Electrical excitability of the heart in a Chondrostei fish, the Siberian sturgeon (Acipenser baerii)

Thomas Eliot Haworth,1 Jaakko Haverinen,2 Holly A. Shiels,1 and Matti Vornanen2

1University of Manchester, Faculty of Life Sciences, Manchester, United Kingdom; and 2University of Eastern Finland, Department of Biology, Joensuu, Finland

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Haworth TE, Haverinen J, Shiels HA, Vornanen M. Electrical excitability of the heart in a Chondrostei fish, the Siberian sturgeon (Acipenser baerii). Am J Physiol Regul Integr Comp Physiol 307: R1157–R1166, 2014.—Sturgeons (family Acipenseridae) are regarded as living fossils due to their ancient origin and exceptionally slow evolution. To extend our knowledge of fish cardiac excitability to a Chondrostei fish, we examined electrophysiological phenotype of the Siberian sturgeon (Acipenser baerii) heart with recordings of epicardial ECG, intracellular action potentials (APs), and sarcolemmal ion currents. Epicardial ECG of A. baerii had the typical waveform of the vertebrate ECG with Q-T interval (average duration of ventricular AP) of 650 ± 30 ms and an intrinsic heart rate of 45.5 ± 5 beats min−1 at 20°C. Similar to other fish species, atrial AP was shorter in duration (402 ± 30 ms) than ventricular AP (585 ± 40 ms) (P < 0.05) at 20°C. Densities of atrial and ventricular Na+ currents were similar (−47.6 ± 4.5 and −53.2 ± 5.1 pA/pF, respectively) and close to the typical values of teleost hearts. Two major K+ currents, the inward rectifier K+ current (I_{Kr}), and the delayed rectifier K+ current (I_{K1}) were found under basal conditions in sturgeon cardiomycocytes. The atrial I_{Kr} (3.3 ± 0.2 pA/pF) was about twice as large as the ventricular I_{Kr} (1.3 ± 0.4 pA/pF) (P < 0.05) conforming to the typical pattern of teleost cardiac I_{Kr}. Divergent from other fishes, the ventricular I_{Kr} was remarkably small (−2.5 ± 0.07 pA/pF) and not different from that of the atrial myocytes (−1.9 ± 0.06 pA/pF) (P > 0.05). Two ligand-gated K+ currents were also found: ACh-activated inward rectifier (I_{K_ACh}) was present only in atrial cells, while ATP-sensitive K+ current (I_{K_ATP}) was activated by a mitochondrial blocker, CCCP, in both atrial and ventricular cells. The most striking difference to other fishes appeared in Ca2+ currents (I_{Ca}). In atrial myocytes, I_{Ca} was predominated by nickel-sensitive and nifedipine-resistant T-type I_{Ca}, while ventricular myocytes had mainly nifedipine-sensitive and nickel-resistant L-type I_{Ca}. I_{CaL}/I_{CaT} ratio of the sturgeon atrial myocytes (2.42) is the highest value ever measured for a vertebrate species. In ventricular myocytes, I_{CaL}/I_{CaT} ratio was 0.09. With the exception of the large atrial I_{CaT} and small ventricular I_{Kr}, electrical excitability of A. baerii heart is similar to that of teleost hearts.

fish heart; action potential; ion currents; electrocardiogram

Electrical excitation of sarcolemma (SL) triggers contraction of the cardiac myocyte and enables orderly spread of contraction over different compartments of the vertebrate heart. Sequential activation and partially overlapping flow of inward and outward currents through Na+, Ca2+, and K+-specific ion channels of the SL generates an electrical signal, the cardiac action potential (AP). The shape of AP waveform varies between species and between parts of the heart within one species (11). Na+ current (I_{Na}) initiates the depolarization of SL and produces the fast upstroke phase of AP. The density of I_{Na} determines the rate of AP propagation over the heart. Efflux of K+ ions via various K+ channels and simultaneous influx of Ca2+ ions through Ca2+ channels maintains the long duration of cardiac AP. Finally, the total outward K+ current (I_{K}) overcomes the inward Ca2+ current (I_{Ca}) and repolarizes membrane potential back to the resting level. This basic sequence of events is similar in all vertebrate hearts, but the underlying ion currents and ion channels may significantly vary between vertebrate species, partly depending on phylogeny and partly because of species-specific physiological demands or adaptation/acclimation of the animal to special environmental conditions (16, 40). Furthermore, under different environmental and physiological stresses (hypoxia, exercise, and heat), ligand-gated ion channels provide protection to cardiac myocytes via ATP-sensitive K+ current (I_{K_ATP}) (33, 36, 53) and ACh-activated inward rectifier K+ current (I_{K_ACh}) (26, 42).

Cardiac function is a key physiological variable in environmental adaptation and acclimation of aquatic vertebrates by delivering oxygen and nutrients to tissue cells and providing homeostatic balance between body parts (14, 38, 50). Excitability of the heart is an integral part of cardiac function, and therefore, understanding of cardiac excitability and its nervous and humoral control may provide clues to the mechanisms of adaptation of animals to their habitat conditions. To date, the majority of studies on fish cardiac function, have been carried out on hearts of teleost fishes, while relatively little research has been done on other fish groups.

The ray-finned fishes (class Actinopterygii) are the most species-rich and diversified group of vertebrate animals, comprising about 27,000 species (about 99% of all fishes). The great majority of the ray-finned fishes belong to the infraclass Teleostei, which by far is the most successful fish group, probably owing to their rich gene supply as a result of the whole genome duplication in this lineage (30). Teleost fishes have successfully inhabited almost all aquatic environments of the earth by evolving numerous physiological adaptations. Chondrostei are a subclass of the ray-finned fishes, but most members of this group became extinct in the late Jurassic, and currently, they comprise only ~50 species. Sturgeons (family Acipenseridae) separated from the other ray-finned fishes more than 300 million years ago and currently constitute about half (25) of the extant Chondrostei species. Sturgeons are often regarded as “living fossils”, since their evolution has been very slow, with little morphological and molecular changes (22).

The current study extends research of cardiac excitability to Acipenser baerii, the Siberian sturgeon. A. baerii are found in all major Siberian river basins, primarily the Ob River and its tributaries (41). By analyzing electrical excitability in A. ba-
erii, we hoped to gain novel insight into electrophysiological characteristics of the Chondrostei and to reveal similarities and differences in ion channel function between Chondrostei and Teleostei, which might be associated with success of these groups in environmental adaptation, i.e., we tested the hypothesis that the evolutionary success of extant sturgeons and sturgeon/cultured species/Acipenser baerii/en). Experiments were started after a 2-wk habituation of the fish under constant temperature and light regimes. The sturgeon were fed on a diet of commercial trout feed (naturaG; EWOS, Floro, Norway) 5 days/wk. The experimental protocols were approved by the Animal Experiment Board in Finland (permission nos. STH998 A and PH472 A).

Recording of epicardial electrocardiogram in vitro and intracellular action potentials. Fish were stunned with a blow to the head, and the spine was cut before the heart was excised. The heart was first used for epicardial ECG recordings in vitro. A metal cannula was inserted into the ventricle of the excised heart through the bulbus arteriosus. The heart was retrogradely perfused with a constant flow (4 ml/min) of physiological saline solution (in mM) [150 NaCl, 5.4 KCl, 1.8 CaCl2, 1.2 MgCl2, 10 glucose, and 10 HEPES (pH 7.7)] for the duration of the ECG recordings, and the solution was continually oxygenated with 100% O2. Two thin electrodes (seven-strand Teflon-coated wire, 0.23 mm in diameter; A-M Systems, Sequim, WA) separated by a short gap were gently placed on the surface of the ventricle, one at the base of the ventricle and the other about 5 mm apically. The reference electrode was submerged in oxygenated external saline solution in a small petri dish where the heart was placed. The ECG activity was continually monitored using LabChart 7 (AD Instruments, Oxford, UK). The heart was allowed to equilibrate for roughly 45 min before the ECG data were collected. The recordings were made at room temperature (~20°C).

The same heart was then used for AP recordings (16). The heart was opened and fixed, using insect pins, to the bottom of a Sylgard-coated 10-ml recording chamber filled with continuously oxygenated (100% O2) physiological saline solution (see above). The temperature of the bath was maintained at 20°C using a circulating water bath and was continually monitored using a thermocouple. The solution was allowed to equilibrate for roughly half an hour before APs were recorded using glass microelectrodes. Pipettes were pulled from borosilicate glass (Garner, Claremont, CA) and were filled with 3 M KCl. Recordings were analyzed off-line using Clampfit software (Axon Instruments, Sunnyvale, CA) to calculate resting membrane potential (RMP), AP overshoot, AP amplitude, and AP duration at 50% of repolarization (APD50).

Whole-cell patch clamp. Ventricular and atrial myocytes were isolated with enzymatic digestion and were used within 8 h from isolation (47, 49). Whole-cell patch-clamp experiments were carried out using an Axopatch 1-D (Axon Instruments) and an EPC-9 amplifier (HEKA Instruments, Lambrecht, Germany). Myocytes were superfused with external saline solution regulated to 18°C by Peltier devices (TC-10; Dagan, Minneapolis, MN). Patch pipettes were filled with electrode solutions, which varied depending on the experiment being carried out (see Solutions). Electrode resistance and series resistance were 2.2 ± 0.03 and 7.2 ± 0.3 MΩ (means ± SE), respectively. Cell capacitance was determined for every myocyte [31.7 ± 0.8 and 37.7 ± 1.2 pF, means ± SE for atrial (n = 101) and ventricular myocytes (n = 126), respectively] for calculation of current densities. The digitized data were recorded using Clampfit 10.3 software (Axon Instruments), and the recordings were analyzed using Clampfit 10.3 and SigmaPlot 11.0 software (SPSS, Chicago, IL). The results of the patch-clamp studies are presented as means ± SE of 5–17 myocytes (n) from three to six animals for each experiment.
Recording of delayed rectifier and inward rectifier K⁺ current. To measure delayed rectifier (I_{Kr}) and inward rectifier (I_{K1}) currents from the same myocyte, a specific sequence of voltage protocols were used. To prevent Na⁺, Ca²⁺, and ATP-sensitive K⁺ currents, TTX (0.5 µM; Tocris Cookson, Bristol, UK), nifedipine (Nif; 10 µM; Sigma, St. Louis, MO) and glibenclamide (10 mM; Sigma) were included in the external solution, respectively. The current-voltage relationship of I_{Kr} tail current was determined at a holding potential (HP) of −80 mV using a two-step protocol. A series of prepulses (3 s in duration) from −80 to +80 mV were used to activate the channels to different degrees, and then the I_{Kr} tail current was determined at −20 mV under a constant driving force for K⁺ efflux (16). For each myocyte used, this protocol was run first in the absence, then in the presence of the specific I_{Kr} blocker E-4031 (2 mM; Tocris Cookson). I_{Kr} was measured as an E-4031-sensitive current (15).

After the prevention of I_{Kr} current using E-4031, I_{K1} current could then be determined. I_{K1} was elicited using a protocol of repolarizing voltage ramps (1 s in duration) from +60 to −120 mV at a HP of −80 mV. The protocol was then run again with the addition of 0.5 mM Ba²⁺ to completely block I_{K1} channels (15). I_{K1} was obtained by subtracting the current after Ba²⁺ addition from the current recorded before Ba²⁺ addition. The entire protocol, from I_{Kc} to I_{K1} typically took between 10 and 15 min to complete.

Recording of sodium current. I_{Na} was elicited from a −120 mV HP. The currents were recorded at a sampling rate of 10 kHz and the recordings were low pass filtered at 5 kHz (17). To ensure sufficient voltage control, a minimum of 80% series resistance compensation was used. To prevent Ca²⁺ currents, 10 µM Nif was included in the external solution. I_{Na} currents were elicited using a protocol that applied 60-ms depolarizing pulses between −100 and +70 mV in 10-mV steps. Steady-state activation and inactivation curves were determined with established voltage protocols and using Boltzmann fits (17). To determine TTX sensitivity of sodium current (I_{Na, TTX}), different concentrations of the drug (10⁻¹⁰–10⁻⁷ M) were cumulatively added into the external saline solution, and the curves were fitted using a three-parameter sigmoid equation with one drug binding site (45).

Recording of calcium currents. Calcium currents (I_{Ca}) were recorded in the presence of 0.5 µM TTX, which completely blocks I_{Na}. Current-voltage relationships were determined by eliciting I_{Ca} from two different HPs (−80 and −60 mV). Because of the length of these protocols a significant run-down of I_{Ca} occurred, making comparison of I-V relationships between the two HPs inaccurate. Therefore, recordings from the two HPs were obtained from separate cells.

To compare relative current densities of L-type and T-type currents (I_{CaL, T}, I_{CaT}) between atrial and ventricular myocytes, a short two-step voltage protocol was used, allowing recordings to be carried out on the same cell without complications from the rundown of I_{Ca}. Using the HP of −80 mV, we first stepped membrane voltage to −40 mV for 300 ms to mainly activate I_{CaT}. This was immediately followed by a second step to 0 mV for 300 ms to mainly activate I_{CaL}. This protocol was performed in the presence of 20 µM Nif to block I_{K1} and in the presence of 20 µM Nif + 300 µM Ni²⁺ to block both I_{CaL} and I_{CaT}. Effects of β-adrenergic on T-type and L-type I_{Ca} was studied by adding 1 µM isoproterenol (Iso) in the external solution.

Recording of ligand-gated ion currents. ATP-sensitive K⁺ current (I_{KATP}) was recorded using the same ramp protocol and the same external saline solution as for I_{Kc}. Pipette solution was the same used for recording I_{Kc} and I_{K1} with the exception that MgATP was omitted (37). I_{KATP} was induced by 3 mM CCCP (carbonyl cyanide m-chlorophenyl hydrazine, a mitochondrial uncoupler), in the external saline (2). I_{KATP} was obtained as a difference current between the recordings in the presence and absence of CCCP. Glibenclamide (10 µM) was used as a specific blocker of I_{KATP}.

ACh-induced inward rectifier K⁺ current (I_{KACCh}) was recorded using the same protocols and solutions as for I_{K1} and I_{Kc}. I_{KACCh} was
induced by 10 μM carbacholine (CCh) in the external saline. \( I_{K_{ACH}} \) was obtained as a difference current in the presence of CCh and CCh + 1 μM atropine, a specific blocker M2 receptors (1, 51).

**Solutions.** Different external and internal saline solutions were used in patch-clamp experiments to prevent unwanted ion conductances. Composition of solutions is given as millimoles per liter, and pH was adjusted at 20°C.

External saline solution used in \( I_{K_{ACH}} \)/\( I_{K_{ACH}} \) recordings consisted of (in mM): 150 NaCl, 5.4 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 10 glucose, and 10 HEPES at pH 7.7. Pipette solution used in \( I_{K_{ACH}} \)/\( I_{K_{ACH}} \) recordings consisted of (in mM): 140 KCl, 1 MgCl₂, 5 EGTA, 4 MgATP, and 10 HEPES at pH 7.2.

External saline solution used in \( I_{Na} \) recordings consisted of (in mM): 20 NaCl, 120 CsCl, 0.5 CaCl₂, 10 glucose, and 10 HEPES at pH 7.7. Pipette solution used in \( I_{Na} \) recordings consisted of (in mM): 5 NaCl, 130 CsCl, 1 MgCl₂, 5 EGTA, 5 Mg₂ATP, and 5 HEPES at pH 7.2.

External saline solution used in \( I_{Ca} \) and \( I_{Ca} \) recordings consisted of (in mM): 130 NaCl, 5.4 CsCl, 1.5 MgSO₄, 0.4 NaH₂PO₄, 2.0 CaCl₂, 10 glucose, and 10 HEPES at pH 7.7. Pipette solution for \( I_{Ca} \) and \( I_{Ca} \) recordings consisted of (in mM): 130 CsCl, 5 Mg₂ATP, 15 tetraethylammonium chloride, 1 MgCl₂, 5 oxaloacetate, 5 sucinate, 5 EGTA, and 10 HEPES at pH 7.2.

In addition, specific blockers were used to prevent selected ion conductances, as detailed above for each ion current.

**Statistics.** A Student’s \( t \)-test for independent samples or a nonparametric Kruskal-Wallis test was used to compare ion current and AP data between atrial and ventricular myocytes. A value of 0.05 was set as the limit for statistical significance.

**RESULTS**

**ECG recordings.** The epicardial ECG of *A. baerii* heart has the typical waveform of vertebrate heart ECG with P, QRS, and T waves (Fig. 1). The biphasic nature of T wave is typical of the typical waveform of vertebrate heart ECG with P, QRS, and T waves (Fig. 1). The biphasic nature of T wave is typical of vertebrate heart ECG with P, QRS, and T waves (Fig. 1). The biphasic nature of T wave is typical of vertebrate heart ECG with P, QRS, and T waves (Fig. 1). The biphasic nature of T wave is typical of vertebrate heart ECG with P, QRS, and T waves (Fig. 1). The biphasic nature of T wave is typical of vertebrate heart ECG with P, QRS, and T waves (Fig. 1).

**Characteristics of atrial and ventricular membrane potentials.** Consistent with the ECG recordings, the shape of ventricular AP showed a long plateau phase. The duration of AP was about 20 ms longer in the ventricle than in the atrium (585 ± 40 vs. 542 ± 33 ms, \( P < 0.05 \)) (Fig. 2). Amplitude of AP was slightly higher in the ventricle (93 ± 7 mV) than in the atrium (79 ± 5 mV) (\( P < 0.05 \)), while no significant difference was found between atrial and ventricular RMP (−75.5 ± 9 vs. −82.7 ± 5 mV) (\( P > 0.05 \)) or AP overshoot (6.5 ± 3 vs. 8.5 ± 2 mV) (\( P > 0.05 \)).

**Potassium currents \( I_{K_{1}} \) and \( I_{K_{11}} \).** Two major K⁺ currents, \( I_{Kr} \) and \( I_{K_{11}} \), were present in sturgeon atrial and ventricular myocytes under basal conditions. When these currents were blocked with E-4031 and Ba²⁺, respectively, only a linear leakage current was left (not shown), suggesting no other major K⁺ currents are activated under basal conditions in sturgeon cardiac myocytes.

A robust outward tail current of the \( I_{K_{1}} \) was present in sturgeon cardiac myocytes (Fig. 3). Furthermore, a prominent difference in current density existed between atrial and ventricular \( I_{K_{1}} \), as the peak tail current density was 2.3 times as high in atrial myocytes (3.3 ± 0.2 pA/pF) as in ventricular myocytes (1.3 ± 0.4 pA/pF) (\( P < 0.05 \)).

The background inward rectifier current (\( I_{K_{11}} \)) was present in both atrial and ventricular myocytes. Interestingly, no significant difference was found between the density of atrial (−1.9 ± 0.06 and 0.4 ± 0.02 pA/pF at −120 and 0 mV, respectively) and ventricular (−2.5 ± 0.07 and 0.3 ± 0.03 pA/pF) \( I_{K_{11}} \) (\( P > 0.05 \)).

**Sodium current, \( I_{Na} \).** The \( I_{Na} \) current activated around −70 mV and peaked at near −30 mV in ventricular cells (−53.2 ± 5.1 pA/pF) and −20 mV in atrial cells (−47.6 ± 4.5 pA/pF) (\( P > 0.05 \)). Activation of the atrial \( I_{Na} \) seemed to occur at slightly more positive voltages compared with the ventricular \( I_{Na} \) (Fig. 4). The differences in current density and steady-state activation were not, however, statistically significant (\( P = 0.06 \)). No differences were evident in voltage dependence of steady-state inactivation. \( I_{Na} \) of atrial and ventricular myocytes was highly sensitive to TTX with half-maximal inhibition at 3.4 and 3.7 nM, respectively.

**Calcium currents \( I_{Ca} \) and \( I_{Ca} \).** In atrial myocytes, amplitude of \( I_{Ca} \) elicited from the HP of −80 mV peaked at around −30 mV (−3.52 ± 0.39 pA/pF). After −30 mV, \( I_{Ca} \), decreased...
steadily until around 0 mV, where the decrease in $I_{\text{Ca}}$ amplitude leveled out before turning close to zero at +60 mV (Fig. 5). The broad and complex $I-V$ relationship suggested the presence of both $I_{\text{CaT}}$ and $I_{\text{CaL}}$ in sturgeon atrial myocytes. When elicited from the HP of −80 mV, voltage dependence of $I_{\text{Ca}}$ was narrower with a single peak at 0 mV ($-1.44 \pm 0.27 \text{ pA/pF}$) ($P < 0.05$). In ventricular myocytes, the $I-V$ relationship of $I_{\text{Ca}}$ was not dependent on the HP. A single peak of $I_{\text{Ca}}$ occurred at 0 mV whether induced from −80 mV ($-2.85 \pm 0.64 \text{ pA/pF}$) or −60 mV ($-2.31 \pm 0.71 \text{ pA/pF}$) ($P > 0.05$), suggesting the presence of only $I_{\text{CaL}}$.

To further elucidate the relative contribution of $I_{\text{CaT}}$ and $I_{\text{CaL}}$ to the total $I_{\text{Ca}}$, the currents were triggered from the HP of −80 mV in successive steps to −40 and 0 mV to elicit mainly $I_{\text{CaT}}$ and $I_{\text{CaL}}$, respectively. The same voltage protocol was then repeated in the presence of 20 μM Nif and 20 μM Nif plus 300 μM Ni²⁺ to preferentially block first $I_{\text{CaL}}$ and then both $I_{\text{CaL}}$ and $I_{\text{CaT}}$. In atrial myocytes, the current at −40 mV was 2.42 times as large as the current at 0 mV (Fig. 6). In contrast to atrial myocytes, in ventricular cardiomyocytes the current at −40 mV was only 9% of the current at 0 mV. These findings suggest predominance of $I_{\text{CaT}}$ in atrial cells and $I_{\text{CaL}}$ in ventricular cells. Consistent with that, Nif blocked only 20% of the atrial $I_{\text{Ca}}$ at −40 mV, but 66% of the $I_{\text{Ca}}$ at 0 mV. In ventricular myocytes, Nif blocked 85% of the $I_{\text{Ca}}$ at 0 mV, and 27% of the $I_{\text{Ca}}$ at −40 mV. In the presence of both Nif and Ni²⁺, $I_{\text{Ca}}$ was almost abolished in both atrial and ventricular myocytes.

Iso (1 μM), a β-agonist, strongly stimulated $I_{\text{Ca}}$ at 0 mV (202 ± 52 and 175 ± 63% in atrial and ventricular cells, respectively) but had much less effect at −40 mV (40 ± 18 and 72 ± 28% in atrial and ventricular cells, respectively) (Fig. 7).

$\text{Ligand-gated K}^+$ currents. CCh (10 μM) induced a prominent $I_{\text{KACH}}$ in atrial myocytes (4.6 ± 0.5 pA/pF) but was without effect on ventricular myocytes (0.4 ± 0.3 pA/pF) ($P < 0.05$). The atrial $I_{\text{KACH}}$ was completely blocked by 1 μM atropine, a blocker of muscarinic cholinergic receptors (Fig. 8A).

A very large $I_{\text{KATP}}$ was induced by 3 μM CCCP in atrial myocytes (54.5 ± 5.4 pA/pF at +50 mV), while ventricular $I_{\text{KATP}}$ was only 6.1 ± 1.1 pA/pF ($P < 0.05$) (Fig. 8B). $I_{\text{KATP}}$ was inhibited 92 ± 12 and 84 ± 16% by 10 μM glibenclamide in ventricular and atrial myocytes, respectively ($P > 0.05$) (not shown).

**DISCUSSION**

The present results show that in most respects electrical excitability of *A. baerii* heart, a chondrostei species, is similar to that of the teleost heart. The major distinctive characteristics of *A. baerii* cardiac ion currents compared with cardiac ion currents of teleost fishes are the predominance of $I_{\text{CaT}}$ in atrial myocytes and the small size of $I_{K1}$ current in ventricular myocytes. The most parsimonious explanation for the functional similarities is that those properties were already present in the common ancestor of Teleostei and Chondrostei over 300 million years ago. On the other hand, the marked differences in $I_{\text{CaT}}$ and $I_{K1}$ might be outcomes from differences in genome evolution between these two fish groups. It remains to be...
shown to what extent the similarities in cardiac ion currents are based on products of homologous genes in Chondrostei and Teleostei and whether convergent evolution of ion channel function is involved.

**Electrocardiogram and action potential.** The waveform of epicardial ECG in *A. baerii* shows P, QRS, and T waves and, therefore, conforms to the general vertebrate pattern (6, 43). However, multielectrode arrays or optical mapping are needed for obtaining more detailed data on transmural conduction and spatial spread of electrical activity over different chambers of the sturgeon heart (35, 45, 52). The intrinsic HR of the Siberian sturgeon with 45.5 beats min$^{-1}$ at 21°C is similar to the value (about 45 beats min$^{-1}$ at 18°C) measured by Maxime at al. (27) for the same species. Furthermore, the duration and shape of cardiac APs in *A. baerii* display similarities with what would be expected in teleost fishes and other vertebrates at comparable temperature. The APD$\text{50}$ of cardiomyocytes being significantly longer in ventricular cells than in atrial cells is also characteristic for vertebrate animals (16). After comparing sturgeon APDs to recordings from teleost species, the length of sturgeon atrial and ventricular APs was found to fit well within typical durations for teleost fish, sitting between the relatively short APD$\text{50}$ of the rainbow trout (*Oncorhynchus mykiss*) and the much longer APD$\text{50}$ of the crucian carp (*Carassius carassius*). Short APs are generally associated with highly active, migrating, and predatory species, such as trout, salmon, and tuna fish (13, 16). Therefore, the APD$\text{50}$ of *A. baerii* fits with its lifestyle characteristics of a migrating but slow-moving benthic feeding fish and with the relatively low metabolic rate of sturgeons (9, 34).

**Sodium current.** No significant difference was found in the density of sturgeon $I_{\text{Na}}$ between atrial and ventricular myocytes. Since $I_{\text{Na}}$ determines the rate of AP propagation over the heart, the present findings suggest a similar impulse velocity in atrial and ventricular muscle. In this regard, the present findings are similar to earlier findings for rainbow trout atrial and ventricular myocytes (17). Also, the density of sturgeon $I_{\text{Na}}$ is similar as in other teleost fishes under comparable experimental conditions, but significantly larger than the $I_{\text{Na}}$ of a cyclostome (*Lampetra fluviatilis*) ventricular myocytes (15a). Furthermore, akin to cardiac $I_{\text{Na}}$ of other teleosts, yet different from mammalian $I_{\text{Na}}$, the sturgeon $I_{\text{Na}}$ is highly sensitive to TTX in both atrial and ventricular myocytes (17, 48).

**Potassium currents.** Similar to other fishes, $I_{\text{K1}}$ and $I_{\text{Kr}}$ are the two major $K^+$ currents of *A. baerii* cardiac myocytes. The background inward rectifier $K^+$ current, $I_{\text{K1}}$, maintains the negative RMP and contributes to the final phase-3 repolarization of AP. It is an indispensable part of atrial and ventricular ion channel composition by maintaining quiescence of these cardiac compartments in the absence of stimulus from the pacemaker tissue. Usually, the density of $I_{\text{K1}}$ is much smaller in atrial than ventricular myocytes of the vertebrate heart, as noted also for a number of fish species (16, 19). The smaller $I_{\text{K1}}$ of atrial myocytes makes the muscle easily excitable by depolarization from a relatively small number of pacemaker cells.

Unlike many other vertebrates, the $I_{\text{K1}}$ of sturgeon ventricular myocytes was equally small as in atrial myocytes and much smaller than the ventricular $I_{\text{K1}}$ of teleost fishes. It would be expected that this characteristic would make ventricular...
This shows that the low larger density of was found to be over twice as high as in atrial myocytes and in a few species (e.g., guinea-pig) also in ventricular myocytes (5, 31) (Table 1). Functionally, ICaT is an important current component in pacemaker tissue and cardiac conduction pathways of mammals, birds, and frogs (25).

The present results show that ICaT is the major Ca2+ current of the sturgeon atrial myocytes, although a substantial ICaL is also present. ICaT/ICaL ratio of the sturgeon atrial myocytes is exceptionally high (2.42) among atrial and ventricular myocytes of the vertebrate heart (Table 1). ICaT was also present in some sturgeon ventricular myocytes giving an average ICaT/ICaL ratio of 0.09. In fishes, ICaT has been previously reported for (cultured) embryonic and adult cardiac myocytes of the zebrafish (Danio rerio) (7, 32). In ventricular myocytes of the shark (Squalus acantias), the spiny dogfish heart, the density of ICaT is similar to the density of ICaL (0.92) (28).

The role of ICaT in pacemaker and conduction cells of the heart is well documented, but its physiological role in working myocytes of atrial and ventricular muscle is still poorly understood. In this respect, it is interesting that ICaT is so strongly expressed in the sturgeon atrial tissue. Because of its activation at negative membrane potentials (the peak around −40 mV) and fast inactivation kinetics, it should not be active at the plateau voltages of cardiac AP (around 0 mV) and, therefore, Ca2+ influx through T-type channels cannot significantly contribute to AP plateau, Ca2+ loading of the sarcoplasmic reticulum and contractile regulation (21). Similar to INa, ICaT is activated at around −70 mV and by this means, it could increase excitability (depolarization power) of atrial myocytes around the threshold potential of AP. However, it’s unlikely to have any large effect on the rate of AP upstroke or propagation, since its density is much lower compared with INa.

Notably, ICa of A. baerii heart is strongly stimulated by Iso. The β-adrenergic effect is probably solely due to the activation of ICaL, since the smaller stimulation of ICa seen at −40 mV is attributable to activation of a few L-type Ca channels as a consequence of leftward shift in voltage dependence of ICaL activation (44, 46). Blood adrenaline and noradrenaline are strongly increased in hypoxic A. baerii, and therefore, ICaL is likely to play significant role in positive inotropic (force increasing) response of the sturgeon heart at initial phases of oxygen shortage (27). However, the response is expected to be weaker in the atrium than in the ventricle, because ICaT is insensitive to β-adrenergic stimulation (29).

Ligand-gated K+ currents. ATP-sensitive inward rectifier channels and ACh-activated inward rectifier channels are intimately involved in hypoxia responses of the vertebrate heart (33, 53). IKATP is activated in hypoxia and under other stressful conditions (e.g., in heat exposure of fish), providing cardioprotection by shortening of AP and consequent reduction in force of contraction (10, 36). IKACH is also involved in hypoxia responses by hypoxic bradycardia and reduction of atrial contraction force in fishes (3, 51). The presence of IKATP in sturgeon atrial and ventricular myocytes indicates that A. baerii are able to reduce electrical excitability during environmental hypoxia or thermal stress. Similarly, the large IKACH of atrial

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**Fig. 7. Effects of β-adrenergic activation with 1 μM isoproterenol on ICa of A. baerii atrial (A) and ventricular (B) myocytes.** Currents were elicited from the holding potential of −80 mV by consecutive 300-ms depolarizing voltage steps to −40 mV and 0 mV. The bar graphs show mean current densities (±SE) of seven ventricular and seven atrial myocytes from three fishes. The voltage protocol and original ICa recordings are shown on right. *Statistically significant differences (P < 0.05) in current density between the pulse potentials (−40 and 0 mV). Different letters (a, b, c) signify differences between treatments at the same pulse potential.*

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This shows a strong phylogenetic link (16).

**Calcium currents.** Two major types of Ca2+ current are present in the vertebrate heart (29). Compared with ICaT, ICaL is activated at more depolarized membrane potentials (peak current around 0 mV), is kinetically slower and is blocked by Nif and other dihydropyridines. ICaL is important in maintaining the long plateau phase of cardiac AP and regulation of excitation-contraction coupling (29). ICaT has the peak current around −30 mV, is inactivated by depolarized holding potentials, and is kinetically faster than ICaL. ICaT is relatively resistant to Nif but can be blocked by lower concentrations of

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myocytes suggests that atrial inotropy and chronotropy can be reduced on demand. These findings are consistent with the previous results indicating that the heart of *A. baerii* has a similar hypoxic response as the heart of teleost fishes (27, 34). Collectively, the analysis of ligand-gated ion currents indicate that atrial myocytes of *A. baerii* have a much larger reserve of outward currents than ventricular myocytes, which raises interesting questions on their physiological significance.

**Perspectives and Significance**

The species richness of the ray-finned fishes is assumed to be due to the 3rd round of whole genome duplication (3R), which occurred after the separation of teleost fishes from the other ray-finned fishes, i.e., the 3R was a teleost-specific event (20). In this scheme, the low species number of the extant Chondrostei could be seen as an outcome of genetic limitations. The major finding of the present study is that cardiac excitation in the Chondrostei fish *A. baerii* is largely controlled by similar electrophysiological mechanisms characteristic of teleost fishes. This suggests that the few extant Chondrostei species have succeeded by using the same physiological adaptations as the flourishing teleost group. Future studies should clarify to what extent this is explained by the common ancestry and what is the role of convergent evolution in adaptation of cardiac excitability. To this end, genetic and molecular similarities/differences in the electrophysiological traits between sturgeon and teleost hearts need to be examined, in particular, whether the ion currents are produced by homologous genes in Teleostei and Chondrostei. Clarification of the genetic basis of cardiac electrophysiology in sturgeons may shed light on those molecular arrangements required for successful environmental adaptation of the few Chondrostei species and physiological plasticity of cardiac excitation under further challenges. In particular, the specific features of *A. baerii* cardiac excitation, including the predominance of *I*ₐₜ in the atrium and the small ventricular *I*ₖ₁, provides an interesting possibility to test the physiological significance of the cardiac T-type Ca²⁺ channels and the inward rectifier K⁺ channels in cardiac function under different physiological/environmental conditions. The results of the present study provide solid background knowledge for future research on cardiac function in sturgeons.

**Table 1. The ratio of type Ca²⁺ current and L-type Ca²⁺ current (Iₐₜ/Iₖ₁) in different cardiac tissues of vertebrate species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>Iₐₜ/Iₖ₁</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig (Cavia porcellus)</td>
<td>Ventricle</td>
<td>0.12</td>
<td>(8)</td>
</tr>
<tr>
<td>Rat (Rattus norvegicus)</td>
<td>Ventricle</td>
<td>0.00</td>
<td>(24)</td>
</tr>
<tr>
<td>Zebrafish (Danio rerio)</td>
<td>Ventricle</td>
<td>0.65</td>
<td>(32)</td>
</tr>
<tr>
<td>Dogfish (Squalus acanthias)</td>
<td>Ventricle</td>
<td>0.92</td>
<td>(28)</td>
</tr>
<tr>
<td>Sturgeon (Acipenser baerii)</td>
<td>Ventricle</td>
<td>0.09</td>
<td>present study</td>
</tr>
<tr>
<td>Rat (Rattus norvegicus)</td>
<td>Atrium</td>
<td>0.26</td>
<td>(24)</td>
</tr>
<tr>
<td>Bullfrog (Rana catesbeiana)</td>
<td>Atrium</td>
<td>0.24</td>
<td>(4)</td>
</tr>
<tr>
<td>Zebrafish (Danio rerio)</td>
<td>Atrium</td>
<td>0.52</td>
<td>(32)</td>
</tr>
<tr>
<td>Sturgeon (Acipenser baerii)</td>
<td>Atrium</td>
<td>2.42</td>
<td>present study</td>
</tr>
<tr>
<td>Rabbit (Oryctolagus cuniculus)</td>
<td>SAN cells</td>
<td>0.35</td>
<td>(39)</td>
</tr>
<tr>
<td>Dog (Canis familiaris)</td>
<td>Parkinje cells</td>
<td>0.58</td>
<td>(18)</td>
</tr>
</tbody>
</table>

SAN, sinoatrial node.
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AUTHOR CONTRIBUTIONS
Author contributions: T.E.H., J.H., and M.V. conception and design of research; T.E.H., J.H., H.A.S., and M.V. performed experiments; T.E.H., J.H., H.A.S., and M.V. analyzed data; T.E.H., J.H., H.A.S., and M.V. interpreted results of experiments; T.E.H., J.H., and M.V. prepared figures; T.E.H. and M.V. drafted manuscript; J.H., H.A.S., and M.V. edited and revised manuscript; M.V. approved final version of manuscript.

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