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Forum Minireview

New Aspects for the Treatment of Cardiac Diseases Based on the Diversity of Functional Controls on Cardiac Muscles: Diversity in the Excitation–Contraction Mechanisms of the Heart

Hikaru Tanaka[1,*], Iyuki Namekata[1], Hideaki Nouchi[1], Koki Shigenobu[1], Toru Kawanishi[2], and Akira Takahara[1]

[1]*Department of Pharmacology, Toho University Faculty of Pharmaceutical Sciences, Chiba 274-8510, Japan*
[2]*Division of Drugs, National Institute of Health Science, Tokyo 158-8501, Japan*

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Abstract. The waveform of the myocardial action potential (AP) triggering contraction differs among the species, developmental stage, and pathological state. The species difference in heart rate, which inversely correlates with body size, originates in the ion-channel mechanisms responsible for diastolic depolarization of the sinoatrial node. In some cases, such as the chronically AV-blocked dog and 11- to 13-day chick embryo, the repolarization reserve is decreased making the heart useful for drug evaluation. The degree of dependence of contraction on sarcoplasmic reticulum (SR) function increases during development. The large SR dependence and short AP of the adult mouse and rat support their rapid contraction under high heart rate. The function of the Na^+/Ca^{2+} exchanger is affected by AP waveform and ion concentrations; its major role is Ca^{2+} extrusion, but under pathological conditions such as ischemia-reperfusion, it allows Ca^{2+} influx and leads to myocardial injury, including loss of mitochondrial function. The role of mitochondria in ATP supply is less in the fetus where glycolysis plays a greater role. The pharmacological properties of the myocardium are affected by all of these factors and also by autonomic innervation and the hormonal status. Such comprehensive understanding is indispensable for the development of novel therapeutic strategies.

Keywords: heart, action potential, contraction, calcium ion, autonomic nervous system, cardiac disease

Introduction

The action potential and the Ca^{2+} transient are the common mechanisms triggering myocardial contraction, but the precise mechanisms for the generation of the action potential and Ca^{2+} handling as well as their neurohormoral regulation differs among the species, developmental stage, and pathological state. In general, the hearts of smaller animals have higher beating rates, which could be ascribed to a difference in the pacemaker depolarization mechanisms of the sinoatrial node. The time course of repolarization and Ca^{2+} transient is faster in the myocardium of smaller animals, which can be ascribed to ion-channel and Ca^{2+}-handling mechanisms. Such difference in the basic excitation–contraction mechanisms underlies the difference in pharmacological properties of the myocardium. In general, difference among species is small in the immature myocardium and become prominent towards adulthood. The pharmacological properties of the myocardium are also affected by the metabolic background and also by the presence of non-myocardial cells in the myocardium such as neurons and endocardial endothelial cells. Here we will overview the factors underlying the diversity in myocardial excitation–contraction mechanisms. For details, refer to recent reviews in each field.

*Corresponding author. htanaka@phar.toho-u.ac.jp
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Pacemaker mechanisms

Cardiac pacemaking is the result of multiple ionic

mechanisms in the sinoatrial node cell (1, 2). Ion channels including the L-type calcium current (I_{CaL}), sustained inward current (I_{st}), hyperpolarization-activated inward current (I_h or I_f), T-type calcium current (I_{CaT}), and sodium calcium exchanger current (I_{NCX}) have been reported to be involved in the depolarization of the sinoatrial node action potential. The contribution of each component appears to vary among animal species. The action potential upstroke is caused by I_{CaL}, whose molecular identity was reported to include two different pore-forming subunits, Cav1.2 and Cav1.3. Most of the I_{CaL} flows through Cav1.2 in the mouse sinoatrial node while that in the porcine sinoatrial node flows exclusively through Cav1.3 (2). I_{CaT} appears to contribute to the pacemaker (phase 4) depolarization but only in smaller animals (2). Results from voltage clamp experiments as well as analysis with the selective I_{CaT} blocker *R*(−)-efonidipine (3) revealed that the I_{CaT} density in sinoatrial node cells is higher in smaller animals such as the mouse and low in larger animals such as the rabbit and pig. There is no report on the species difference of I_h and I_{st}. I_h may rather be involved in the regional variation in the maximum diastolic potential within the sinoatrial node region: the density is higher in the peripheral region of the sinoatrial node, which may function to protect the pacemaking activity at the central region from the hyperpolarizing influence of the surrounding atrial cells (1).

The rate of repolarization is determined by the density and type of potassium currents present in the sinoatrial node cells (2). The delayed rectifier potassium current I_K consists of two components, the rapidly activating I_{Kr} and the slowly activating I_{Ks}. Selective blockade of I_{Kr} by E-4031 inhibits spontaneous activity of the rabbit, guinea-pig and mouse sinoatrial node cells, and blockade of I_{Ks} by chromanol 293B inhibits those in porcine and guinea-pig sinoatrial node cells. These results suggest that the sinoatrial node automaticity is driven by I_{Kr} in smaller animals with high sinus rate and by I_{Ks} in larger animals with slower sinus rate. Some researchers postulate that pacemaking is also controlled by an intracellular clock; Ca^{2+} released from the sarcoplasmic reticulum during the diastolic period is pumped out of the cell through the forward-mode Na^+/Ca^{2+} exchanger that generates an inward current and may contribute to pacemaker depolarization (4). However, others reported that the automaticity of the sinoatrial node cells was not abolished by ryanodine, which interferes with sarcoplasmic reticulum function (5). Such discrepancy may reflect the regional variations in pacemaker mechanisms; the contribution of the intracellular clock may be larger in the peripheral region of the sinoatrial node (6). The myocardium present in the pulmonary vein, which is attracting attention as the source of ectopic automaticity responsible for the generation and maintenance of atrial fibrillation, was shown to have action potential properties different from those of the atrium (7). It shows spontaneous and ouabain-induced automaticity, which can be inhibited by either ryanodine or the Na^+/Ca^{2+} exchanger blockade. Thus, acceleration of pacemaker depolarization by the intracellular clock may be prominent in certain regions of the myocardium and/or under pathological conditions.

Action potential properties in the working myocardium

Concerning the action potential of the working myocardium, diversity is observed in the repolarization phase rather than in the depolarization phase (8). The rapid upstroke of the action potential is caused by a large I_{Na} current density that guarantees propagation of the action potential through the myocardium (9). In the adult mouse ventricular myocardium, the upstroke velocity is more than twofold higher than those observed in most other working myocardia. This might be a mechanism to compensate for the extremely short action potential duration in the adult mouse ventricle; shorter action potential duration means less electrotonic depolarizing support from behind at the activation wave front. A large diversity exists in the rate of the repolarization and the ionic currents involved among animal species and the developmental stage (ref. 8 and Fig. 1). The adult mouse ventricular myocardium has an action potential with extremely short duration at depolarized potentials of only a few ms and a late slowly repolarizing phase. Voltage clamp studies showed that the I_{Ca} density of the mouse and rat myocardium is not less than that of other species such as the guinea pig and rabbit, which has an action potential lasting as long as 200 ms. The repolarizing potassium current of the mouse and rat ventricle is the transient outward current (I_{to}), which activates much faster than I_K, the major repolarizing current of ventricular myocardia in many other animals. Thus, the diversity in repolarizing potassium currents appears to be the major cause of species difference in myocardial repolarization.

The potassium currents responsible for repolarization are influenced by the endocrine hormones and are reduced under pathological conditions and in the immature myocardium. A cardiac ion channel responsible for repolarization is known to be affected by sex hormones (10). This can at least partly explain the observed gender difference in susceptibility to the arrhythmogenic effects of QT-prolonging drugs. The hearts of the chronic AV block dog were shown to undergo structural remodeling including myocardial hypertrophy and in-

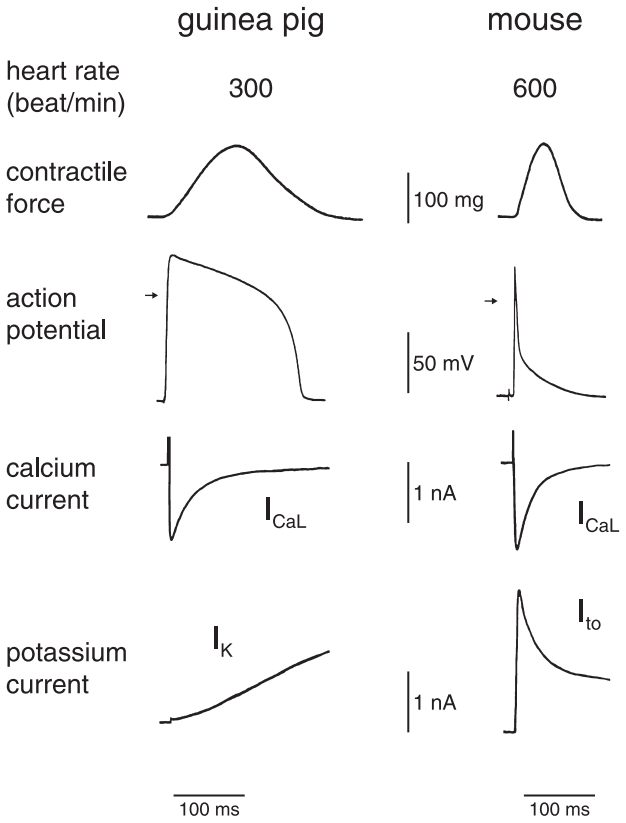

Fig. 1. Species difference in myocardial excitation–contraction properties. Typical records for the contractile force, action potential, and membrane currents of isolated myocardial tissue and cells were compared between the guinea pig and mouse.

crease in collagen fibers (11). This was accompanied by decreased expression level of potassium channels, resulting in increased action potential duration in the AV-blocked dog myocardium (12). It was also shown to have an increased sensitivity to the action potential prolonging and/or arrhythmogenic effect of drugs with potassium channel–blocking activity. Reduced repolarizing currents have been reported in immature myocardia from many animal species. In case of the guinea-pig ventricle, the densities of I_{Ca} and I_K are both decreased in the fetus compared to those in the adult (13). The fetal ventricle has an action potential with longer duration and a higher sensitivity to the prolonging effect of pharmacological agents (14). Ventricular myocardium from the 11- to 13-day chick embryo also has an increased sensitivity to action potential prolonging drugs (15). I_{Kr} blockade with E-4031 prolongs the action potential duration at this age and induces early afterdepolarization. Terfenadine, which is known for its lack of action potential–prolonging activity on isolated myocardial tissue preparations despite its QT-prolonging and arrhythmogenic activity in vivo, was shown to produce action potential prolongation in the 11- to 13-day chick embryo ventricle. Thus, myocardia from certain pathological models and from immature animals appear to have decreased repolarization reserve and may be a useful model for the investigation of arrhythmogenic mechanisms and a sensitive assay system for the action potential prolonging activity of drugs.

Ca^{2+} handling

Ventricular myocytes from the adult mammalian heart have a well-developed T-tubular system throughout the cell. During the action potential plateau, Ca^{2+} influx through the sarcolemma and T-tubules triggers Ca^{2+} release from ryanodine receptors located on the adjacent sarcoplasmic reticulum membrane. These results in a higher Ca^{2+} concentration at the Z-band region of the ventricular myocyte only for several milliseconds during the early phase of contraction, and at about 10 ms after the onset of the action potential, Ca^{2+} concentration is uniform throughout the whole cytoplasm (16). Variations exist in the relative contribution of the transsarcolemmal Ca^{2+} influx and Ca^{2+} release from the sarcoplasmic reticulum (ref. 8 and Fig. 2). The potency order for the negative inotropic effect of nicardipine, which reflects dependence on transsarcolemmal Ca^{2+} influx, was mouse < rat < guinea pig. This correlated with the species

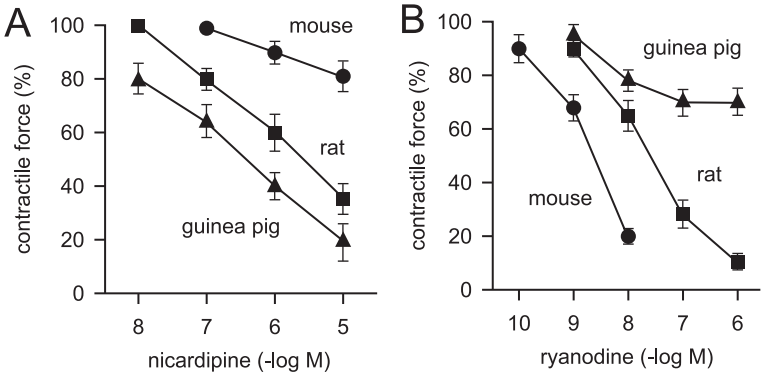

Fig. 2. Species difference in the calcium source for contraction. Negative inotropic effects of nicardipine (A), an inhibitor of transsarcolemmal Ca^{2+} influx, and ryanodine (B), an inhibitor of Ca^{2+} release from the sarcoplasmic reticulum, were compared between isolated ventricular preparations from the mouse (circles), rat (squares), and guinea pig (triangles).

difference in action potential duration. On the other hand, the potency order for the negative inotropic effects of ryanodine and cyclopiazonic acid, which reflects dependence on Ca^{2+} release from the sarcoplasmic reticulum, was mouse > rat > guinea pig. This was the same as the order of the myocardial relaxation rate. Comparative studies with various mammals revealed that hearts of smaller weight have higher resting sinus rate and higher sarcoplasmic reticulum Ca^{2+} ATPase activity. Hearts with higher beating rate require faster contraction and relaxation for sufficient refilling before the next heartbeat, which can be accomplished by increasing sarcoplasmic reticulum function. Such species difference in excitation–contraction mechanism is less prominent in immature myocardia. In the fetal myocardia, the size of the cardiomyocytes is small and the sarcoplasmic reticulum and T-tubular system are scarcely developed (17). This results in a higher dependence of contraction on transsarcolemmal Ca^{2+} influx.

In contrast to the adult ventricular myocardia where a large species difference was observed, the atrial myocardia of the mouse, rat, and guinea pig were shown to have similar high inotropic sensitivity to ryanodine, which could be explained by the atrial excitation–contraction coupling mechanisms (18). In atrial cardiomyocytes that lack a T-tubular system, transsarcolemmal Ca^{2+} influx triggers Ca^{2+} release only at the subsarcolemmal sarcoplasmic reticulum. This Ca^{2+} triggers Ca^{2+} release from the neighboring sarcoplasmic reticulum and a wave of Ca^{2+}-induced Ca^{2+} release propagates towards the cell interior. Ca^{2+} waves were more sensitive to ryanodine and cyclopiazonic acid than Ca^{2+} transients evoked by action potentials. Involvement of Ca^{2+} waves in normal atrial excitation–contraction coupling can explain the high sensitivity of atrial contraction to these agents regardless of the animal species.

Sympathetic regulation

The sympathetic nervous system is the major accelerator of myocardial function, which increases myocardial contractile force through β-adrenoceptor–mediated activation of adenylate cyclase in most animal species (19). The inotropic sensitivity to the sympathetic neurotransmitter noradrenaline is known to be affected by various factors. It is increased by hyperthyroidism and decreased under diabetic conditions. Sympathetic innervation itself exerts long-term influence on the myocardium including maintenance of its sensitivity to its own neurotransmitter. Sympathetic denervation of the heart results in supersensitivity of the myocardium not only to noradrenaline but also to other transmitters such as acetylcholine (20, 21). The fetal heart is initially devoid of sympathetic innervation. In the case of the rat, sympathetic innervation occurs at fetal day 17 at the sinoatrial node and from late embryonic period in the ventricle. The development of sympathetic innervation is followed by a tenfold decrease in the chronotropic and inotropic sensitivity of the β-adrenoceptor–mediated mechanisms (22). The time course of the functional development of the sympathetic nervous system as well as the sensitivity to autonomic transmitters are altered in pathological models such as the spontaneously hypertensive rat (23). Disturbance of the normal development of the sympathetic innervation results in alterations in responsiveness to β-adrenergic stimulation and increased susceptibility to arrhythmogenic stimuli (24). Recent studies on the roles of adenylate cyclase isoforms in the myocardium suggest that isoform-specific manipulation of the enzyme may be of value in the treatment of heart failure (25).

α-Adrenoceptors are present in the myocardium and their stimulation results in no or weak positive inotropy through mechanisms different from those of β-adrenoceptor stimulation (19). Sustained positive inotropy is observed in the rabbit, guinea pig, and rat ventricle, but in the guinea pig and rat, a transient decrease in contractile force is observed before the increase. The mouse ventricle shows a sustained negative inotropy in response to α-adrenoceptor stimulation (26) that is accompanied by decreased calcium transient amplitude (27). Electrophysiological and pharmacological analysis revealed that α-adrenoceptor stimulation activates the Na^+/Ca^{2+} exchanger and decreases the Ca^{2+} released from the sarcoplasmic reticulum (28). Interestingly, activation of the Na^+/Ca^{2+} exchanger following α-adrenoceptor stimulation was also observed in guinea-pig ventricular myocytes. The extremely short action potential of the mouse ventricular myocyte favors the Na^+/Ca^{2+} exchanger to function in the forward (Ca^{2+} extrusion) mode and decrease the Ca^{2+} transient amplitude and contractile force. In fact, in the neonatal mouse ventricle, which has an action potential of longer duration, α-aderenoceptor–mediated inotropy is positive. Similar developmental conversion of inotropy from positive to negative is observed in the mouse ventricle with endothelin I and angiotensin II (29). These results suggest that the inotropic responses of the myocardium to various active substances are largely affected by the basic excitation–contraction properties of the myocardium.

Muscarinic receptor–mediated regulation

In the ventricular myocardium, the parasympathetic neurotransmitter acetylcholine is generally considered to

show no inotropy under basal conditions and negative inotropy after elevation of adenylate cyclase activity by adrenoceptor stimulation (19). In some cases, direct negative inotropy by muscarinic receptor stimulation has been reported in ventricles from the ferret, rat, dog, and hatched chick. The negative inotropy was mediated by hyperpolarization and decrease in action potential duration in the case of the ferret, while inhibition of an intrinsically activated adenylate cyclase activity was the likely mechanism in the case of the hatched chick (30). In mammalian atria, acetylcholine is considered to produce negative inotropy through inhibition of adenylate cyclase and activation of the G-protein coupled potassium current I_{KACh}. However, there are cases in which acetylcholine induces positive inotropy. In the chick embryonic atria, acetylcholine induces positive inotropy through stimulation of M_1 receptors and activation of phosphlipase C. Muscarinic receptors are known to be present not only in cardiomyocytes but also in other cell types in the myocardium. In mouse atria, a biphasic inotropic response to acetylcholine was observed: a transient negative inotropy was followed by positive inotropy that were both inhibited with atropine (31). The positive inotropy in this case was revealed to be mediated by prostaglandins released from the endocardial endothelium (32). In other cases, muscarinic agonist–induced positive inotropy was mediated by noradrenaline released from sympathetic nerve terminals. There seems to be a large variation in the polarity and mechanism for the acetylcholine-induced inotropy, which reflects both the intrinsic properties of the cardiomyocyte and the influence of other cell types in the myocardium. Whether these mechanisms are related to the pathophysiology of the heart and the effects of therapeutic agents awaits further investigation.

Effects of ischemia

The adult myocardium has a high mitochondrial content and capacity for oxidative metabolism to produce ATP. Ischemia or hypoxia, resulting from a disruption or reduction of the coronary blood flow in vivo or its simulation in experimental systems, results in myocardial injury including Ca^{2+} overload, decreased resting membrane potential and action potential duration, loss of mitochondrial function and decreased ATP content, and marked reduction of the contractile force. Such damage is only partially recovered even if the coronary perfusion is recovered. Many agents have been reported to show protective effects against myocardial ischemia–reperfusion damage (33). In most cases, the protection appeared to be accompanied by drug-induced reduction of myocardial performance. Some agents such as Cl^--channel blockers (34) and Na^+/Ca^{2+}-exchanger inhibitors (35) showed protection against myocardial ischemia–reperfusion injury with no evidence of cardiosupression. Evidence available at present suggests that preservation of mitochondrial function is involved in the protective effect of these agents (36). Various ion channels and transporters are present on the mitochondrial membrane and their modulation has been reported to affect mitochondrial function (37). Agents that open mitochondrial K^+ channels prevents mitochondrial Ca^{2+} overload and preserves its function through partial depolarization of the mitochondrial inner membrane.

There seem to be some variations in the susceptibility to injury during ischemia and reperfusion. The hypertrophied myocardium exposed to sustained pressure- or volume-overload is known to have increased susceptibility, which may be the result of altered metabolic properties (38). Myocardial long-chain fatty acid oxidation and coupling between glycolysis and glucose oxidation are lower than normal, resulting in enhanced H^+ production in hypertrophied myocardium. Some researchers postulate that agents that alter myocardial energy metabolism through inhibition of fatty acid oxidation may be beneficial for cardioprotection against ischemic insult. The susceptibility of the myocardium to ischemic insult is also different between mature and immature myocardium. In the adult guinea-pig ventricle, experimental hypoxia results in marked shortening of the action potential duration and complete loss of contractile activity (39). In contrast, both the action potential duration and contractile force were partly maintained in the fetal ventricle. The ATP-sensitive K^+ channel, which is considered to be the primary cause of action potential shortening under hypoxic conditions, was present in similar densities in cardiomyocytes from the adult and fetus. The relative resistance to hypoxia of the fetal myocardium could rather be explained by its high dependence on glycolysis. In general, the developmental conversion of metabolism from anaerobic to aerobic appears to parallel the increase in susceptibility to ischemic insult. Thus, the most effective strategy for cardioprotection against ischemia–reperfusion may vary depending on the metabolic status of the myocardium.

Conclusion

A large diversity exists in the excitation–contraction mechanism of the myocardium that reflects diversity in the ultrastructure of the cardiomyocyte, type and amount of ion channels and various functional proteins expressed, and the metabolic status of the myocardium. These basic properties are influenced by non-cardiac cells present in the myocardium such as neurons and endocardial endo-

thelial cells, and they are altered under pathological conditions. Diversity in the chronotropic and inotropic responses to neuronal and hormonal stimuli and to various pharmacological agents can be partly explained by the underlying excitation–contraction properties. Such comprehensive understanding of the myocardium is indispensable for the extrapolation of experimental findings to the human heart and for the development of novel therapeutic strategies.

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