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Short communication

Developmental conversion of inotropism by endothelin I and angiotensin II from positive to negative in mice

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Abstract

Inotropic effects on isolated neonatal and adult mouse myocardium of endothelin I and angiotensin II were examined. Endothelin I produced a sustained positive inotropic response in the neonate but a sustained negative response in the adult. Both were concentration-dependent and were inhibited by the endothelin ET_A receptor antagonist, BQ-123 (Cyclo(D-a-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl)). Angiotensin II produced a sustained positive inotropic response in the neonate while a sustained negative response in the adult. Both were concentration-dependent and were inhibited by the angiotensin AT_1 receptor antagonist, YM358 (2,7-diethyl-5-((2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl-5*H*-pyrazolo(1,5-b)(1,2,4)triazole potassium salt monohydrate). These results indicate that inotropic responses of the mouse heart to cardioactive peptides are unique among experimental animal species and may be reversed during development.

Keywords: Myocardium; Contractile force; Endothelin I; Angiotensin II

1. Introduction

Selective overexpression or knock-out of specific genes using transgenic technology has begun to provide useful information on various physiological processes including cardiovascular regulation. Overexpression or knockout of genes encoding proteins involved in the regulation of myocardial contraction such as α- and β-adrenoceptors has been reported (Ben-Yehuda and Rockman, 1996; Rockman et al., 1997). Although development of model animals with inheritable cardiac disorders would be useful for studying the pathophysiology of the heart, little information is available on the physiological and pharmacological properties of the mouse heart per se.

In the present study, we examined the inotropic responses of isolated neonate and adult mouse myocardium to two cardiovascular-active peptides, endothelin I and angiotensin II, whose positive inotropic effects have been extensively investigated in other experimental species [reviewed in (Endoh, 1995); Goto et al., 1996] Positive inotropic effects of these peptides have been reported in rabbit, guinea-pig, rat and human myocardium while absence of inotropic effects was reported for canine myocardia. Our objective was now to compare the inotropic effects of these peptides on mouse ventricular myocardium with those in other experimental animal species, and to clarify whether the responses change with development.

2. Materials and methods

Right ventricular free wall strips were rapidly isolated from 1-day-old and 2-week-old neonate and adult (4 to 5 weeks old) *ddy* strain mice. The approximate length and width of adult preparations were 3 mm and 2 mm, respectively, and those of neonate preparations were 2 mm and 1 mm, respectively. The preparations were placed horizontally in a 20-ml organ bath containing physiological salt solution of the following composition (mM concentration): NaCl 135, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, $NaHCO_3$ 15 and

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glucose 5.5 (pH 7.4). The nutrient solution was aerated with 95% O_2:5% CO_2 and maintained at 36.5°C. The preparations were driven by a pair of platinum plate electrodes (field stimulation) with rectangular current pulses (1 Hz, 5 ms, 1.2 × threshold voltage) generated from an electronic stimulator (Dia Medical System, DPS-165B). The resting tension applied to each preparation was adjusted so that the muscle was stretched to the peak of its length–tension curve. Contractile force was recorded isometrically with a force-displacement transducer (Nihon Kohden TB-611T) connected to a minipolygraph (Nihon Kohden RM-6100). The basal contractile force of adult preparations was 42.0 ± 4.1 mg ($n = 42$) and that of neonate preparations was 9.2 ± 0.8 mg ($n = 42$).

Peptides and drugs were added after the preparations had been allowed to equilibrate for more than 30 min, after which the developed tension of the preparations was well maintained. All drugs solutions were prepared in distilled water immediately before the start of the experiments. Small aliquots of a solution were added to the bath to give the desired final concentrations. The preparations were incubated with antagonists for 15 min before exposure to endothelin I or angiotensin II. Each preparation was exposed to only a single concentration of peptides to avoid desensitization or exhaustion of preparations. Peptides and drugs used were endothelin I (Peptide Institute), angiotensin II (Research Biochemicals International), BQ-123 (Cyclo(D-a-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl); Research Biochemicals International), BQ-788 (*N*-[*N*-[*N*-[(2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl) monosodium; Research Biochemicals International), YM358 (2,7-diethyl-5-((2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl)-5*H*-pyrazolo(1,5-*b*)(1,2,4)triazole potassium salt monohydrate; Yamanouchi), PD123319 (*S*(+)-1-[[4-(Dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid ditrifluoroacetate; Sigma), prazosin hydrochloride (Sigma), propranolol hydrochloride (Sigma) and atropine sulfate (Wako). All experiments comply with ''the Guiding Principles for the Care and Use of Laboratory Animals'' approved by the Japanese Pharmacological Society. Data were expressed as the means ± S.E.M.

3. Results

Endothelin I produced a negative inotropic effect in adult myocardium (Fig. 1). The contractile force decreased to $63.4 \pm 7.2\%$ ($n = 5$) of the basal value at 10 min after the addition of 10^{-8} M endothelin I. The effect was sustained and no spontaneous recovery of contractile force was observed; 10^{-7} M endothelin I did not produce a larger decrease in contractile force and 10^{-10} M had no

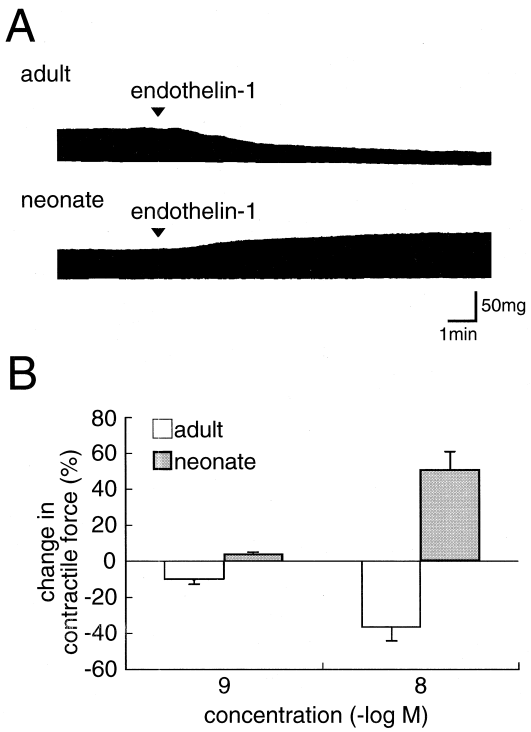

Fig. 1. Inotropic responses of ventricular myocardia from adult and neonatal mice to endothelin I. (A) Traces of changes in contractile force elicited by endothelin I in adult and neonate preparations; triangles indicate the addition of 10^{-8} M endothelin I. (B) Summarized inotropic responses to endothelin I in adult and neonate preparations. Columns and vertical bars represent the mean and S.E.M. from five to eleven experiments.

effect. In contrast, in the neonatal myocardium, endothelin I produced a positive inotropic effect. The contractile force increased to $150.9 \pm 10.1\%$ ($n = 6$) of the basal value at 10 min after the addition of 10^{-8} M endothelin I. The contractile force remained elevated for at least 60 min; 10^{-7} M endothelin I did not produce a larger increase in contractile force and 10^{-10} M had no effect. The negative and positive inotropic responses to endothelin I were not antagonized by 10^{-6} M propranolol, 10^{-6} M prazosin or 10^{-6} M atropine. At 2 weeks after birth, endothelin I had a minimum inotropic effect; the contractile force in the presence of 10^{-8} M endothelin I was $112.2 \pm 8.7\%$ ($n = 5$) of the basal value.

The endothelin ET_A receptor antagonist, BQ-123 (10^{-6} M), but not the endothelin ET_B receptor antagonist, BQ-788 (10^{-7} M), antagonized both negative and positive inotropic effects of endothelin I. In the presence of BQ-123, the contractile force of adult preparations at 10 min after addition of 10^{-8} M endothelin I was $85.7 \pm 3.2\%$ ($n = 11$) of the basal value and that of neonatal preparations was $112.4 \pm 5.9\%$ ($n = 8$).

Angiotensin II produced a negative inotropic effect in adult myocardium (Fig. 2). The contractile force decreased to $61.6 \pm 5.1\%$ ($n = 5$) of its basal value at 10 min after

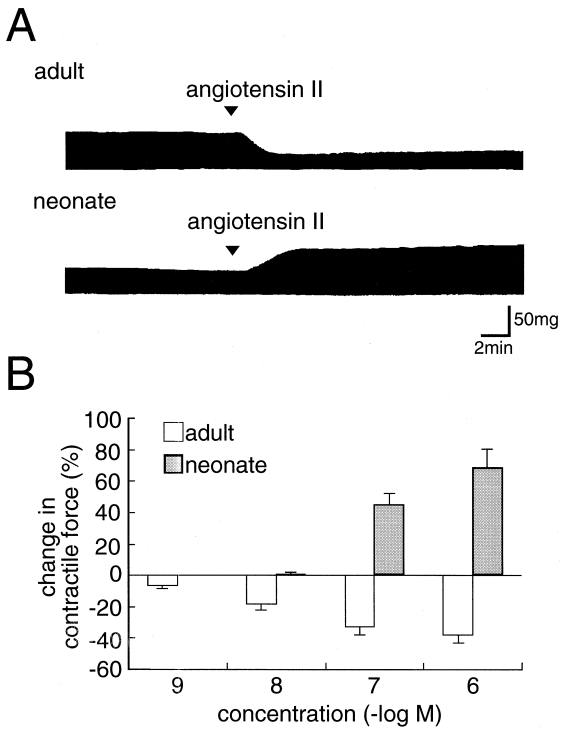

Fig. 2. Inotropic responses of ventricular myocardia from adult and neonate mice to angiotensin II. (A) Traces of changes contractile force elicited by angiotensin II in adult and neonatal preparations; triangles indicate the addition of 10^{-6} M angiotensin II. (B) Summarized inotropic responses to angiotensin II in adult and neonate preparations. Columns and vertical bars represent the mean and S.E.M. from five to eleven experiments.

the addition of 10^{-6} M angiotensin II. The effect was sustained and no spontaneous recovery of contractile force was observed; 10^{-5} M angiotensin II did not produce a larger decrease in contractile force and 10^{-10} M had no effect. In contrast, in the neonatal myocardium, angiotensin II produced a positive inotropic effect. The contractile force increased to $169.0 \pm 1.2\%$ $(n = 7)$ of the basal value at 10 min after the addition of 10^{-6} M angiotensin II. The contractile force remained elevated for at least 60 min; 10^{-5} M angiotensin II did not produce a larger increase in contractile force and 10^{-10} M had no effect. The negative and positive responses to angiotensin II were not antagonized by 10^{-6} M propranolol, 10^{-6} M prazosin or 10^{-6} M atropine. At 2 weeks after birth, angiotensin II had a minimum inotropic effect; the contractile force in the presence of 10^{-6} M angiotensin II was $93.9 \pm 7.7\%$ $(n = 5)$ of the basal value.

The angiotensin AT_1 receptor antagonist, YM358 (10^{-6} M), but not the angiotensin AT_2 receptor antagonist, PD133129 (10^{-6} M), antagonized both negative and positive inotropic effects of angiotensin II. In the presence of YM358, the contractile force of adult preparations at 10 min after addition of 10^{-6} M angiotensin II was $96.6 \pm 5.0\%$ $(n = 5)$ of the basal value and that of neonatal preparations was $98.8 \pm 1.8\%$ $(n = 5)$.

4. Discussion

Almost no basic pharmacological data on the mouse myocardial contraction are available in the literature, probably because the small size, extremely short action potential duration and high oxygen demand of the mouse heart make it relatively difficult to obtain isolated preparations free from arrhythmic activity (Binah et al., 1987; Barth et al., 1992). However, data on the effects of endogenous regulator substances and of basic pharmacological agents on the mouse myocardium are essential for the interpretation of data from transgenic mice and their application to the development of novel therapeutic agents. We have, therefore, been performing pharmacological studies on mouse myocardium and have found characteristics both similar to and different from those of myocardium from other experimental species. β-Adrenoceptor stimulation of the mouse myocardium, like that in most other species, results in positive inotropic responses and the sensitivity appears to be regulated by sympathetic innervation (Tanaka et al., 1994, 1995b). In contrast, α-adrenoceptor stimulation of the adult mouse myocardium results in a sustained negative inotropic response (Tanaka et al., 1995a,b), which is different from the results obtained with many other species in which positive or no responses were observed (Ishikawa et al., 1994; Endoh, 1996; reviewed by Endoh, 1995; Endoh et al., 1998).

In the present study, we examined the inotropic effects of two cardiovascular-active peptides, endothelin I and angiotensin II, and found that both peptides produced sustained decreases in contractile force in the adult mouse myocardium (Figs. 1 and 2), which was similar to the effect of α-adrenoceptor stimulation on adult mouse ventricle (Tanaka et al., 1995a,b). The effects of these peptides as well as of α-adrenoceptor stimulation on the myocardium have been extensively investigated in experimental species other than the mouse (Endoh, 1996; reviewed by Endoh, 1995; Endoh et al., 1998) and in most cases, positive or no inotropic effects have been reported. In the dog ventricular myocardium, sustained negative inotropic responses to endothelin I were observed only in the presence of β-adrenoceptor stimulation, which was not accompanied by the inhibition of cAMP accumulation (Zhu et al., 1997). The common features of α-adrenoceptor stimulation and the effects of endothelin I and angiotensin II are that they produce an increase in blood pressure through their potent vasoconstrictor action. In the myocardium, they all produce acceleration of phosphoinositide hydrolysis. Other mechanisms, such as activation of L-type Ca^{2+} channels and the Na^+/H^+ exchange, changes in intracellular pH and Ca sensitivity of contractile proteins, have also been suggested, but the causal relationship with inotropic effects has not yet been com-

pletely established (Ikenouchi et al., 1994; Endoh, 1996; reviewed by Endoh, 1995; Endoh et al., 1998).

Understanding of the mechanisms and functional significance for the negative inotropic responses to these stimuli of the mouse ventricle awaits accumulation of basic information. Pieces of evidence suggest that the mouse heart has unique properties compared with the heart of other experimental animal species. The mouse heart exhibits an extremely high beating rate which could be ascribed to the fact that, unlike other species, sympathetic, but not parasympathetic, innervation of the heart is dominant in the mouse (Ishii et al., 1996; Desai et al., 1997). The mouse myocardium is known to have a high oxygen consumption and mitochondrial content compared with those of other experimental animal species (Barth et al., 1992). It was recently reported that the mouse cardiac muscle has Ca^{2+} cycling and contractile activation properties different from those of other species (Gao et al., 1998). We have also reported pharmacological evidence that contraction of the mouse myocardium is highly dependent on sarcoplasmic reticulum function compared with that of other species (Tanaka et al., 1998). These features may explain the rapid contraction and relaxation of mouse myocardium. The reason why the inotropic responses to these three vasoconstrictive stimuli are negative in the adult mouse myocardium is not clear at present. It might be a mechanism to prevent the heart from being overaccelerated under increased total peripheral resistance. The absence of negative inotropic responses to these stimuli in neonatal mice, which have less functional sympathetic innervation (Tanaka et al., 1994), appears consistent with this view. Further investigation of the mouse heart may reveal the functional relation between its unique inotropic responsiveness to vasoconstrictive agents and the unique characteristics of its cardiovascular system.

In the neonate mouse myocardium, endothelin I and angiotensin II produced sustained increases in contractile force, an effect which is similar to the neonate response to α-adrenoceptor stimulation (Tanaka et al., 1995a). Developmental changes in the positive inotropic response to endothelin have also been reported for rat ventricular tissue; the response was much larger in the neonate than in the adult (Ishikawa et al., 1991). There is a report that ventricular cardiomyocytes from the chick, adult rabbit and neonate rats respond differently to endothelin (Kohmoto et al., 1993). These observations indicate that when interpreting the data from transgenic mice, not only the species difference but also the developmental change in myocardial responsiveness, both quantitative and qualitative, must be considered. We have recently reported that the excitation–contraction mechanisms of the mouse ventricular myocardium change during development to increase its dependence on sarcoplasmic reticulum function (Tanaka et al., 1998). This parallels the developmental shortening of the ventricular action potential duration. Whether these developmental changes are functionally related to the changes in the inotropic responses to cardioactive substances such as endothelin I and angiotensin II remains to be investigated. Thus, investigation of the mouse myocardium is not only essential for the interpretation of experimental results from transgenic mice, but would also provide an insight into myocardial excitation–contraction mechanisms and their regulation.

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