Physiol. Res. 42: 293 – 299, 1993

Are There any Differences in the Excitation-Contraction Coupling of the Working Myocardium of Adult and Newborn Guinea-Pigs?

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Received February 20, 1992
Accepted March 19, 1993

Summary

Action potentials (APs) and the force of isometric contractions of the right ventricular papillary muscles were measured in adult and newborn guinea-pigs. The measurements were carried out in the steady state with the rate of stimulation of 0.1, 1, and 2 Hz, and further some measurements were done in which Sr^{2+} was substituted for Ca^{2+}. The duration of APs of the newborn animals without pharmacological treatment was significantly shorter in comparison with that of the adults at all the used stimulation frequencies. An analogous sensitivity was found in the contractile force to increased stimulation frequency and when the steady state stimulation was discontinued by the insertion of interpolated extrasystoles in papillary muscles of adult or newborn animals. The biphasic contractions of papillary muscles were evoked in both groups of animals by the incomplete substitution of Sr^{2+} by Ca^{2+} in the presence of isoprenaline. The early component of the biphasic contractions had a faster course as compared to the late component and disappeared in the presence of caffeine in both groups of animals. Our results suggest that the heart cells of newborn guinea-pigs probably possess the sarcoplasmic reticulum (SR), whose function does not differ in quality from that of the adult guinea-pigs. The postnatal prolongation of APs is therefore not probably the result of postnatal development changes of the functions of SR, but could be related to changes in the relations between the surface and volume of the heart cell during its growth.

Key words

Guinea-pig – Ontogeny – Action Potential – Contractility – Excitation-contraction Coupling

Introduction

The development of heart cell structures is not terminated by the time of birth. This results from the fact that the myocardium of newborn mammals usually exhibits different features as compared with the adults. The volume of heart cells of newborn animals is smaller than that of the adults and increases postnatally more rapidly than their surface (Friedman 1972, Sheridan *et al.* 1979). The difference in the surface/volume relation between adult and newborn mammal diminishes in consequence of the development of T-tubules in most of them (Hoerter *et al.* 1981, Maylie 1982). In case of the guinea-pig myocardium, the development of the T-tubules can not play a significant role in the surface/volume ratio, since the T-tubules are already developed at the time of birth (Forbes and Sperelakis 1976).

The sarcoplasmic reticulum (SR) of many mammals is not yet fully developed in the perinatal period and it does not therefore function as an intracellular store of Ca^{2+} (Seguchi *et al.* 1986). This fact is of great significance in the postnatal development of excitation-contraction coupling. The result of the morphological and functional immaturity of the SR is the smaller sensitivity of the contractile force of immature myocardial fibres to the drugs which decrease SR function (Hoerter *et al.* 1981, Tanaka and Shigenobu 1989) and, on the other hand, the greater dependence of the contractile force on the transmembrane Ca^{2+} influx (I_{Ca} and Na^{+}-Ca^{2+} exchange) (Artman *et al.* 1985, Králíček *et al.* 1987). The functional SR immaturity is characterized by the rate insensitivity of the newborn cat myocardium

(Maylie 1982) and newborn rabbits respectively (Pučelík and Fiala 1990).

On the other hand, the morphological study of Forbes and Sperelakis (1976) suggests that the ventricular myocardium of newborn guinea-pigs possess a developed system of T-tubules, myofibrils and SR. In the present paper, we have tried to demonstrate whether the morphological maturity of these heart cell structures is also accompanied by functional maturity.

Methods

The experiments were carried out on papillary muscles from the right heart ventricles of twenty adult guinea-pigs (body weight more than 300 g, older than 3 months) and eighteen newborn ones (younger than 3 days). The diameter of papillary muscles was 0.8±1.2 mm in adult animals and 0.6±1.4 mm in those of newborn guinea-pigs. After decapitation of the animals the chest was opened and the heart was rapidly removed and further preparation was done in a warm oxygenated Tyrode's solution of the following composition (mmol.l^{-1}): NaCl 137, KCl 4.5, $CaCl_2$ 2, $MgCl_2$ 1.0, Na_2HPO_4 0.5, $NaHCO_3$ 11.0, glucose 10.0. The solution was saturated with a mixture of 96 % O_2 and 4 % CO_2, its pH was 7.4; during the experiment it flowed through the measuring bath at a rate of 6–10 ml.min^{-1}, its temperature was 36 °C. In case of incomplete substitution of Sr^{2+} for Ca^{2+} the concentration of Sr^{2+} was 1.8 mmol.l^{-1}, the concentration of Ca^{2+} was simultaneously decreased to 0.2 mmol.l^{-1}. Isoprenaline was applied in the concentration of 10^{-6} mol.l^{-1}. The stimulation of beta-adrenergic receptors increases the sarcolemmal permeability for Ca^{2+} and further enhances the uptake of Ca^{2+} into the SR (Callewaert *et al.* 1988). The utilization of this effect was shown necessary in respect to the weak contractile force in preceding experiments which resulted from the lower concentration of Ca^{2+} in the incubation medium. Caffeine was used in the concentration of 10^{-2} mol.l^{-1} in theses experiments, which is sufficient to rule out the SR from its function of storing intracellular Ca^{2+} (Rasmussen *et al.* 1987). After each change in the composition of the solution, the preparation was always stimulated for at least 30 min before starting the actual measurement.

The preparations were stimulated with rectangular electric pulses of 0.1 ms duration and double the threshold stimulation voltage. In the introductory stabilization phase, the preparation was stimulated one hour at a frequency of 1 Hz and then we proceeded with the actual measurements. In case of the steady state stimulation programme the preparation was stimulated at a frequency of 0.1, 1 and 2 Hz. In case of the extrasystole programmean impuls was inserted always after 10 regular APs in the defined time interval. This interval was designed as T_E (Fig. 2). These evaluations were beeing done: 1) the last regular AP and contraction of the previous serie, 2) extrasystolic AP and beat, 3) postextrasystolic AP and beat – the first of the following serie.

The course of membrane voltage changes was recorded with glass microelectrodes. Using a mechano-electrical transducer, the force of isometric contractions (MG) was measured. The derivation of contraction and relaxation dT/dt was obtained electronically. After amplification, electrical membrane manifestations, together with the mechanoelectrical transducer output were recorded by the graphic unit of a conventional polygraph (NEK RTF 6 G).

From the recording we obtained: 1) the duration of the action potential at electrical zero level (D_0; ms), 2) the duration of AP at repolarization level to - 60 mV (D_{-60}; ms), 3) the peak of the contractile force (MG; r.u.). The contractile force in the steady state was equal to that at the stimulation frequency of 0.1 Hz. In the stimulation programme of interpolated extrasystoles, the force of contraction was equal to that at the basal stimulation frequency of 1 Hz. In case of biphasic contractions we evaluated: 1) the time from the beginning of AP to the peak of the early component of contraction (ms) and the time from the beginning of AP to the peak of contractile force in the presence of caffeine (ms).

The results are presented as the mean values ± S.E.M.

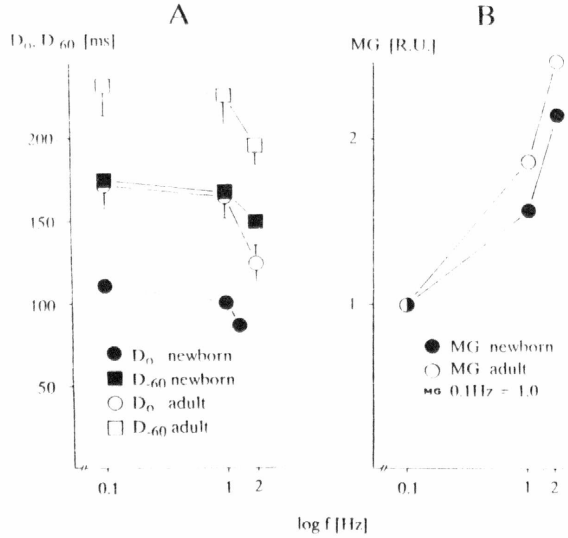

Fig. 1
The dependence of the duration of AP (A) and the contractile force (B) on the stimulation frequency (expressed as log frequency) in adult (empty symbols) and newborn (full symbols) guinea-pigs. The duration of AP is plotted on the zero electric level (D_0, ms) and on the level of - 60 mV (D_{-60}, ms). The number of papillary muscles was 9 in adult animals and 8 in newborn animals.

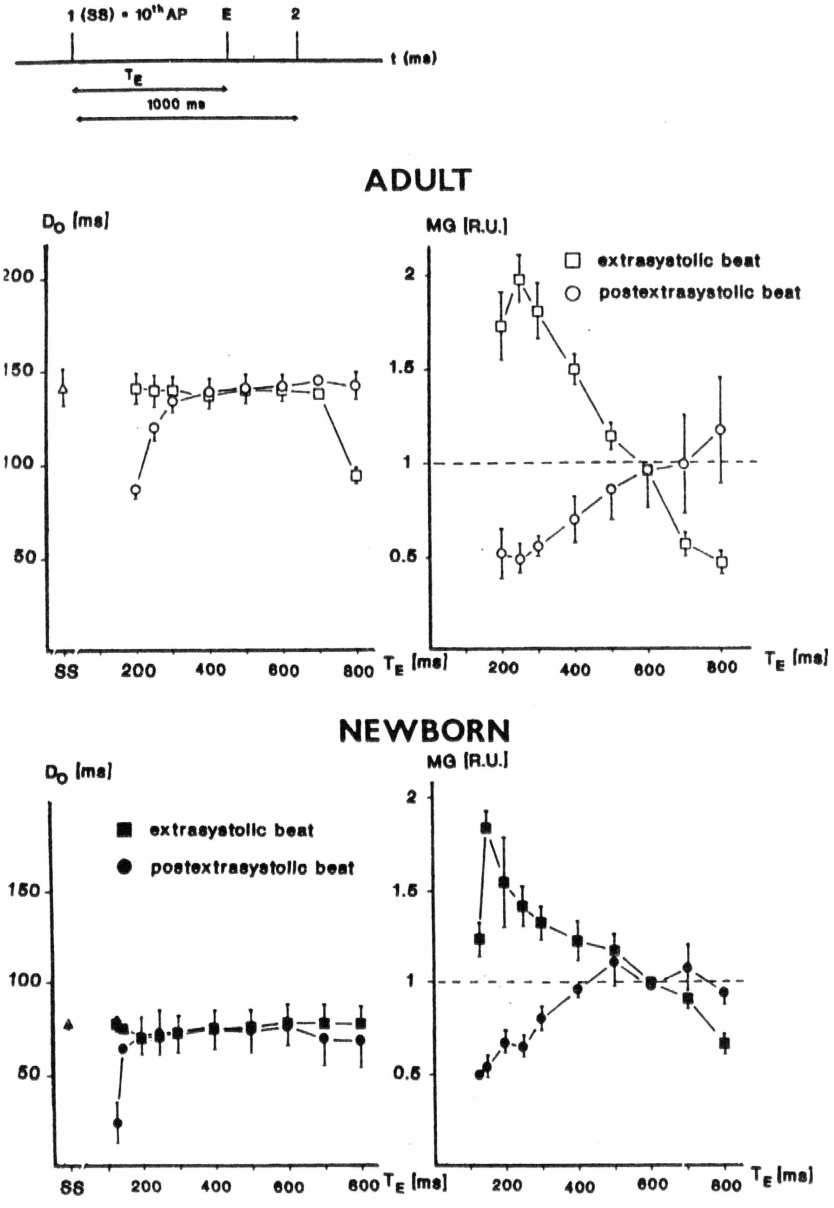

Fig. 2
The dependence of the duration of AP on the zero electric level (D_0, ms) and of the force of isometric contractions (MG, r.u.) on the extrasystolic interval (T_E) in papillary muscles of 7 adult and 6 newborn guinea-pigs. The experimental protocol is shown above. The values of adult animals are indicated by empty symbols and that of newborn animals by full symbols. The magnitude of the contractile force in the steady state was equal to one.

Results

An increase in the stimulation frequency (0.1, 1, 2 Hz) induced a slight shortening of the action potential in the phase of AP plateau (D_0 – the duration of AP at the zero electrical level) as in the phase of terminal repolarisation (D_{-60} – the duration of AP at the level of – 60 mV) both in the adult and newborn guinea-pigs (Fig. 1A). It may be seen from this figure that the duration of AP was shorter at all the used stimulation frequencies in the newborn animals in the agreement with the results of Pučelík (1983). It is shown in Fig. 1B that the contractile force increased simultanously with the increase of stimulation frequency analogically in both the animal groups, while the duration of AP slightly decreased with increasing stimulation frequency and the contractile force was markedly enhanced.

It is accepted for the ventricular myocardium of most adult mammals that the insertion of an extrasystole in the steady state results in characteristic changes of the course of AP and contraction of extrasystolic and postextrasystolic beats. There is a conspicious increase of the contractile force following an extrasystole – postextrasystolic potentiation. Fig. 2 shows that this phenomenon is present both in adult and newborn animals. At the short T_E the contractile force of extrasystolic beats was small in comparison with the regular beats and became progressively enhanced with increasing T_E; it reached values of the steady state with the T_E duration of 500 ms in both groups of animals. The course of changes of the contractile force was quite analogous in the newborn and adult guinea-pigs. The dependence of D_0 on T_E is shown in the left part of Fig. 2. At very short T_E (shorter than the duration of D_0 of regular beats), the duration of APs was shorter than in the steady state and it increased with the prolongation of T_E. At T_E longer than the duration of AP there was no difference between D_0 of the extrasystolic and regular beats. We

found no prolongation of APs of extrasystolic beats either in the adult or in the newborn guinea-pigs as was found in the papillary muscles of adult rabbits (Pučelík *et al.* 1983). This is probably due to the absence of the transient outward current of potassium (I_{to}) in the ventricular cells of guinea-pigs (McDonald *et al.* 1984). This assumption is supported by the finding of Šimurda *et al.* (1988) that after the blockade of I_{to} the prolongation of extrasystolic AP disapeared in the ventricle fibres of the dog.

The changes of dT/dt were the result of increased stimulation frequency and insertion of an extrasystole were analogous to the changes of contractile force in both groups of animals. For technical reasons, we could not determine dT/dt in all the preparations and therefore these values are not shown.

Using incomplete substitution of Sr^{2+} for Ca^{2+} in the presence of isoprenaline we evoked biphasic contractions. Fig. 3 shows the curves drawn from the original APs, contractile force and time derivation of contraction and relaxation. The parameters in the absence of caffeine are designed as a and in the presence of caffeine as b. It is evident from this figure that in newborn and as well as adult guinea-pigs two components of contraction can be determined (Fig. 3a). The early component reached the peak in 59±2 ms from the beginning of the AP in the adult and in 45±2 ms in newborn guinea-pigs respectively. The second, late phase of contraction had a substantially smaller amplitude and, since it fluently followed the early phase, its peak can not be determined with sufficient accuracy. The addition of caffeine caused the early component of contraction to disappear in both groups of animals (Fig. 3b). The late phase of contraction had a clearly differentiable peak, which is reached within 222±2 after the beginning of AP in adult and in 152±6 ms in newborn guinea-pigs. The course of the early component of contraction is also more rapid than that of the late one in adult and newborn guinea-pig papillary muscles (see dT/dt in Fig. 3).

It is further evident from Fig. 3 that duration of APs also remained longer in the adult guinea-pigs in comparison with the newborn animals after incomplete substitution of Sr^{2+} for Ca^{2+}. After the early component of contraction had disappeared under the influence of caffeine the AP in both groups of animals became prolonged. While Sr^{2+} was substituted for Ca^{2+} the value of resting membrane potential decreased by about 8 mV, similarly as in the experiments of King and Bose (1983).

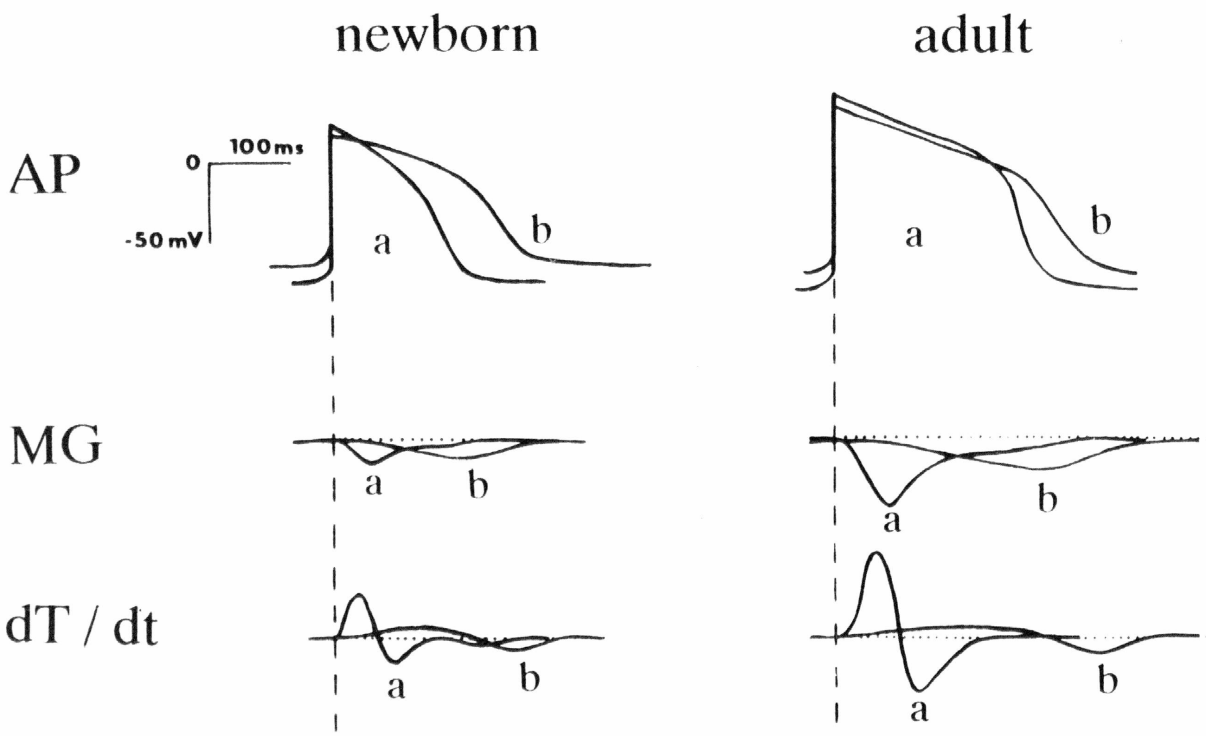

Fig. 3
The drawn original record of AP, biphasic contractions and time derivation of contraction during the incomplete substitution of Sr^{2+} for Ca^{2+} in the presence of isoprenaline without caffeine (a) and in the presence of caffeine (b). Closely analogous traces were obtained in three other experiments in a group of adult and newborn animals.

Discussion

A special feature of the myocardium of adult mammals is the dependence of the contractile force on the interval between individual beats (Kruta and Bravený 1961). The increase of the contractile force following the increase of stimulation frequency is in accord with this (Fig. 1B). The positive inotropic effect of the increase of stimulation frequency is probably caused by the accumulation of Ca^{2+} in SR (Blaustein 1989). The increase of contractile force due to this accumulation of Ca^{2+} in the heart cells is associated with a decrease of the slow inward current (Arlock and Wohfart 1990). The shortening of D_0 in our experiments, is in agreement with this fact, since the duration of the phase plateau of APs depends on the duration of the opening of the I_{Ca} channels (Carmeliet and Vereecke 1979). By its frequency sensitivity, the myocardium of adult mammals differs markedly from the myocardium of amphibians, whose myocytes do not contain a functionally significant SR (Morad and Cleemann 1987). It also differs from the myocardium of newborn mammals, which is similar to the myocardium of the amphibians with the character of excitation-contraction coupling (the predominance of voltage control of contraction above reticular control). Since the increase of the contractile force due to increased stimulation frequency in the steady state (Fig. 1B) requires the presence of a functional SR, the presence of this phenomenon serves as evidence for the functional maturity of SR in the papillary muscles of newborn guinea-pigs.

The interpolated extrasystole is an other manifestation of the frequency sensitivity of the mammalian myocardium. This influence on the steady state causes a marked increase of contractile force of the postextrasystolic contraction of heart fibres in adult mammals (Wohlfart and Noble 1982, Pučelík *et al.* 1983, Anderson 1987). The postextrasystolic potentiation depends on the ability of SR to accumulate Ca^{2+}, because this phenomenon disappears in the presence of various drugs blocking this accumulation (Wier and Yue 1985, Pučelík and Fiala 1990). The prominent postextrasystolic potentiation of approximately equal size in the adult as well as newborn guinea-pigs serves as evidence of the maturity of SR in this animal species already in the perinatal period of development.

By using various methods, biphasic contractions could be evoked in heart fibres (Rieter 1988, Beyer *et al.* 1988, Honoré *et al.* 1987). Irrespectively of the way in which the biphasic contractions are evoked, it has been shown that the magnitude of the early component of contraction is determined by Ca^{2+} released from the SR, while the magnitude of the late one depends on the transmembrane influx of Ca^{2+} into the heart cell (Honoré *et al.* 1987). The early phase of contraction is present in adult as well as in newborn guinea-pigs and it can be abolished with caffeine in both cases.

The slow course of contraction in the presence of caffeine (dT/dt, see Fig. 3) shows a retardation of the saturation process of the contractile apparatus of heart cells, since the first derivation of the time course of contraction (dT/dt) reflects the offer of calcium as the activator of contraction (Blinks and Jewel 1972). The peak of both components of contraction is reached later in the adult than in the newborn animals. The longer diffusion distance for Ca^{2+} between the sarcolemma and myofibrils could be the reason for this fact, respecting the greater volume of the heart cells of adult animals, while the difference in the diffusion distance between SR and myofibrils is less significant. Our unpublished microscopic observations suggest that in the guinea-pig the volume of the heart cells of the adult is also longer than that of newborn guinea-pigs, similarly as in the other mammals (Friedman 1972, Sheridan 1979, Hoerter *et al.* 1981). The increase of the late component of contraction in the presence of caffeine can be result of the effect of caffeine on intracellular structures other than SR (Clusin 1985, Chiu *et al.* 1989).

If the function of the SR does not change appreciably in the guinea-pig myocardium and the intracellular economy does not differ from the myocardium of adults, it is not clear why the APs become prolonged postnatally (Fig. 1A). This seems to be analogous to the change of AP duration which results from artificially induced hypertrophy of the myocardium of the guinea-pig (Nordin *et al.* 1989) or cat (Kleiman and Houser 1988). However, this has a number of limitations, since in the case of the hypertrophic myocardium there is not only an increase in the size of cells (Anderson 1987). Nevertheless, the increased size of heart cells, which occurs during postnatal development (Friedman 1972, Maylie 1982) and during the hypertrophy of heart cells is connected with the prolongation of APs. Kleiman and Houser (1988) found that the hypertrophy of the heart cells induces the prolongation of APs as a consequence of decreased outward current sensitive to cesium and to the slowing down of the voltage dependent inactivation of I_{Ca}. This seems to be, at least in part, a mechanism independent of the intracellular accumulation of Ca^{2+} and its release from the SR during the contraction. Our results also suggest that the AP prolongation is independent of the function of SR. The substitution of Sr^{2+} for Ca^{2+}, even if partial, does not result in changes between the duration of APs in adult and newborn guinea-pigs. Furthermore, the functional exclusion of the SR evoked by caffeine does not cause differences in prolongation of APs between adult and newborn animals. Nevertheless, the relationship between the volume of heart cells and the duration of their APs requires further detailed analysis.

Acknowledgments

I would like to thank doc. Dr. P. Pučelík, Ph.D. and Ing. F. Barták for the helpful discussion of these results and to M. Bláhová for excellent technical assistance.

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