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Immediate-early gene induction by repetitive mechanical but not electrical activity in adult rat cardiomyocytes

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Mechanical factors are thought to play an important role in the induction of myocardial hypertrophy. Yet, it is not known whether active contraction induces genes that probably represent initial steps in the hypertrophic response in the adult myocardium – and if so, whether the mechanical or the electrical component of the twitch governs this response. We therefore investigated whether electrical stimulation of contraction was able to induce the immediate-early genes (IEGs) *egr-1* and *c-fos* in adult rat cardiomyocytes. Cyclical contraction led to an increase in *egr-1* and *c-fos* mRNA levels within 30 min. Full inhibition of contraction during electrostimulation by the Ca^{2+}-desensitizer 2,3-butanedione monoxime (BDM) totally blocked this IEG-response without altering membrane potential. These data suggest that in adult myocardium, the mechanical rather than the electrical activity is responsible for the IEG-response during active twitch.

Heart; *egr-1*; *c-fos*; Contraction; 2,3-Butanedione monoxime

1. INTRODUCTION

Load is a main determinant of cardiac hypertrophy, in both whole heart [1] and passively stretched isolated myocytes [2–6]. This demonstrates that cardiac myocytes themselves are able to sense mechanical forces and to transform these signals into a hypertrophic response. One of the first changes in growth induction is the expression of a specific set of immediate-early genes (IEGs) [3,5,7] which encode a limited number of regulatory proteins critical for signal transduction pathways and may thereby control cardiac growth [8]. These changes are followed by an increase in protein and RNA synthesis [4], isoform switching of contractile proteins [5,9] and ventricular reexpression of fetal genes such as the atrial natriuretic factor [5].

Another mechanical stimulus capable of inducing cardiac hypertrophy is contractile activity. Repetitive contraction of neonatal cells has been demonstrated to cause myocardial growth as evidenced by an increase in protein and RNA synthesis [10] as well as by the induction of atrial natriuretic factor and myosin light chain-2 [11]. In adult feline myocytes it has been shown that β-adrenergic-induced beating was followed by an increase in protein synthesis and by a significantly greater development of myofibrillar structures than in quiescent cells at the same age in culture [12].

However, it remains to be elucidated whether the mechanical component of contraction or associated depolarization (i.e. the electrical activity) triggers the hypertrophic response. This question is relevant since electrical activity alone, i.e. in the absence of mechanical action, has been shown to induce a hypertrophic (or a proliferative) response in (1) skeletal muscle where the MyoD family of myogenic factors is regulated by electrical activity [13], and (2) neuronal cells (i.e. non-contractile, yet excitable cells) which react upon electrical stimulation with an IEG-program [14], which is thought to play a major role in synaptic plasticity. Furthermore, it has recently been shown that electrical activity of axons was sufficient to induce the proliferation of surrounding oligodendrocytes probably by the production and release of growth factors [15], thus supporting the idea of an auto-/paracrine mechanism of growth control by electrical activity.

In the present study, we attempted to distinguish between electrical and mechanical components of contraction in the induction of an immediate-early gene program in adult cardiomyocytes using 2,3-butanedione monoxime as an electromechanical uncoupler. This agent is known to reversibly inhibit contraction mainly by desensitizing the myofilaments against calcium [16]. The results show that it is mechanical, not electrical activity that causes the IEG-response in the adult myocardium.

2. MATERIALS AND METHODS

Cardiomyocytes were isolated from adult (8–10 weeks), male Wistar–Kyoto rats (200–250 g) as described by Powell [17] with slight modifications [18]. Cells were diluted to a final concentration of approximately 10^5 cells/ml in a medium containing Dulbecco's modified

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Eagle's medium (DMEM) 6.76 g/l, Ham's F12 5.35 g/l and HEPES 20 mM (all reagents from Serva). Cells were stimulated by biphasic electrical stimuli (40 V/cm, 5% above threshold). The stimulation system used has been described in detail previously [19]. Contraction analysis was performed at 37°C using a computerized videomicroscopic system. Cell shortening was expressed as percentage of total diastolic cell length. Total RNA for Northern blot analysis was isolated as described by Chomczynski [20]. A 2.1 kb fragment of *egr-1* [21], a 1.0 kb fragment of *v-fos* (Oncor) and a 0.77 kb fragment of β-actin (Oncor) were used as probes. Labeling was performed by the random oligohexanucleotide method. All blots are representative of at least 3–4 experiments. Electrical recordings were performed using the patch-clamp technique in whole cell recording mode [22]. Patch-electrodes had a resistance from 3–5 MΩ, those used for potential recordings exclusively had higher resistances. The electrodes were filled with (in mM): KCl 135, EGTA 0.2, HEPES 10 titrated with NaOH to pH 7.2.

3. RESULTS

Electrical stimulation of contraction for 30 min at 120 beats per minute (bpm) caused a rapid increase in the mRNA-levels of the two IEGs *egr-1* and *c-fos* as detected by Northern blot (Fig. 1). This induction was 5-fold for *egr-1* and 4-fold for *c-fos*. The induction of the IEGs was transient, after 60 min there was no significant induction detectable as compared to the control (Fig. 2). The expression of *egr-1* was maximal at 30 min whereas *c-fos* showed a somewhat earlier peak at 15 min (Fig. 2). Fig. 3 shows that BDM caused a dose-dependent inhibition of contraction, with full inhibition at 10 mM BDM, which was reversible on redilution. Patch-clamp recordings revealed a resting potential (RP) of rat ventricular cells of −63 mV ± 5 mV (n = 9). Perfusion with BDM 10 mM did not shift the RP significantly (shift 0.7 ± 1.8 mV; n = 6), nor did the N-shaped steady state current/voltage relation as elicited by a ramp clamp from −100 to 50 mV show signifcant differences. At 10 mM BDM the IEG-induction after electrostimulation was totally blocked (Fig. 2). This demonstrates that mechanical activity and not the triggering depolarization is the major determinant of contraction-induced *egr-1* and *c-fos* expression in adult rat cardiomyocytes.

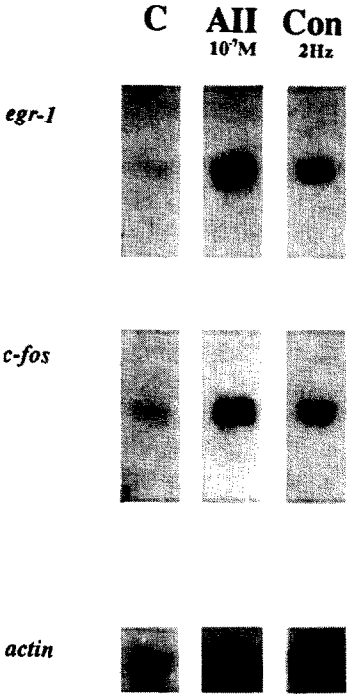

Fig. 1. Northern blot: induction of the immediate-early genes *egr-1* (3.4 kb transcript) and *c-fos* (2.2 kb transcript) after electrical stimulation of contraction (Con) (30 min at 120 bpm). Angiotensin II (AII) was chosen as a reference ([AII] = 10^{-7} M). (C = unstimulated control). Lower part: actin-control.

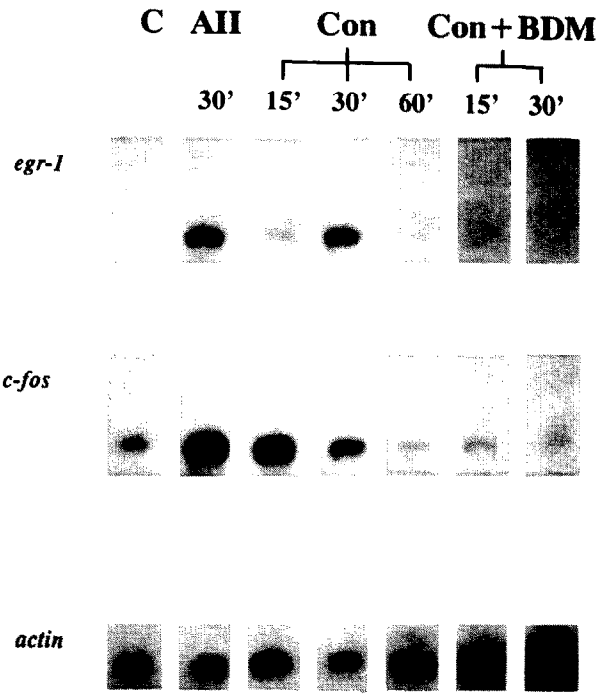

Fig. 2. Northern blot: total inhibition of twitch-induced IEG-expression by BDM ([BDM] = 10 mM). (C = unstimulated control; AII = Angiotensin II, 10^{-7} M; Con = Contraction (120 bpm)). Lower part: actin-control. 8 μg total RNA/lane except in: contraction 15 min and 60 min, contraction + BDM 15 min and 30 min. A higher amount (15 μg) was loaded in these lanes to bring out eventual signals.

To elucidate the possible effects of permanent (as opposed to repetitive) depolarization we investigated whether depolarization by high extracellular potassium induced the IEG-program. Fig. 4a shows that raising the extracellular potassium concentration from 4 to 25 mM resulted in an induction of the two IEGs after 30 min. Patch-clamp recordings revealed a reversible depolarization from RP to −31 ± 1.2 mV (n = 5) using 25 mM KCl. Cell length showed no significant changes by KCl (Control 100%; KCl 101.2 ± 2.1%; n = 8) indicating that mechanical factors were not responsible for this IEG-induction (Fig. 4b).

4. DISCUSSION

This study shows that the induction of an immediate-early gene (IEG) program by repetitive electrical stimu-

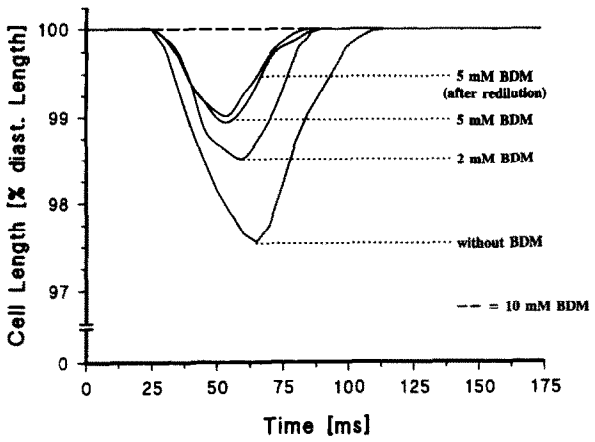

Fig. 3. Contraction analysis of electrically stimulated cardiomyocytes in the absence and presence of 2,3-butanedione monoxime (BDM) ([BDM] 0–10 mM). Redilution to 5 mM BDM after full inhibition of contraction (10 mM BDM) demonstrated the reversibility of the BDM effect on contraction. Values are mean, $n = 5$. $P < 0.05$ for all BDM-curves vs. control.

lation in adult cardiomyocytes is due to the mechanical rather than the electrical component of the twitch. Because of the impossibility to induce contraction without changing membrane potential, we made use of the electromechanical uncoupler 2,3-butanedione monoxime (BDM) to test whether abolishing mechanical activity would leave the IEG-response unchanged. In agreement with earlier observations we were able to show that 10 mM BDM leaves membrane potential of rat cardiomyocytes unchanged [23,16]. The results show that BDM in the lowest concentration which abolished contraction also abolished the IEG-response. According to the literature 10 mM BDM mainly results in a Ca^{2+}-desensitation of myofilaments, but in addition, a decrease in the L-channel Ca^{2+}-current by about 30% [23,24] and a comparable reduction in the amplitude of $[Ca^{2+}]_i$-transients [23] have been described. Although unlikely, it cannot be excluded that these alterations in intracellular Ca^{2+} may interfere with the IEG-response, e.g. via a Ca^{2+}-sensitive kinase [25]. In any case, the conclusion holds that the electrical component of the twitch is not responsible for the IEG-program. In contrast to the ineffectiveness of repetitive short time depolarization, our results show a strong IEG-induction by high potassium, i.e. permanent depolarization. This finding is in agreement with reports in neuronal cells [25], yet in contrast to the situation in neonatal cardiomyocytes [6] and may be important in designing future experiments in this field, especially when growth responses in KCl- arrested cells are investigated.

The mechanisms by which stretch or contraction induce an IEG-response in the heart remain largely unknown. Recently, Sadoshima et al. performed an extensive series of experiments in neonatal rat cardiomyocytes using the IEG-response as a marker similarly to the approach we take here [6]. They were able to exclude microtubules, microfilaments and a special strech-sensitive ion-channel as transducing elements of the stretch-induced IEG-response. On the other hand, Komuro et al. showed that permanent stretch induced *c-fos* in iso-

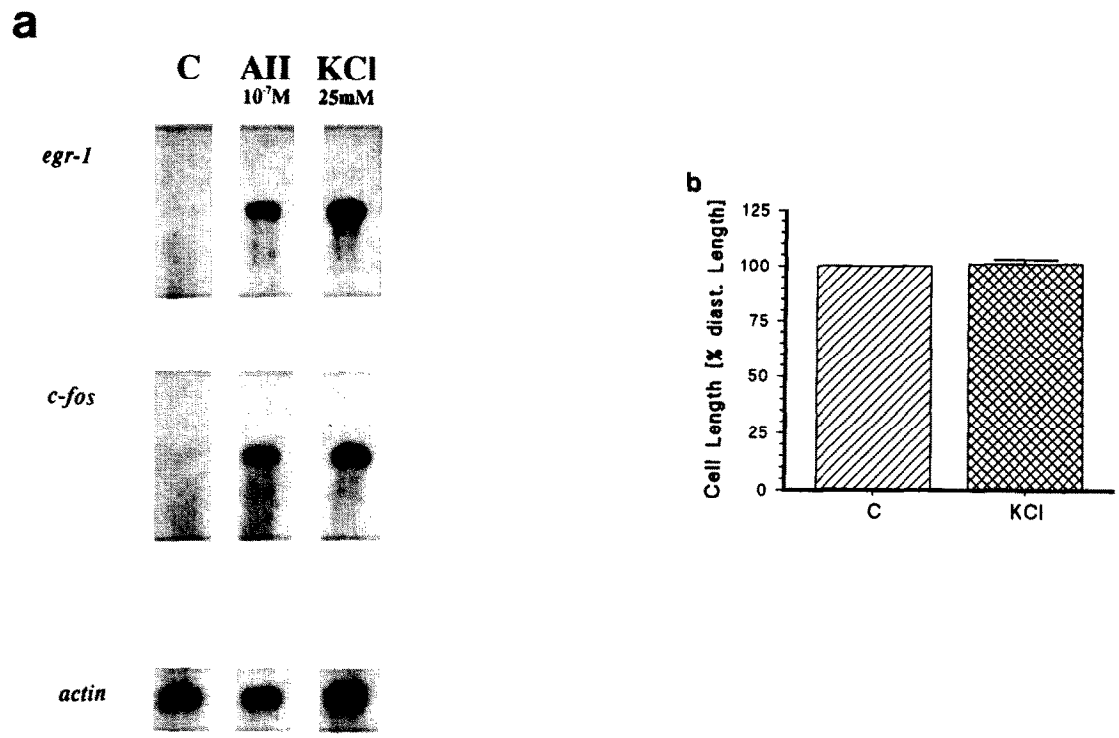

Fig. 4 (a) Northern blot: effect of permanent depolarization (30 min) by KCl (25 mM) on *egr-1* and *c-fos* mRNA-levels. (b) Cell length during KCl-depolarization (C = unstimulated control). Values are mean ± SD, $n = 8$, differences not significant, $P < 0.05$.

lated neonatal cardiomyocytes, probably via protein kinase C and a serum response element on the *c-fos* promoter [3]. In addition, Kim recently reported the existence of an extremely sensitive atrial, cation-selective ion channel which is activated by stress or hypotonic swelling [26], and which may play a role in stretch-induced IEG-expression. The present study largely eliminates the working hypothesis that 'mechano-transcriptional' coupling in heart is triggered by short time membrane depolarization. Three hypotheses for the mechanism of contraction-induced IEG-expression come to mind: (1) changes in intracellular Ca^{2+} may make a small contribution to the induction of an IEG-program; (2) stretch sensing mechanisms in the cytoplasmic cytoskeleton may play a role. This may be valid, even though the participation of some of the cytoskeletal structures in stretch-induced IEG-programs has been excluded [6]; (3) more appealing seems the assumption that a structure in the sarcomere itself is the stretch sensor. This may be the simplest explanation for the fact that there is a direct correlation between stretch and IEG-induction [3]. It would also explain that inhibition of various membrane phenomena [6] did not influence the IEG-response towards stretch.

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