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Changes in α_1-adrenoceptor coupling to Ca^{2+} channels during development in rat heart

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Abstract

It has been reported in the literature that α_1-adrenoceptor activation in adult rat heart does not cause an increase in Ca^{2+} current but involves a decrease in I_t. This may explain in part the positive inotropic effect of α_1-adrenoceptor activation. In this study, the effect of phenylephrine, an α-adrenergic agonist, on L-type Ca^{2+} channel current was compared in young and neonatal rat myocytes. In the presence of propranolol, phenylephrine increased the Ca^{2+} current (reversed by prazosin) in neonatal but not in young rat myocytes suggesting that the coupling of the α_1-adrenoceptor to Ca^{2+} channels may switch during development.

Key words: α_1-adrenoceptor; Ca^{2+} channel current; Neonatal rat ventricular cell; Young rat ventricular cell

1. Introduction

In addition to β-adrenoceptor-induced positive inotropism and chronotropism, cardiac α-adrenoceptor stimulation also induces positive inotropism and chronotropism. Although a mechanism underlying the α_1-adrenoceptor mediated effect in the adult rat heart has been reported to involve the reduction of I_t (transient outward potassium current) [1–2], the ionic mechanisms underlying α_1-adrenergic activation in neonatal rat ventricular cells are not known. It has been suggested that there are significant differences in α_1-adrenoceptor mechanisms in the neonatal and adult rat heart. There are, for example, differences in α_1-adrenergic receptor density [3–4], chronotropic responsiveness to α_1-agonists [5], the G protein coupling to α_1-adrenoceptors [6] and the role of sarcoplasmic reticulum in releasing Ca^{2+} [7]. There have also been reports of differences in the distribution and the density of the ion channels during various stages of development. For example, I_t is often absent from, and appears to have little funtional importance in, newborn cells whereas the density of Ca^{2+} current is greater in neonatal rat myocytes than that seen in adult rat myocytes [8–9]. This suggests that different ionic mechanisms may exist in neonatal and adult heart for the positive inotropic responses induced by α_1-adrenergic agonists. The present study investigates the effect of α_1-adrenoceptor activation on the L-type Ca^{2+} channel current at two stages of development. This study may further our understanding of the pathology of the heart since altered α_1-adrenergic receptors may have critical importance in the pathogenesis of arrhythmias and may influence myocardial cell viability after ischaemic insult [10].

2. Materials and methods

Single ventricular cells were isolated from the heart of either young (1–2-month) or neonatal (3–4-day-old) Sprague–Dawley rats by enzymatic dispersion and mechanical disruption. The heart, in each case, was removed rapidly under sterile conditions from the chest cavity of the anaesthetized rat.

2.1. Neonatal rat myocytes

Collagenase (0.1 mg/ml) (type IV, Sigma) was used to digest the tissue [8]. The heart was cut into pieces which were placed in six successive changes of the enzyme solution. The tissue pieces were triturated with a pasteur pipette after each incubation. After the remaining tissue pieces were removed, the enzyme solution was collected and mixed with DMEM. The solution was then centrifuged and the pellet was resuspended. The solution was filtered through a 200 μm nylon mesh. After filtration, the dispersed cells were incubated at 37°C in an atmosphere of 5% CO_2/95% room air for 2 h to facilitate the attachment of the fibroblasts to the culture dishes. After the fibroblasts were attached, the ventricular myocytes were removed and dispersed into 35 mm Petri dishes and kept in an incubator at 37°C in an atmosphere of 5% CO_2 and 95% room air until use.

2.2. Young rat myocytes

The cells were dissociated according to the method of Ravens et al. [2]. After the heart was removed from the chest, the aorta was cannulated and the heart was perfused via the coronary arteries for 5 min with a Ca^{2+}-free solution containing (in mM), NaCl 100, KCl 10, $MgSO_4$ 5.0, KH_2PO_4 1.2, glucose 20, taurine 50, MOPS[3-(*N*-morpholino)propanesulfonic acid] 10 and 1 mg/ml bovine serum albumin. The pH of the solution was adjusted to 7.2 and it was aerated with a mixture of 95%

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O_2/5% CO_2 at 37°C. This was followed by a 10–15 min perfusion with Ca^{2+}-free solution containing 1.0 mg/ml collagenase (type IV, Sigma) at a rate of 10 ml/min at 37°C. Following collagenase digestion, the aorta and atria were trimmed away, the ventricles were cut open and the ventricular tissues were incubated in fresh collagenase solution at 37°C, and gently agitated. The myocytes were harvested by decanting the solution from the remaining ventricular tissue. The myocytes were then washed and centrifuged. The solution was filtered through a 200 μm nylon mesh and diluted to about 3×10^5 cells/ml. The Ca^{2+} concentration in the myocyte suspension solution was increased, in steps, to 2 mM. After addition of Ca^{2+}, the ventricular myocytes were dispersed into 35 mm Petri dishes and kept in an incubator at 37°C in an atmosphere of 5% CO_2 and 95% room air until use.

2.3. Culture time

Neonatal rat myocytes were maintained in culture for about 8 h after plating. They were used for experiments within 10 h. The myocytes from young rats were maintained in culture for 4 h before use. They were used for experiments within 8 h.

2.4. Ca^{2+} current recordings

The currents were recorded using the whole cell version of the patch clamp technique. Patch electrodes were pulled and heat polished using borosilicate glass capillary tubes (OD 1.2 mm, ID 0.9 mm, FHS, Brunswick, ME, USA), and filled with a solution containing (in mM): Cs_2-aspartate 70, HEPES 20, EGTA 11, $CaCl_2$ 1, $MgCl_2 \cdot H_2O$ 5, glucose 5 and ATP-Na_2 2. Cs_2-aspartate was synthesized by Dr. H. J. Liu, Department of Chemistry, University of Alberta. The osmolarity was adjusted to 320 mOsm and the pH to 7.2. The recording solution for the measurements consisted of the following (in mM): Tris-Cl 130, $MgCl_2$ 0.8, KCl 5.4, $BaCl_2$ 20, tetrodotoxin 0.01 and HEPES 10. The membrane Ca^{2+} currents were measured using an Axopatch 1B patch clamp amplifier (Axon Instruments, Foster City, CA, USA). Series resistance was compensated using the Axopatch 1B. The data were sampled using pClamp software (5.5) and a Labmaster analogue-to-digital interface (Axon Instruments) and stored on floppy discs using a Zenith computer. Leakage and capacitative currents were subtracted using the same software. All voltage clamp data were obtained at room temperature (20–22°C).

The cells were depolarized from a holding potential of −40 mV to various potentials. The drug was usually added after the current reached the steady-state. The effects of the drugs were measured every 5 min using depolarizing test pulses (indicated in the text) applied at a frequency of 0.05 Hz.

3. Results

Electrophysiological experiments using dissociated young rat and neonatal rat ventricular myocytes were carried out under identical conditions. Ba^{2+} (20 mM) was used as the charge carrier and the cells were depolarized from a holding potential of −40 mV to a test potential of +10 mV. The inward currents activated in both cells were sensitive to dihydropyridines (nifedipine), indicating that these currents were L-type Ca^{2+} channel currents [11] (Fig. 1). The characteristics of the L-type Ca^{2+} channel current from young rat myocytes are similar to those obtained from adult rat myocytes [1–2]. The absolute current amplitude from the young rat myocytes was much larger than the current amplitude from neonatal rat myocytes. This was in part due to the larger cell size and hence the larger membrane surface area of the myocytes from young rats. This study compares the effect of phenylephrine on Ca^{2+} channel currents in rat myocytes at two stages of development and not Ca^{2+} channel currents per se. Therefore, the current density was not used.

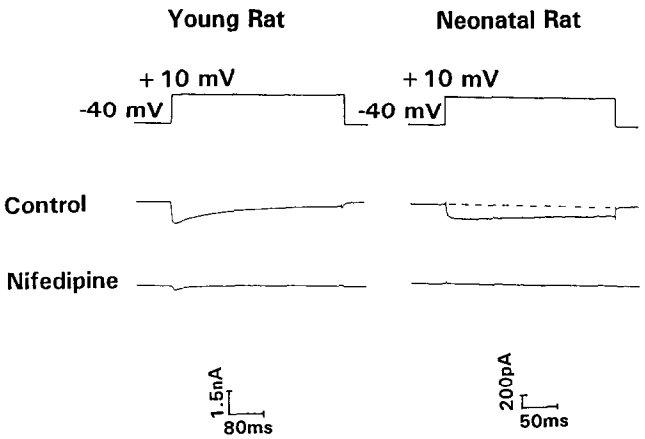

Fig. 1. Current records from young (left) and neonatal (right) rat ventricular cells. The L-type Ca^{2+} channel currents were activated by depolarizing the cell to +10 mV from a holding potential of −40 mV. Nifedipine (10 μM) blocked this inward current in both neonatal and young rat myocytes.

The effect of phenylephrine (10 μM) in the presence of propranolol (1 μM) on L-type Ca^{2+} channel current in neonatal rat ventricular myocytes is shown in Fig. 2. The inward Ca^{2+} channel current was activated by depolarizing the cell to various test potentials (250 ms duration) from a holding potential of −40 mV. In the presence of a β-adrenergic antagonist, propranolol, addition of 10 μM phenylephrine to the bath solution increased the L-type Ca^{2+} current (Fig. 2A,B). This increase was larger at more negative potentials. In addition, it was also observed that the inactivation of the current during the test depolarization after phenylephrine was faster and the tail current kinetics also changed (Fig. 2A). Administration of phenylephrine shifted the *I–V* relationship 10 mV towards more negative potentials (Fig. 2B). After addition of phenylephrine, the L-type Ca^{2+} channel current was enhanced. The subsequent application of prazosin (1 μM), an α_1-adrenergic antagonist, completely reversed the effect of phenylephrine on the Ca^{2+} current (Fig. 2C). These results suggest that phenylephrine-induced L-type Ca^{2+} channel current increase is mediated by α_1-adrenoceptors in neonatal rat ventricular cells.

A similar protocol was used for Ca^{2+} channel current recording from single young rat ventricular cells (Fig. 3). Phenylephrine (10 μM) did not affect the L-type current during a 30 min experiment. However, Bay K 8644 (1 μM) increased L-type current and La^{3+} (2 mM) completely blocked this current after phenylephrine. Bay K 8644 not only increased the Ca^{2+} channel current, but also shifted the peak current in the *I–V* relationship towards more negative potentials, as has been reported previously [12]. These results suggest that the L-type Ca^{2+} channel in young rat ventricular myocytes is not modulated by α_1-adrenoceptor activation.

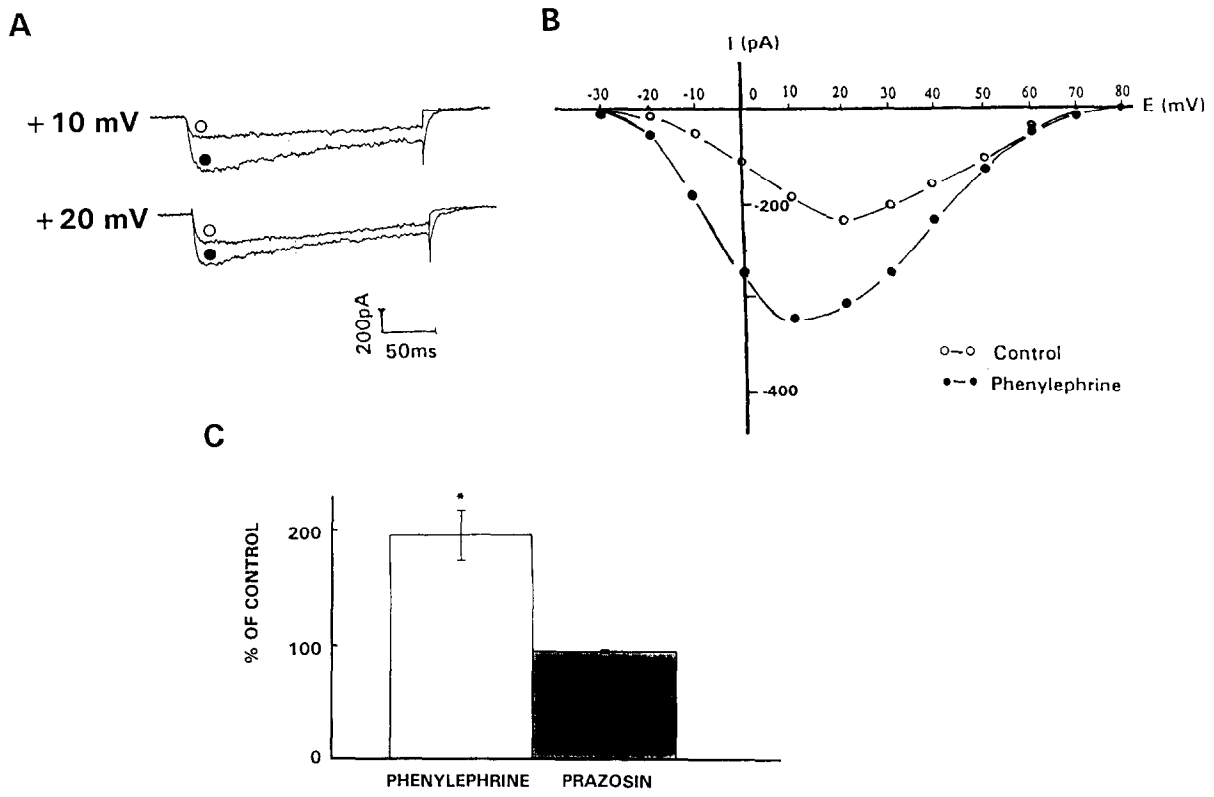

Fig. 2. Effect of phenylephrine (10 μM) on the L-type current in a neonatal rat ventricular cell. The cells were depolarized to various potentials from a holding potential of −40 mV. In the presence of propranolol (1 μM), phenylephrine increased L-type Ca^{2+} channel current. The original current records (A) and the I–V relationship (B) before and after administration of phenylephrine (10 μM) are shown. After the phenylephrine-induced increase in the L-type Ca^{2+} channel current occurred, prazosin (1 μM) was added into the bath. Prazosin reversed the effect of phenylephrine (n = 3) (C). Data are expressed as percentage of the control, mean ± S.E.M. *$P < 0.05$ (Student's t-test).

Fig. 4 shows a comparison of the responses of two groups of cells to phenylephrine. Phenylephrine increased the L-type Ca^{2+} channel current significantly in neonatal rat myocytes (n = 9) while it did not affect the L-type Ca^{2+} channel current in young rat myocytes (n = 6). The absolute control amplitude of Ca^{2+} current from neonatal rat ventricular cells was −300.58 ± 48.1 pA (n = 9) and that from young rat ventricular cells was −1601.90 ± 221.8 pA (n = 6). The data were compared to control values and plotted as a percentage of the control values. Therefore, our results clearly show that the increase in the L-type Ca^{2+} channel current in response to phenylephrine is different during the two stages of development, i.e. the neonatal (3–4 day) and young (1–2 month) rat.

4. Discussion

L-type Ca^{2+} channels can be demonstrated in both neonatal and young rat ventricular myocytes. These channels are sensitive to dihydropyridines (nifedipine). The current records from young rat myocytes showed some inactivation during a 400 ms depolarization. The inactivation shown in Fig. 1 was not as prominent as in previous reports and the current amplitude in our case was larger [2]. This was due to the fact that 20 mM Ba^{2+} was used as the charge carrier in these experiments while Ca^{2+} was used in other experiments [2]. Twenty mM Ba^{2+} was used as the charge carrier in the neonatal rat ventricular myocytes to increase the magnitude of the L-type Ca^{2+} channel current and to reduce Ca^{2+}-dependent Ca^{2+} current inactivation. Twenty mM Ba^{2+} was also used in the experiments with young myocytes in order to make a comparison between neonatal and young rat ventricular myocytes under similar conditions.

In contrast to the results obtained with the young/adult rat, phenylephrine increased Ca^{2+} channel current in neonatal rat ventricular myocytes in the presence of propranolol, a β-adrenergic antagonist. This effect was blocked by prazosin, an α_1-antagonist, indicating that phenylephrine increased the Ca^{2+} current via α_1-adrenoceptor activation in neonatal rat heart.

Phenylephrine did not affect the L-type current in young/adult rat ventricular myocytes. The results from these experiments are similar to those of other studies using cardiac tissue from different species. In adult rat myocytes, 10 μM phenylephrine or methoxamine had no effect on the Ca^{2+} channel current [1–2,13–14]. In rabbit, guinea-pig and cat myocytes, phenylephrine did not af-

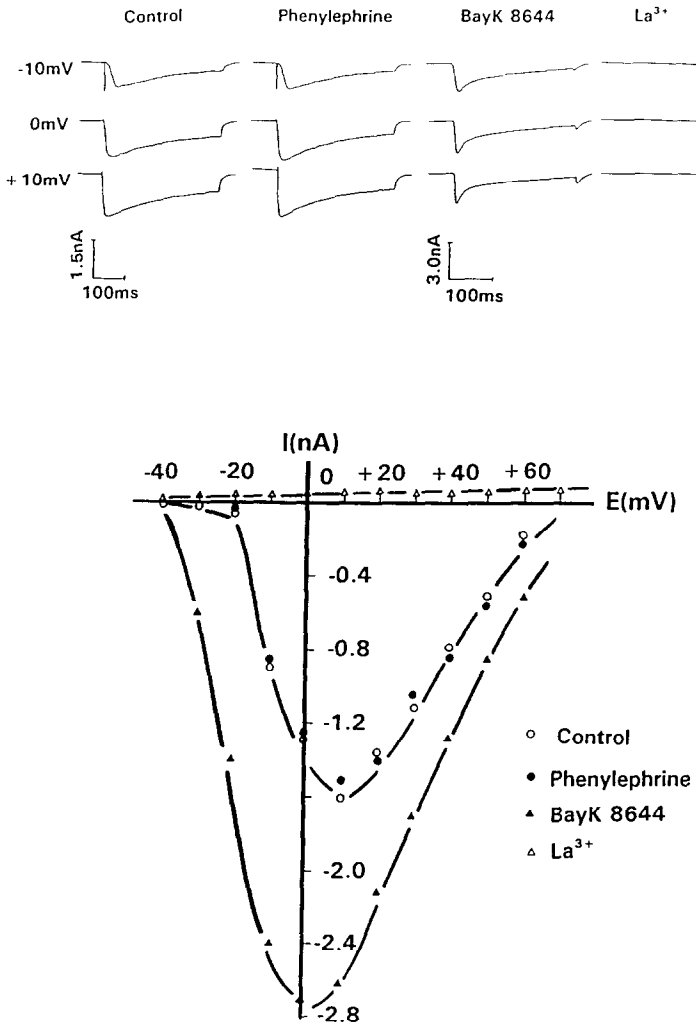

Fig. 3. Effect of phenylephrine (10 μM) on the L-type current in a single ventricular cell (young rat). Upper panel: current records (L channel) from one cell with the magnitude of the test potentials indicated next to each record. Phenylephrine had no effect on the L-type Ca^{2+} channel current. Bay K 8644 (1 μM) increased the current and La^{3+} (2 mM) completely blocked the current. Note that the calibration bars are identical for the control and phenylephrine experiments, but different for the Bay K 8644 experiments. Lower panel: *I–V* relationship plotted from the current records shown in the upper panel. Bay K 8644 not only increased the current amplitude but also shifted the peak current towards more negative potentials. La^{3+} completely blocked the L-type current.

fect Ca^{2+} channel currents [15–17]. In contrast, an increase in I_{Ca} was reported in frog myocytes [18] and bovine Purkinje fibers [19]. However, recent studies have demonstrated that the stimulation of α_1-adrenergic receptors in the heart results in a decrease in the transient outward K^+ current, which might account for the prolonged action potential and positive inotropic response to α_1-agonists [15]. It is not clear why there are differences in the effects of phenylephrine on ion channels, but this discrepancy may be related to tissue as well as species differences, as has been suggested by Endoh et al. [20].

This study shows that different ionic mechanisms may be responsible for the α_1-adrenoceptor activation in rat heart during different stages of development. There is evidence from the literature which supports the hypothesis of a developmental switch in the coupling of the α_1-adrenoceptors to ion channels. I_{k1}, the inwardly rectifying background potassium current, decreased in density whereas I_t, the transient outward current, increased in density during development [8]. I_t which is decreased by the α_1-adrenergic activation in the adult is often absent, or apparent only at positive potentials, in newborn rat myocytes [8]. In contrast, the density of the Ca^{2+} channel current in neonatal rat myocytes was observed to be greater than that seen in the adult [9]. Therefore, it is very likely that the α_1-adrenoceptors are coupled to Ca^{2+} channels at an early stage of development and then shift to the I_t in the mature state. Another point is that ventricular myocytes from prenatal and newborn rats have an action potential with an elevated plateau similar to that of other mammalian species [21], while the action potential in adult rat ventricular cells has a duration of less than 100 ms [1]. The duration of the action potential is

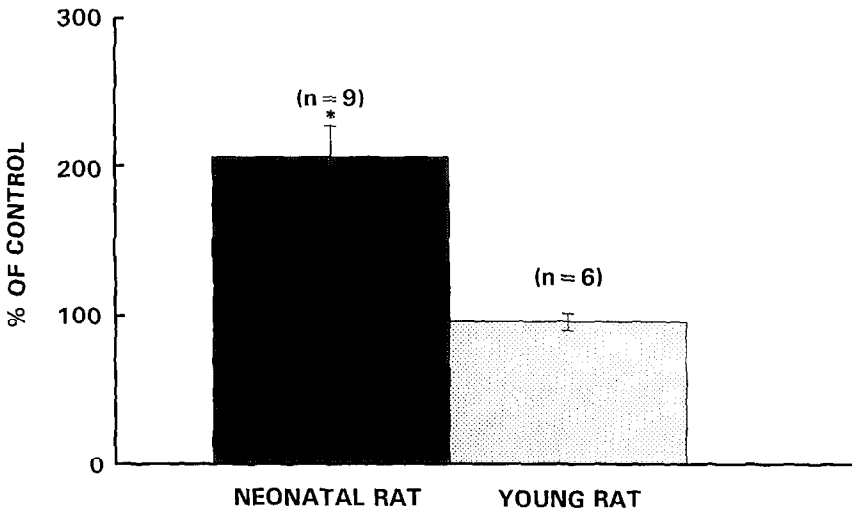

Fig. 4. Effect of phenylephrine (10 μM) on the L-type Ca^{2+} channel current in two groups of cells. The cells were depolarized from a holding potential of −40 mV and the I–V relationships before and after the drug were recorded. The peak currents in the I–V were calculated and compared (test potential was 10 to 20 mV). Phenylephrine did not increase the L-type Ca^{2+} channel current in young rat ventricular myocytes (n = 6) but significantly increased the L-type Ca^{2+} channel current in neonatal rat ventricular myocytes (n = 9). Data are expressed as percentage of control, mean ± S.E.M. *$P < 0.05$ (Student's t-test).

in part due to the influence of Ca^{2+}. It has been reported that α_1-adrenoceptor stimulation by phenylephrine had both a negative and a positive chronotropic effect on isolated ventricular cells from the adult rat. However, only a positive chronotropic effect was observed in neonatal ventricular cells [22–23]. These results are related to the maturation of the autonomic innervation of the neonatal rat myocytes [4]. In addition, recent data indicate that the sarcoplasmic reticulum (SR) plays an important role in the contraction of the adult myocardium but has a much smaller role in the contraction of neonatal myocardium [7]. Thus, other mechanisms such as the entry of Ca^{2+} via Ca^{2+} channels may have a greater influence on the contractility of the neonatal myocardium. As may be seen from the above discussion, neonatal and adult rat hearts vary in many aspects. This is the first report to show that phenylephrine, an α-adrenergic agonist, increases the Ca^{2+} channel current in neonatal rat ventricular cells. The results described here suggest that the ionic mechanism of the α_1-adrenoceptor activation may change from the modulation of Ca^{2+} channels to the modulation of K^+ channels during development. Although ion channel modulation plays a role in the inotropic effect, it is only one of the possible mechanisms.

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