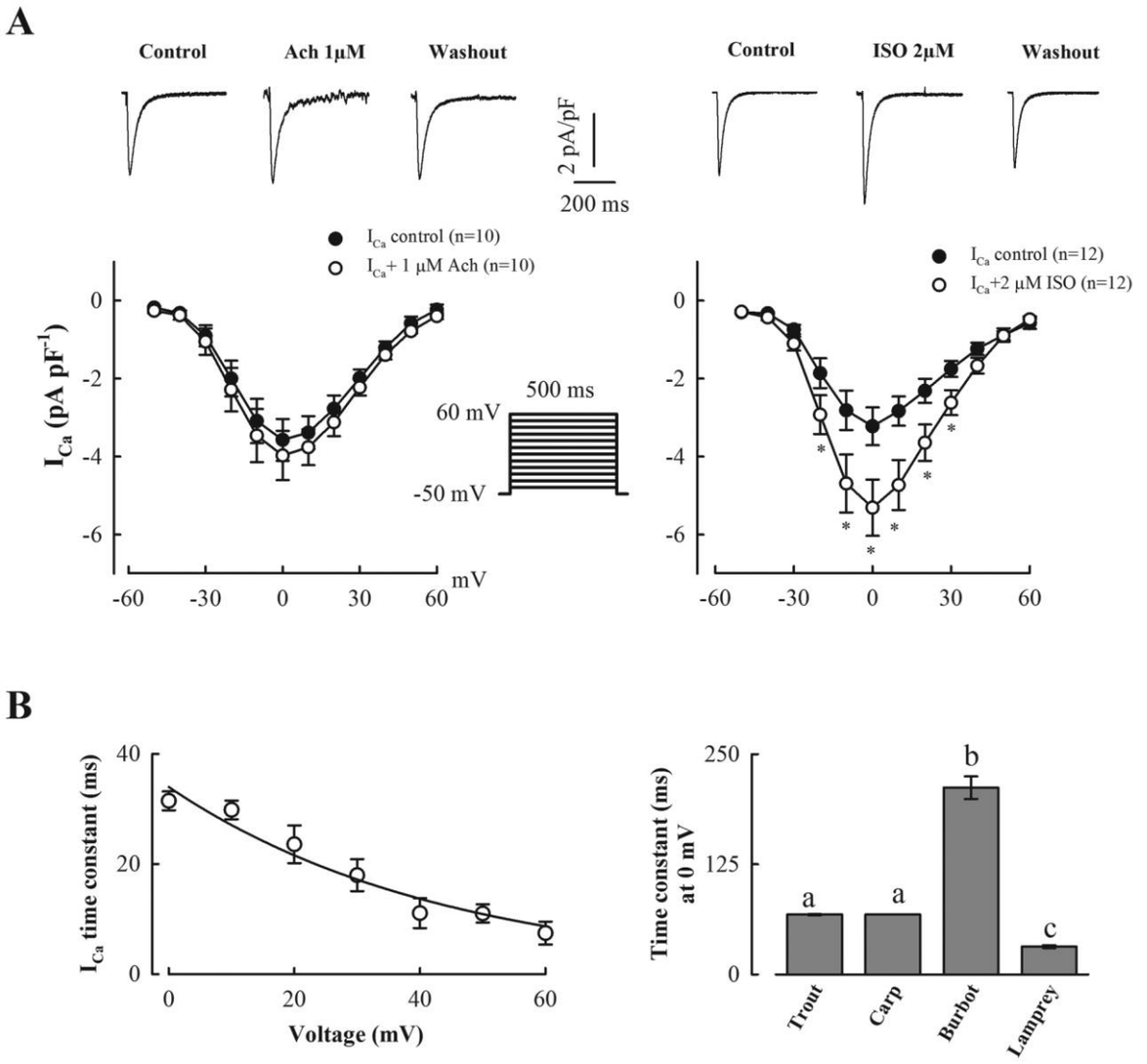

Figure 4. L-type Ca^{2+} current (I_{CaL}) of lamprey ventricular myocytes at 5°C. *A*, Mean results (±SEM) of current-voltage relationship in the absence and presence of 2 μM isoprenaline or 1 μM acetylcholine. The voltage protocol is shown between the two graphs. Representative recordings of I_{CaL} at +10 mV in the absence and presence of 2 μM isoprenaline (ISO) or 1 μM acetylcholine (Ach) and after the washout of the drugs are shown above the graphs. *B*, Voltage dependence of the inactivation time constant (τ) of lamprey I_{Ca} (*left*) and comparison of inactivation time constants between lamprey and teleost I_{Ca} at 0 mV (*right*). The results are means (±SEM) of 10–12 cells. Dissimilar letters indicate a statistically significant difference ($P < 0.05$) between species. Data for trout (*Oncorhynchus mykiss*), crucian carp (*Carassius carassius*), and burbot (*Lota lota*) I_{Ca} are from Shiels et al. (2000), Vornanen (1998), and Shiels et al. (2006), respectively.

rectifier channels are active ATP-sensitive K^+ channels and background inward rectifier (Kir2) channels.

Delayed Rectifier K^+ Current (I_{Kr})

The final rapid repolarization of fish cardiac AP is produced by inactivation of I_{CaL} and by activation of I_{K1} and the delayed rectifier K^+ currents, which may have two major components, slow (I_{Ks}) and fast (I_{Kr}; Hassinen et al. 2008*a*, 2011). Lamprey ventricular myocytes demonstrated a small E-4031-sensitive (2 μM) I_{Kr} current at 5°C with a peak tail current density of 1.3 ± 0.33 pA pF^{-1} (fig. 6). Very little outward current remained in the presence of E-4031, suggesting that the slow component of the typical delayed rectifier (I_{Ks}) is not present in lamprey ventricular myocytes.

Discussion

This study provides the first patch-clamp analysis of cardiac ion currents in a cyclostome vertebrate, the European river lamprey. It is demonstrated that the shape of ventricular AP and its ion current basis are in many respects similar to those of teleost fishes. However, electrical excitation shows several quantitative and qualitative differences from the teleostean

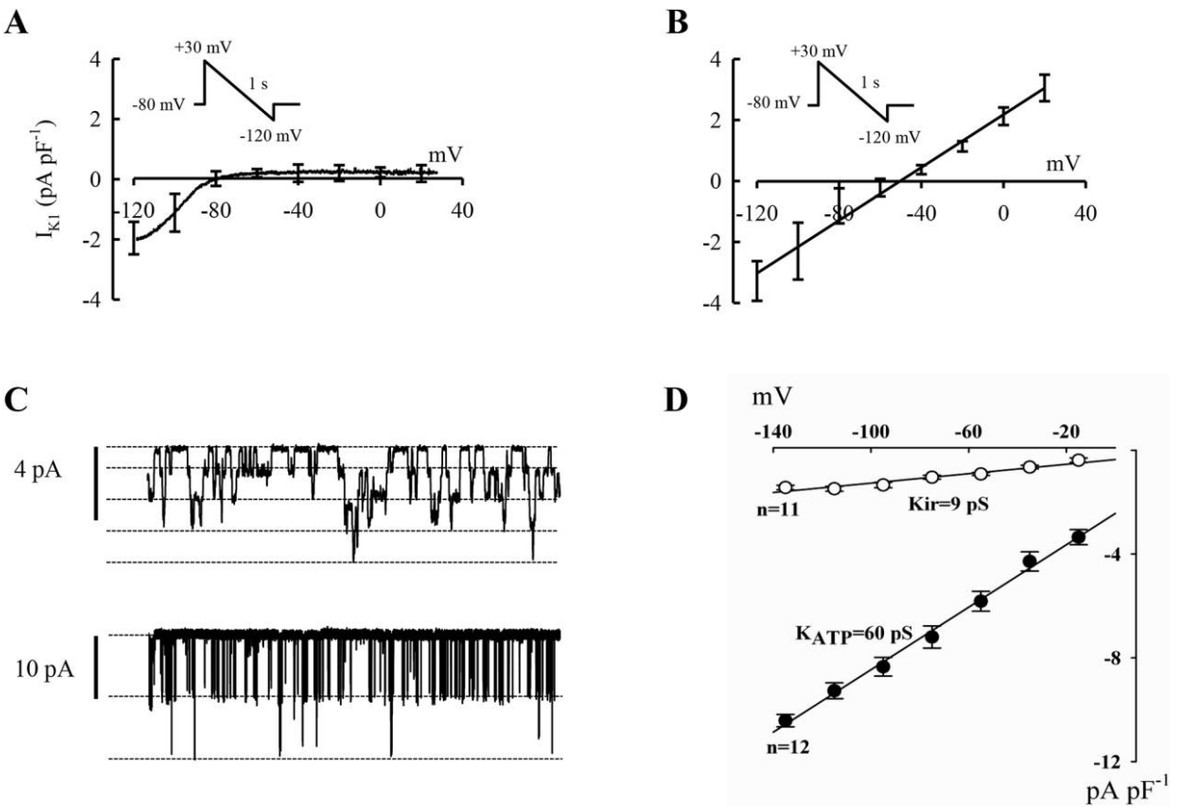

Figure 5. The inward rectifier potassium current (I_{K1}) and the ATP-sensitive K^+ current of the lamprey ventricular myocytes at 5°C. *A*, Current-voltage relation of the Ba^{2+}-sensitive inward rectifier K^+ current (I_{K1}). *B*, Voltage dependence of the Ba^{2+}-resistant current ($I_{K,ATP}$). The results are means ± SEM from 10 myocytes. *C*, Cell-attached single-channel recordings of K^+ currents in ventricular myocytes of the lamprey heart. Representative recordings from a membrane patch containing either inward rectifier channels (*top*) or ATP-sensitive K^+ channels (*bottom*). *D*, Mean current-voltage relations of inward rectifier potassium channels and ATP-sensitive K^+ channels of the lamprey ventricular myocytes at 5°C. The results are means ± SEM from 11 or 12 cells, as indicated.

type of cardiac activity in regard to ECG waveform, AP shape, and composition and densities of SL ion currents. Our findings on ventricular ion currents provide mechanistic explanations for the physiological characteristics of the lamprey heart and insights into the early evolution of vertebrate heart function.

Electrocardiogram and HR

The waveform of ECG in *Lampetra fluviatilis* is similar to what has been reported for the hagfish *Eptatretus cirrhatus* (Davie et al. 1987). The lamprey (cyclostome) ECG is different from the teleostean ECG in that it includes the PT wave, an electrical signal from the repolarization of the atrial tissue. This difference can be explained by two distinctive features of cardiac excitation in the European river lamprey: the very short duration of the atrial AP and the relatively slow velocity of impulse conduction over the heart. At similar temperatures, AP duration of the lamprey atrial muscle (122 ms) is only about 30%–45% of the atrial AP duration of the teleost heart (Haverinen and Vornanen 2009). Because of the short AP duration, depolarization of the atrial muscle is almost immediately followed by atrial repolarization and is not, therefore, masked below the ventricular depolarization (QRS complex), as happens in teleost hearts. Furthermore, the slower rate of impulse conduction (see "Resting Membrane Potential") in the lamprey heart extends the time delay between atrial and ventricular depolarizations, preventing the overlap of the PT wave and the QRS complex.

The in vivo basal HR of the liver lamprey at 5°C agrees well with previous measurements from the same species of 25 beats min^{-1} at 7°C for *L. fluviatilis* (Claridge and Potter 1975), while 25 beats min^{-1} at 5°C is reported for another species, *Entosphenus tridentatus* (Johansen et al. 1973). The HR of the river lamprey is similar to that of cold-active teleosts at similar temperatures, for example, rainbow trout (*Oncorhychus mykiss*; 32.5 beats min^{-1} at 6.5°C), and markedly higher than in more sluggish species such as goldfish (*Carassius auratus*; 10–14 beats min^{-1} at 5°C), crucian carp (*Carassius carassius*; 10–14 beats min^{-1} at 5°C), common carp (*Cyprinus carpio*; 8.8 beats min^{-1} at 5°C), eel (*Anquilla anquilla*; 9–13 beats min^{-1} at 5°C), and hagfish (*Eptatretus stautii*; 8.3 beats min^{-1} at 10°C; Priede 1974; Seibert 1979; Tsukuda et al. 1985; Matikainen and Vornanen 1992; Stecyk and Farrell 2006; Cox et al. 2010). These findings are consistent with the relatively high metabolic rate of this cyclostome subgroup, with standard oxygen consumption

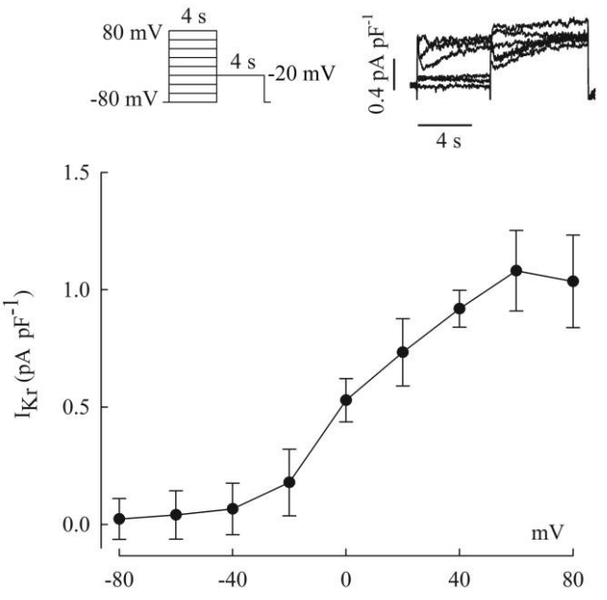

Figure 6. Density of the rapid component of the delayed rectifier K^+ current (I_{Kr}) in lamprey ventricular myocytes at 5°C. The current was measured as an E-4031-sensitive (2 μM) tail current using the voltage protocol shown above the graph. The results show the mean current density (±SEM) of 10 ventricular myocytes as a function of membrane voltage.

of adult lamprey (*Petromyzon marinus* and *L. fluviatilis*) being several times higher than that recorded at comparable temperatures for the hagfishes (Hardisty et al. 1989).

Rhythm of the vertebrate heart is characterized by marked HR variability. Similar to higher vertebrates, there is significant variability of HR in the resting lamprey, as shown by the elliptical shape of the Poincaré plot (Tulppo et al. 1996). Width and length of the Poincaré plots represent short- and long-term variability of HR, which are considered to result from the balance of parasympathetic and sympathetic regulation of HR. High vagal tone of the resting animal is generally considered to cause the short-term variability of HR (SD1) in mammals. It is interesting that in the lamprey, which is devoid of inhibitory cholinergic innervation, HR variability is largely similar to that of higher vertebrates, including a prominent short-term variability. Acceleration/deceleration of HR in lampreys is considered to be mediated via nicotinic stimulation of catecholamine release from cardiac chromaffin tissue (Augustinsson et al. 1956). Experiments using blockers of nicotinic acetylcholine and adrenergic adrenaline receptors are needed to solve the autonomic regulation of HR variability in lampreys.

Resting Membrane Potential

RMPs (−68 to −71 mV) of the lamprey atrium and ventricle are 11–14 mV more positive than the theoretical reversal potential of K^+ ions (−82 mV) and only slightly less negative than in many teleost fishes (Haverinen and Vornanen 2009). However, the RMP values measured in this study for *L. fluviatilis* are much more negative than previously reported for hagfish (*Myxine glutinosa*, *Myxine circifrons*, *Eptatretus stoutii*, and *Eptatretus deani*) hearts (−30 to −50 mV at 20°–23°C; Jensen 1965; Arlock 1975). RMP of the cell is measured relative to the external solution and is largely dependent on K^+ ion concentration of the bathing solution (assuming that RMP is determined by K^+ currents). Ion compositions of the saline solutions were not given in the previous studies, but assuming that they were similar to the K^+ concentration of hagfish blood (10 mM; Robertson 1954), the RMP of hagfish heart should be close to −65 mV, that is, much more negative than the reported values. A depolarized RMP would mean that a greater portion of the Na^+ channels are inactivated (unable to open), and, consequently, the rate of AP rise and velocity of AP propagation will be slow. At the reported RMP values of the hagfish hearts, I_{Na} would be completely abolished. The rate of AP upstroke in *L. fluviatilis* is more than five times faster (5.4–6.3 V s^{-1} at 5°C) than in *Myxine* (1 V s^{-1} at 20°–22°C; Arlock 1975), consistent with the relatively negative RMP values. On the other hand, the rate of upstroke in *L. fluviatilis* atrium and ventricle is somewhat slower than in teleost hearts under similar experimental conditions (Haverinen and Vornanen 2006), suggesting a slower AP transmission.

Cardiac Action Potentials

AP of the vertebrate heart is characterized by a long plateau phase, which is particularly prominent in ventricles and clearly shorter in the atrial chamber (Rosati et al. 2008; Haverinen and Vornanen 2009). Similarly, atrial AP of *L. fluviatilis* was markedly shorter than ventricular AP (see "Electrocardiogram and HR"). Functionally, the short atrial AP duration adjusts electrical excitation of the myocytes to high myofibrillar ATPase activity of atrial myocytes and thus to short atrial systole (Minajeva et al. 1997; Aho and Vornanen 1999), providing a rapid and powerful boost to ventricular filling. In this regard, the lamprey heart seems to be no exception.

Although the shape of the lamprey cardiac AP is similar to that of other vertebrates, there are several quantitative differences. In comparison to several teleost fishes under similar experimental conditions, the duration of ventricular AP is remarkably short (table 2; Haverinen and Vornanen 2009). Short APs and short contraction durations are typical of vertebrates that display an active lifestyle, relatively high HRs, and powerful cardiac contractions, as exemplified by salmonid and tuna fish hearts (Haverinen and Vornanen 2009; Shiels et al. 2011). The brevity of atrial and ventricular APs of the lamprey heart is consistent with the high resting HR values measured in vivo and the marked contribution of SR Ca^{2+} stores to contractile activation (Vornanen and Haverinen 2012).

There seems to be a contradiction between ventricular AP duration measured with microelectrodes (550 ms) from the excised tissue and that derived from the Q-T interval (831 ms) of the ECG. This difference is explained by the slow AP transmission over the ventricle (duration of QRS complex,

Table 2: Comparison of ventricular action potentials and cardiac ion current densities between the river lamprey (*Lampetra fluviatilis*) and selected teleost fishes

Species	RMP (mV)	APD_{50} (ms)	I_{Ca} (pA pF^{-1})	I_{Na} (pA pF^{-1})	I_{Kr} (pA pF^{-1})	I_{K1} (pA pF^{-1})
Lampetra fluviatilis	−67.7 ± 9.8	550 ± 44.6	3.0 ± .4	14.9 ± 3.5	1.0 ± .2	2.5 ± .3
Lota lota	−71.4 ± 3.3[c]	1,002.3 ± 39.8[c]	.8 ± .1[b]	33.2 ± 2.8[d]	1.8 ± .2[c]	2.3 ± .3[c]
Oncorhynchus mykiss	−82.7 ± 1.0[c]	702.2 ± 8.2[c]	.6 ± .2[b]	25.9 ± 1.4[d]	2.2 ± .1[c]	5.4 ± .5[c]
Carassius carassius	−74.4 ± 1.3[c]	1,477.3 ± 55.5[c]	1.1 ± .2[a]	9.4 ± 1.0[d]	.7 ± .1[c]	16.1 ± 1.2[c]
Perca fluviatilis	−69.4 ± 1.5[c]	815.6 ± 115.2[c]	.8 ± .2[e]	27.5 ± 4.5[e]	.9 ± .05[c]	2.6 ± .2[c]
Esox lucius	−71.0 ± 1.1[c]	794.8 ± 20.5[c]	ND	ND	.7 ± .04[c]	6.2 ± 1.0[c]
Rutilus rutilus	−70.0 ± 1.9[c]	890.5 ± 53.0[c]	ND	ND	1.0 ± .2[c]	8.0 ± 1.7[c]

Note. All ion current measurements are from patch-clamp experiments on isolated ventricular myocytes at 4°–5°C, except for the rainbow trout (*Oncorhynchus mykiss*) I_{Ca}, which is from atrial myocytes at 7°C. Resting membrane potential and action potentials were measured with sharp microelectrodes from multicellular ventricular preparations at 4°–5°C. Results are means ± SEM. ND, not determined. Species are *L. lota*, burbot; *C. carassius*, crucian carp; *P. fluviatilis*, perch; *E. lucius*, pike; *R. rutilus*, roach.

[a]Data are from Vornanen and Paajanen 2004.

[b]Data are from Shiels et al. 2003.

[c]Data are from Haverinen and Vornanen 2009.

[d]Data are from Haverinen and Vornanen 2004.

[e]J. Haverinen and M. Vornanen, unpublished data.

218 ms). Summing the impulse transmission time and the average AP duration of the ventricle gives a value of 550 + 218 = 768 ms, which is close to the measured Q-T value (831 ms).

Ion Current Basis of Cardiac Excitation

For single-cell patch-clamp experiments, cardiac myocytes were isolated from the lamprey with methods shown to produce large numbers of myocytes from teleost hearts. An adequate number of viable and Ca^{2+}-tolerant myocytes were obtained from lamprey ventricles but only a few viable myocytes from atria. Therefore, these patch-clamp results represent ion current properties of ventricular myocytes.

Electrical excitation of a cardiac myocyte is the result of concerted activity of Na^+, K^+, and Ca^{2+} ion channels generating the AP (Rosati et al. 2008). It is shown that lamprey ventricular myocytes express at least I_{Na}, I_{Ca}, I_{Kr}, I_{K1}, and $I_{K,ATP}$ currents, which are also present in teleost cardiac myocytes (Paajanen and Vornanen 2001; Vornanen et al. 2002*a*; Haverinen and Vornanen 2006; Hassinen et al. 2008*a*, 2008*b*, 2011). Current densities, inactivation kinetics, and the relative importance of those currents in cardiac excitation differ from those of the teleost ventricular myocytes, in particular in regard to I_{Ca}, I_{Na}, and $I_{K,ATP}$.

The density of cardiac I_{Na} in *L. fluviatilis* is lower than in teleost fishes, which (in addition to the less negative RMP) will contribute to the slow rate of AP upstroke and propagation. Also different from the teleost I_{Na}, the kinetics of I_{Na} inactivation in lamprey is remarkably slow. Unusually, this means that I_{Na} could contribute to the plateau duration of the ventricular AP. The distinct properties of the I_{Na} are probably related to the Na^+ channel composition of the lamprey ventricle, with prominent contribution by an ortholog of the vertebrate $Na_v1.1$ channel instead of $Na_v1.4$ and $Na_v1.5$ in teleosts and $Na_v1.5$ in mammals (Vornanen et al. 2011). A closer comparison of I_{Na} between lamprey and teleost hearts is needed to clarify the physiological importance of these differences.

Outward K^+ currents are repolarizing; that is, they maintain the negative RMP and contribute to final repolarization of AP. Of the three K^+ currents identified in lamprey, the density of I_{K1} is similar to that in teleost ventricular myocytes, while the density of I_{Kr} is lower in comparison to the I_{Kr} of several teleost species (table 2; Haverinen and Vornanen 2009). The total repolarizing power of these K^+ currents is less in lamprey than teleost myocytes and probably unable to generate the very short APs of the lamprey heart. On the other hand, ATP-sensitive K^+ channels seem to be open under normoxic conditions, generating the strongly repolarizing $I_{K,ATP}$. While this may explain the missing repolarizing power in lamprey myocytes, the finding is quite unexpected. Normally ATP-sensitive K^+ channels open in hypoxic insults when the intracellular ATP level drops, protecting myocytes against energy insufficiency via reductions in AP duration and cardiac contractility (Noma 1983). The high constitutive activity of ATP-sensitive K^+ channels in normoxic cardiac myocytes is a novel finding and suggests that participation of $I_{K,ATP}$ in regulation of AP duration may represent the original function of this channel.

Adrenergic and Cholinergic Control of the Cardiac I_{Ca}

L-type Ca^{2+} channels are phosphorylated and dephosphorylated by excitatory adrenergic and inhibitory cholinergic signaling, respectively, resulting in powerful control of cardiac contraction via I_{Ca} (McDonald et al. 1994; Mery et al. 1997). Considering that the lamprey heart completely lacks adrenergic innervation and that the cholinergic innervation is excitatory and mediated via nicotinic receptors, adrenergic and cholinergic regulation of the lamprey I_{Ca} is of interest. The isoprenaline-induced increase of I_{Ca} indicates that, despite the absence of adrenergic innervation, β-adrenergic receptors exist

on lamprey ventricular myocytes and that they are coupled to SL Ca^{2+} channels. On the other hand, insensitivity of I_{Ca} to acetylcholine shows that cholinergic stimulation of the lamprey heart is not mediated by the SL I_{Ca}. Collectively, these findings conform to the proposal that acetylcholine indirectly stimulates cardiac contractility by releasing catecholamines from the cardiac chromaffin cells (Augustinsson et al. 1956). The released catecholamines subsequently bind to the β-adrenergic receptors of cardiac myocytes and augment I_{Ca}.

Conclusions

Fairly high HR rate and short AP duration at low ambient temperature (5°C) are characteristic features of the river lamprey heart in common with cold-active teleost fishes, while the slow rate of impulse propagation separates the lamprey heart from teleost hearts. The electrophysiological phenotype of the lamprey cardiac myocytes (short AP) is consistent with the E-C coupling of the lamprey heart, which relies strongly on intracellular Ca^{2+} stores of the SR (Vornanen and Haverinen 2012). The above features of the lamprey myocardium are appropriate to generate short and powerful (localized) contractions, which propagate relatively slowly over the heart and squeeze blood into the ventral aorta. In this respect the lamprey heart might share features of cardiac function with its invertebrate predecessors, the urochordate tunicates. In the tubular heart of the sea squirt (*Ciona intestinalis*), contraction proceeds as a peristaltic wave over the heart, which is considered to provide almost complete emptying of the heart at each contraction (Kriebel 1967). Clearly, the cardiac function of this basal vertebrate includes both primitive and advanced traits. It remains to be shown to what extent the distinct properties of the lamprey heart (constitutive opening of ATP-sensitive K^+ channels, slow AP propagation) are shared with other basal vertebrates and whether the advanced features (e.g., short AP and reliance on SR Ca^{2+} stores) are restricted to lampreys and might be absent in hagfishes (Thomas et al. 1996). Studies on the molecular background of the physiological traits are needed to solve these issues (Wilson et al. 2014).

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