

# Interspecies Variation in Myocardial Physiology: the Anomalous Rat

by Glenn A. Langer\*

The heart of the adult rat has long been recognized to be anomalous in at least two respects: an absent or negative inotropic response to an increase in rate of electrical stimulation (negative staircase); resistance to digitalis glycosides. The heart of the neonatal rat (less than 2 weeks old), on the other hand, demonstrates a markedly positive staircase and a large increase in force upon application of glycoside. It is significant that the action potential of the neonate ventricle demonstrates a prolonged plateau component which progressively decreases with age. The shortening of the plateau correlates with the disappearance of the positive staircase and glycoside responses. Previous studies indicated that a major factor contributing to the prolonged plateau of the neonate was a high level of sodium (Na) conductance. Thus transmembranous Na movement associated with excitation is considerably greater in the neonatal heart as compared to the heart of the adult rat. The higher level of intracellular Na would produce increased activity of a proposed sodium-calcium (Na-Ca) carrier. This is believed to mediate the augmented influx of Ca which is responsible for positive staircase and glycoside responses.

Ventricular muscle from the hearts of most mammalian species demonstrates a prolonged plateau with maintenance of a "slow" channel for Na. It appears that in the rat this channel closes with age. It follows that there would be a reduced tendency for the adult rat heart to accumulate intracellular Na,  $[Na]_i$ , when excitation frequency is increased or the Na pump is inhibited by digitalis. Since elevation of  $[Na]_i$  is the stimulus for the proposed Na-Ca carrier, this system would not be activated, Ca influx would not increase and contractility would not be augmented.

In many biological investigations it is the mutant organism or the anomalous species which frequently provides additional insights into basic mechanisms of function. Definition of the basis for an anomaly may further define the normal condition. In the area of mammalian myocardial physiology the rat has provided just such an anomalous condition. The rat heart demonstrates anomalies with respect to its action potential (1, 2), force-frequency response (1-3), ionic exchange parameters (2, 4-6) and response to digitalis glycosides (2, 7, 8). Investigation of these characteristics has provided further insight into fundamental properties of myocardial function not only in the rat but in other mammalian species.

I will first review the anomalies and then attempt to correlate them in such a manner as to indicate how the information has provided further definition of a model for control of myocardial force development.

## Action Potential

As compared to most other mammalian species, the action potential (AP) recorded from ventricular cells of the adult rat is considerably shortened (1, 2, 9) with total duration in the range of 100 msec. The AP demonstrates an essential absence of the plateau (phase 2) component and this accounts for the major part of the shortening and the fact that the effective refractory period is less than 50 msec in duration (9). It is interesting that the short AP develops as the rat ages. Prenatally and during the early neonatal period the duration is much increased (2, 10, 11). At 1 day of age a prominent plateau is present with a total AP duration of approximately 300 msec. There is then a progressive loss of the plateau through the next 3-4 weeks such that the adult configuration is achieved (Fig. 1).

It is likely that the changes in the plateau are secondary to changes in both sodium and potassium conductance ( $g_{Na}$  and  $g_K$ ). Diminution in both delayed and anomalous rectification for K would shorten the plateau as would loss of a slow conducting Na channel. There is experimental evidence for the latter (2). If the Na concentration in the per-

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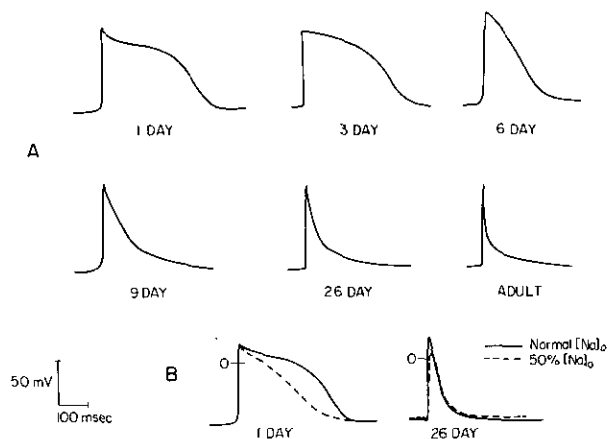


FIGURE 1. Action potentials: (A) Sequence of action potential configuration with age from 1 day to adult. Note the progressive shortening attributable to abbreviation of the plateau. (B). Effect of a 50% reduction in  $[Na]_o$  at 1 day and 26 days. Note that at 1 day the effect was primarily to reduce the level and duration of the plateau but at 26 days the amplitude of the spike and  $dV/dt$  were principally affected. Reproduced with permission of Circ. Res.

fusion medium is reduced to one-half normal (choline chloride substituted to maintain isosmolality) for the 1 day old ventricle the AP plateau amplitude and duration decrease markedly (Fig. 1). This suggests the presence of a significant slow inward Na current during the early neonatal period with progressive closing of the slow-channel as the rat ages.

It should be noted that the configuration of the AP in the early neonatal rat is similar to that found in adults of other mammalian species. Thus the rat heart AP becomes anomalous with age. One might question, in a teleological sense, what the rat gains from the progression. The adult rat has a heart rate of 400–500 beats/min. A short plateau with its attendant short refractory period obviously permits such high heart rates to be established. As important, perhaps, is the closure of the slow Na channel system. In other mammalian species with longer APs and prominent plateau, the slow Na channel admits approximately 75% of the Na which influxes intracellularly with each AP (12). At high heart rate, this large influx places a large load on the Na pump of the cell. In the adult rat heart most of this slow channel Na influx is eliminated, leaving the Na pump to handle the influx associated with the spike component (phase 0) of the AP which is dependent on the fast Na channel. Therefore a large component of the energy required for active Na transport is eliminated.

## Force-Frequency Response

In most mammalian species an increase in stimu-

lation frequency induces an increase in force development known as the Bowditch staircase phenomenon (13). Within similar frequency range the rat heart shows a decline in contractile force with increasing stimulation frequency (1–4).

As indicated with the AP, in the neonate as compared to the adult, the force-frequency response differs markedly in the young as compared to the old rat. A markedly positive staircase is present during the first week of life which then declines by three weeks to give the negative response typical of the adult rat (Fig. 2). This temporal sequence of mechanical function matches the sequence of the electrophysiological changes so that as the AP shortens the positive staircase declines and becomes negative.

Again, the staircase response of the neonatal rat is the response found in the hearts from adults of other species. Therefore the force-frequency response, as does the AP progression, becomes anomalous with age.

## Ionic Exchange

Upon an increase in stimulation frequency most mammalian species demonstrate a net loss of K from the heart (14–16). In contrast, the adult rat heart demonstrates little or no loss of cellular K. Instead the increase in frequency is associated with an increase in the steady-state rate of cellular K exchange, i.e., increased turnover rate without a change in the quantity of K within the cell. This increased steady-state exchange with higher stimulation frequencies is consistent with the absence of a plateau component of the action potential and, more specifically, the absence of an anomalously rectifying channel for K. Though the studies have not been done, it would be expected that the early neonatal heart would demonstrate considerably less increase in steady-state K exchange upon increase in stimulation frequency. This expectation is based on the probable presence of anomalous rectification in the neonatal heart which acts to reduce  $g_K$  below resting level during the plateau phase and cancel, so to speak, the augmented outward K current which develops during repolarization. This combination acts to maintain steady-state K exchange unaltered over a large range of stimulation frequency. The absence of a net loss of K will be discussed further below.

There are other interventions to which the rat responds anomalously in terms of its ionic exchange properties. One is the response of myocardial K exchange to respiratory acidosis. Upon induction of a respiratory acidosis the beating rabbit ventricle demonstrates a net increase in its K content. This is

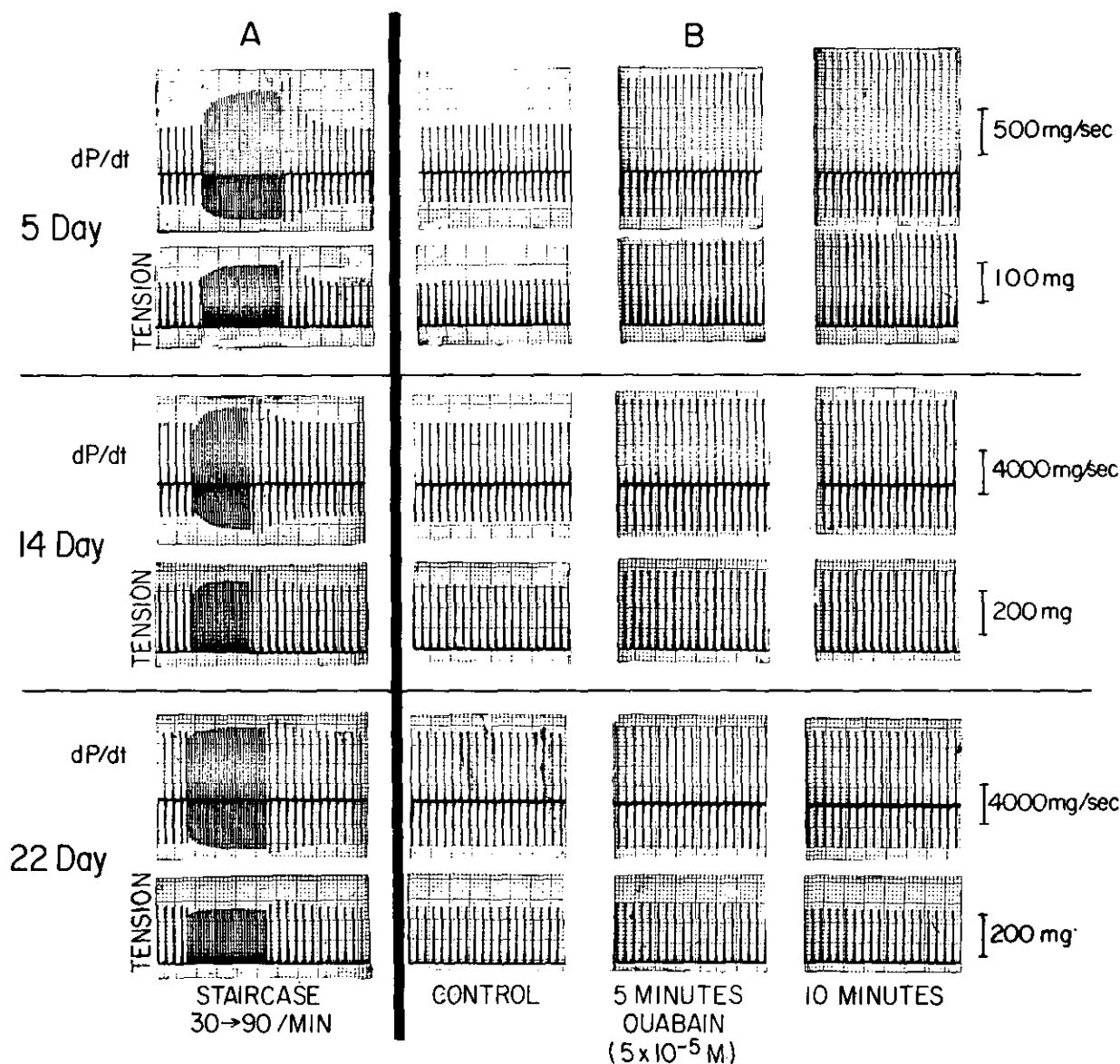


FIGURE 2. Tension and  $dP/dt$  responses: (A) following an increase in stimulation frequency from 30/min to 90/min of a papillary muscle from a 5-day-old, a 14-day-old, and a 22-day-old neonatal rat; (B) the same papillary muscles 5 and 10 min after the administration of  $5 \times 10^{-5} M$  ouabain, stimulation frequency 30/min. Reproduced with permission of Circ. Res.

contrasted to the absence of increased uptake in the quiescent, nonelectrically stimulated rabbit heart and in the stimulated rat heart—i.e., the rabbit's response is made ratlike by the absence of membrane depolarization.

The response of K exchange to magnesium is also dissimilar in the rabbit and the rat (6, 17). In the rabbit, Mg did not affect ventricular K exchange under normal physiological perfusion conditions, whereas K exchange rate in the rat ventricle was markedly depressed by Mg. In order that an effect of Mg be demonstrated in the rabbit, K efflux had

first to be increased by administration of a digitalis compound. Mg would then abolish the glycoside-induced net K loss. In this case, then, the rabbit was made ratlike by prior administration of digitalis.

Mg also affects force development very differently in the rat and rabbit. Perfusion of the rabbit ventricle with  $20 mM$  Mg decreases the rate of tension development ( $dP/dt$ ) to 84% of control whereas the same concentration reduces  $dP/dt$  of the rat ventricle to 15% of control (6, 17). Obviously the rat demonstrates a great deal more sensitivity to the excitation-contraction uncoupling effects of Mg.

## Digitalis Glycosides

It is well known that the adult rat heart is remarkably resistant to the action of the digitalis glycosides (7, 8). Doses of glycosides which are markedly toxic to most mammalian hearts produce little or no effect in the rat. The resistance to the drug has been proposed to be due to its more rapid dissociation from binding sites in the rat heart as compared to the dissociation from hearts of other species (3, 4, 18).

As with AP and force-frequency the neonatal rat myocardium presents a different response which is much less anomalous (2). Papillary muscle from a rat 5 days old demonstrated an increase of 160% in  $dP/dt$  upon perfusion with  $5 \times 10^{-5}M$  ouabain. A muscle from rats 22 days old demonstrated an increase in  $dP/dt$  of only 12% given the same dose of glycoside (Fig. 2). It is notable that the sensitivity of the target enzyme of the glycoside, Na-K ATPase, was exactly the same in the hearts from rats 5 days old and from adult rats (personal communication, K. D. Philipson). This indicates that the different response cannot be attributed to a change in the characteristics of the enzymes with age. The reason for the progressive resistance to glycoside must be related to another system which is operative in the control of contractile force.

The anomalies of the rat heart discussed above have provided important clues leading to the further definition of the systems important to control of contractility in the mammalian heart.

## Model for Myocardial Contractile Control

Details of a model (Fig. 3) for the ionic control of myocardial contractility have been developed in a number of recent publications (19, 20), but a brief summary is necessary here in order to understand how the anomalous rat is the exception that may lead to proving the rule or, in this case, the model.

Mammalian hearts, including the rat, are dependent upon a rapidly exchangeable source of calcium (Ca) for the support of contractile force. It remains uncertain as to whether the major portion of Ca involved in E-C coupling moves across the sarcolemma with each beat or whether a portion is released from internal sarcotubular sites by the mechanism of Ca-induced Ca release (21). It is possible that a combination of the two mechanisms is operative, but it remains, nevertheless, certain that modulation of the amount of Ca which crosses the sarcolemma upon excitation is of critical importance in control of the contractile response. This Ca is probably derived from sites at the surface of the cell within the surface-coat/external lamina com-

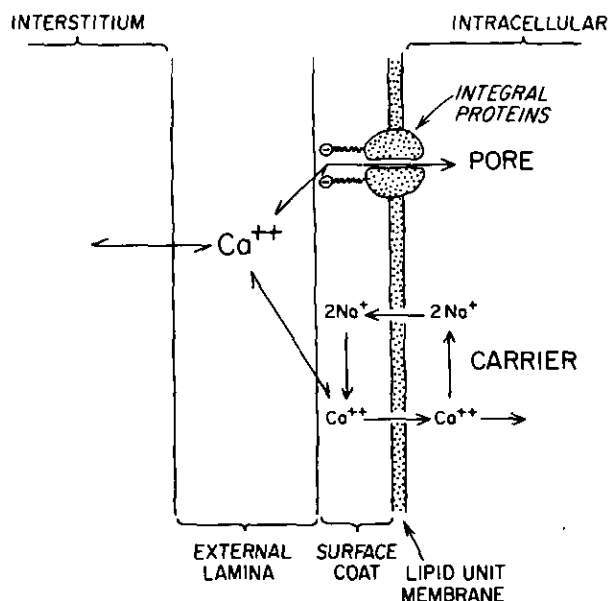


FIGURE 3. Model for Ca movement. Ca is bound to negatively charged sites in the external lamina of the membrane. These sites are in rapid equilibrium with Ca in the interstitium. The external lamina is proposed to supply the Ca that moves across the sarcolemma via two routes: (1) through a pore system formed by integral proteins embedded in the lipid membrane; movement through this system would be electrogenic; (2) with a carrier (coupled to outward Na movement) such that movement via this system is electroneutral. Reproduced with permission of International Review of Physiology, Copyright, 1976. University Park Press, Baltimore.

plex (22). This complex contains large amounts of glycoprotein and glycolipid and carries a large quantity of negatively charged sites which are believed to represent the loci for Ca binding. Although extensive interspecies studies have not been done it is likely that the surface coat/external lamina complex functions similarly in mammals, including the rat. It should be noted however, that the rat's sensitivity to uncoupling by Mg (see above) may indicate some differences in the structure of the surface complex.

The next component of the model is concerned with the movement of Ca from its binding sites across the sarcolemmal complex. Evidence now indicates that a major portion of this movement occurs via a carrier system which couples the movement of one Ca ion inward to the movement of two Na ions outward (23). The rate at which the carrier moves or the number of carriers which are activated (the models are equivalent) depends upon the concentration of a component of intracellular sodium  $[Na]_i$ . An increase in  $[Na]_i$  causes increased carrier activity which results in greater Na movement outward and greater Ca movement inward. The latter

would be expected to bring additional Ca to the myofilaments and result in a positively inotropic response (Fig. 3).

If such a Na-Ca carrier is operative, any intervention which results in increased  $[Na]_i$  would result in increased contractile force. In most mammalian hearts, except the adult rat, increased frequency of stimulation and administration of digitalis clearly produce a significant increase in  $[Na]_i$  and a significant increase in contractile force. The response fits and is predicted by the Na-Ca carrier system. As I have discussed, the adult rat does not demonstrate a positive inotropy upon an increase in stimulation frequency or when given digitalis. Neither does the heart show a gain in  $[Na]_i$  (as indicated by absence of a net K loss) with the above interventions and the reason for this may be found in the configuration of its action potential. As shown (Fig. 1), the plateau is progressively lost with age. A component of the plateau was shown to be dependent upon a slow Na channel which seemed to be present and operative in the young neonatal rat (and in other adult mammals) but lost in the adult. The closure of this Na channel would be expected to greatly reduce the Na influx with each excitation. It would follow that there would be a reduced tendency for the adult rat to accumulate  $[Na]_i$  when the excitation frequency is increased or the Na pump is inhibited by digitalis. Since elevation of  $[Na]_i$  is the stimulus for the Na-Ca carrier activation, this activation would not occur, Ca influx would not increase and contractility would not be augmented. The sequence as described for the neonate to adult is consistent with progressive closure of the slow Na channel, accounts for the anomalous behavior described and adds support to the model proposed for the control of Ca movement in the mammalian heart.

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