Are α-Adrenoceptors Involved in Positive Inotropic Effects of Phenylephrine in Chick Ventricles?

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Abstract—Effects of phenylephrine on contraction and Ca-action potentials were investigated to clarify whether the α-adrenergic mechanism may play a role in chick ventricles. Phenylephrine increased the contractile force of the ventricles isolated from both embryonic and hatched chicks, while methoxamine did not affect their contractility. Developmental changes in the sensitivity to phenylephrine, i.e., increase with age from late embryonic to early neonatal stages, were quite similar to those to a (β-agonist, isoproterenol. The positive inotropism of phenylephrine was antagonized by phentolamine and sotalol, but not antagonized by prazosin or yohimbine. Isobutylmethylxanthine augmented the effect of phenylephrine. Maximum upstroke velocity of Ca-action potentials recorded in partially depolarized ventricles were enhanced by phenylephrine, and the enhancement was eliminated by sotalol but not by phentolamine. The results suggested that the β-adrenergic action of phenylephrine may be involved in part of its positive inotropic effect, which is mediated by increased Ca-influx through sarcolemma. Another mechanism may also participate in the effects of phenylephrine, but may not necessarily be classified as an "α-adrenergic effect".

It has long been believed that actions of sympathomimetic amines on cardiac tissues are mediated only through β-adrenoceptors. Recently, several groups of investigators reported that α-adrenergic agonists can exert their positive inotropic effects even in the presence of β-adrenergic antagonists, and they classified such effects as "α-adrenergic effects in hearts" (see reviews 1–3). However, mechanisms underlying the positive inotropism of α-agonists were not sufficiently investigated. Moreover, in electrophysiological studies, some contradictory results were obtained concerning the effects of α-agonists. Miura et al. (4) reported that phenylephrine enhanced Ca-action potentials by an α-mechanism, while Ledda et al. (5) concluded that the enhancement of Ca-action potential by phenylephrine was due to its β-effect.

In the present study, we investigated effects of phenylephrine on developing chick ventricles pharmacologically and electrophysiologically. The reason why we selected developing chick ventricles in the late embryonic and early postnatal periods was because possible developmental changes in the effect of α-adrenergic stimulation can be concomitantly investigated, the results of which might give us some information on the contribution of α-adrenoceptors in this tissue. We found that Ca-influx through sarcolemma was increased by the β-effect of phenylephrine and that a "non-β-effect" may also participate in the positive inotropic action of this compound.

Materials and Methods

1. Measurement of contractile force: Small strips of trabeculae (about 2×2 mm) were dissected from right ventricular free wall of 15- to 20-day old chick embryos or 0- to 7-day young chicks. One end of the preparation was pinned down on the bottom of
the experimental chamber and the other end was connected to the force displacement transducer (Nihon Kohden, TB612T). The tissue was superfused with a physiological salt solution (PSS) (135 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 15 mM NaHCO₃, 5.5 mM glucose; oxygenated with 95% O₂-5% CO₂) at 37±1 °C and stimulated with bipolar platinum electrodes at 3 Hz. The stimulus intensity was set to just above the threshold level. After a 45 min pre-incubation, adrenergic agonists were administered in a cumulative manner to obtain dose-response curves. When investigating the influence of other drugs on the effect of adrenergic agonists, the tissues were treated with the drugs 15 min before the administration of the agonists.

2. Measurement of membrane potentials:
The ventricular free wall was stimulated at 1 Hz and membrane potentials were measured by conventional glass microelectrode methods. Electrical responses were displayed on a dual beam cathode ray oscilloscope (Nihon Kohden, VC-9) via a microelectrode amplifier with capacity neutralization (Nihon Kohden, MEZ 7101). Ca-action potentials were induced in high potassium PSS ([K⁺]₀=30 mM) (6).

3. Drugs: Drugs used were L-isoproterenol D-bitartrate (Sigma), L-phenylephrine hydrochloride (Sigma), sotalol hydrochloride (Mead Johnson), phentolamine mesylate (Ciba), and 3-isobutyl-1-methylxanthine (IBMX, Aldrich Chemical).

Results

1. Developmental changes in sensitivities of chick ventricle to phenylephrine: Effects of phenylephrine were examined in embryonic and early postnatal ventricles. Phenylephrine (10⁻⁶ to 10⁻³ M) increased the contractile force of the ventricles from 15- to 20-day embryonic and 1- to 7-day neonatal chicks without changing the time to peak tension or duration of contraction. The sensitivity of the ventricles to phenylephrine was relatively low during the embryonic stages and became higher after hatching (Fig. 1A). The ventricles from hatched chicks were about 5-fold more sensitive to phenylephrine than those from embryonic chicks, when judged from the pD₂ values. A similar increment in the sensitivity with age was reported in rat atria (7) and dog cardiac Purkinje fibers (8).

Similar changes were observed in the sensitivity to isoproterenol (Fig. 1B), which was basically the same as those already reported by Higgins and Pappano (9).

2. Pharmacological characterization of positive inotropic effect of phenylephrine: Several adrenoceptor antagonists were tested to determine the receptor type for the positive inotropic effect of phenylephrine. Phentolamine and sotalol were selected as representatives of an α-antagonist and a β-antagonist, respectively.

Dose-response curves for phenylephrine were shifted to the right by 10⁻⁶ M phentolamine or sotalol, and a combined application of both antagonists produced a further rightward shift of the curves (Fig. 2). Blockades by these antagonists were about the same in 15- to 20-day embryonic and 1- to 7-day old neonatal ventricles. The observed blockade by phentolamine might suggest some contribution of the α-adrenergic mechanism to the positive inotropism of phenylephrine. However, other α-antagonists, prazosin (an α₁-antagonist, 10⁻⁷ to 10⁻⁶ M) and yohimbine (an α₂-antagonist, 10⁻⁶ M), failed to inhibit the effect of phenylephrine (Table 1). Failure of these antagonists to block the positive inotropic effect of phenylephrine was inconsistent with the results reported in mammalian ventricles for prazosin (10) or yohimbine (11).

As for α-agonists, methoxamine never exerted positive inotropic action at concentrations up to 10⁻³ M. Norepinephrine augmented the contraction in a dose-dependent manner. The augmentation was blocked by 10⁻⁶ M sotalol, but not by 10⁻⁶ M phentolamine; pD₂ values for norepinephrine were 7.50±0.08 under the control condition (n=3), 7.09±0.18 in the presence of sotalol (n=4) and 7.56±0.06 (n=3) in the presence of phentolamine.

Figure 3 shows the effect of IBMX, a phosphodiesterase inhibitor, on the positive inotropic effects of phenylephrine. IBMX at 3×10⁻⁷ M per se did not change the basal contraction, but enhanced the positive in-
Positive Inotropism of Phenylephrine

Fig. 1. Developmental changes in sensitivities to phenylephrine (A) and isoproterenol (B) in ventricles isolated from 15- to 20-day embryonic and 0- to 7-day neonatal chicks. pD₂ values were calculated from the increase in contractile force when the agonists were administered in a cumulative manner. Each symbol denotes the mean value from 4 to 6 ventricles. Vertical bars are standard errors of mean (S.E.M.).

Theotropic effect of phenylephrine. The results suggested that the effect of phenylephrine was at least partly mediated through a β-adrenergic mechanism.

3. Effect of phenylephrine on Ca-action potential: Figure 4 shows the Ca-action potentials elicited in partially depolarized ventricles from 1-day old hatched chicks. Maximum upstroke velocity (+V_{\text{max}}) was measured as an index for Ca influx flowing through voltage-gated Ca channels. Phenylephrine (3×10^{-5} M) increased +V_{\text{max}} about 2-fold in this representative case. Sotalol antagonized the increment of +V_{\text{max}} in a dose-dependent manner, i.e., +V_{\text{max}} fell to the range of the control level after the addition of 10^{-4} M sotalol, although 3×10^{-5} M phenylephrine was continuously present.
Fig. 2. Antagonistic effects of phentolamine and sotalol on positive inotropism of phenylephrine in ventricles from 19- to 20-day embryos (A) and 0- to 1-day neonatal chicks (B). Ordinates indicate relative increase in contractile force of the tissues in response to phenylephrine. Phentolamine at $10^{-6} \text{M}$ or sotalol at $10^{-6} \text{M}$ slightly shifted the dose-response curves for phenylephrine to the right and a combined application of both antagonists produced further shifts of the curves. Vertical bars are the S.E.M., and number of experiments are in parentheses.

In contrast, phentolamine (up to $10^{-4} \text{M}$) did not affect $+V_{\text{max}}$ enhanced by phenylephrine. The augmentation by phenylephrine of $+V_{\text{max}}$ of Ca-action potentials was also blocked by sotalol but not by phentolamine in the ventricles isolated from embryonic chicks (data not shown). The results were in accordance with those reported in guinea-pig ventricles (5). However, in rabbit papillary muscles, Miura et al. (4) reported that Ca-action potentials restored by phenylephrine were suppressed by phentolamine but not by a $\beta$-antagonist, bufetolol. The discrepancy in sensitivities to phentolamine might be derived from the different animals used. As for the lack of blocking effects of bufetolol, it might be due to the relatively low doses (up to $3 \times 10^{-6} \text{M}$) they used.

Discussion

The main purpose of the present study was to clarify whether $\alpha$-adrenoceptor-mediated
Table 1. Effects of adrenergic antagonists on pD2 values of phenylephrine

|          | 19 to 20-day embryo | 0 to 1-day hatched |
|----------|---------------------|-------------------|
| Phenylephrine alone | 4.76±0.08 (4) | 5.38±0.01 (6) |
| +Phentolamine 10^-6 M | 4.60±0.08 (4) | 5.08±0.22 (4) |
| +Sotalol 10^-6 M | 4.61±0.08 (5) | 5.01±0.07** (5) |
| +Phentolamine and Sotalol 10^-6 M | 4.06±0.11** (4) | 4.77±0.13** (4) |
| +Prazosin 3x10^-9 M | 4.64±0.16 (4) | – |
| +Yohimbine 3x10^-9 M | 4.69±0.11 (4) | – |

mean=±S.E.M., ( )=number of experiments, –; not tested. **=0.01, significantly different from phenylephrine alone.

Positive Inotropism of Phenylephrine

Mechanisms are involved in the positive inotropism of one of the well-known α-adrenergic agonists, phenylephrine, in chick ventricles. We did not find any indications that might support the idea of "cardiac α-adrenceptors" as suggested in cardiac tissues of other species (1-3), but instead, we confirmed the contribution of β-mechanisms to the positive inotropic effect of phenylephrine.

The results supporting the involvement of β-mechanisms are as follows: 1) development changes in the sensitivity to phenylephrine were similar to those to isoproterenol, 2) sotalol antagonized and IBMX enhanced the positive inotropic effects of phenylephrine, 3) phenylephrine augmented Ca-action potentials and the augmentation was eliminated by sotalol. It is well-known that β-agonists increased cyclic AMP level and consequently, enhanced Ca-current (12, 13). The present study indicated that a considerable part of the excitatory effect of phenylephrine may be produced through the same process as that by β-agonists.

Phentolamine at 10^-6 M slightly antagonized the positive inotropism by phenylephrine and augmented the blocking effect of 10^-6 M sotalol. The inhibitory action of phentolamine is unlikely to be due to possible β-antagonistic effects of this compound, since the increase in +Vmax of Ca-action potentials by phenylephrine was not antagonized by phentolamine at concentrations, up to 10^-4 M. The possibility that the effect of phenylephrine is partly mediated through α-adrenceptors which are antagonized by phentolamine may well be excluded by the following findings: 1) other α-agonists, prazosin and yohimbine, did not block the effect of phenylephrine in contrast to the results obtained in mammalian hearts (10, 11) and 2) methoxamine, an α-adrenergic agonist, failed to produce the increase in contractile force. Therefore, the results obtained
Fig. 4. Ca-action potentials elicited in partially depolarized 1-day old chick ventricles (upper traces in each panel) and their differentiated wave forms (lower traces). Maximal upstroke velocity of the Ca-action potentials (indicated by arrows) was increased by $3 \times 10^{-5}$ M phenylephrine. The increment was not affected by a successive application of phentolamine up to a concentration as high as $10^{-4}$ M (A), but it was eliminated by sotalol (B).

may be explained by speculating that some unknown mechanism, in addition to $\beta$-adrenoceptors, may contribute to a small part of the positive inotropism of phenylephrine, which may be sensitive to phentolamine. Although it may tentatively be called an "$\alpha$-adrenergic effect", it is difficult to consider that this "apparent" $\alpha$-adrenergic mechanism may play an essential role in chick ventricles under physiological conditions, because the positive inotropic effects of norepinephrine, a sympathetic neurotransmitter, were inhibited by sotalol but not by phentolamine.

In contrast to the present results, Tayo (14) reported that norepinephrine but not isoproterenol produced an increase in the contractile force of atria isolated from 15-day old hatched chicks; the increase in force was reduced by phentolamine but not affected by propranolol or atenolol. The differences between the results reported by Tayo and by the present authors might come from 1) the use of different preparations (atria vs. ventricles), 2) hearts of different developmental stages (15-day old chicks vs. chicks up to 7-day old), and/or 3) different experimental conditions (spontaneously beating atria vs. electrically driven ventricles, rate-dependent changes in contractile force are well-known phenomena).

In conclusion, phenylephrine exerted its positive inotropic action largely by increasing Ca-influx in chick ventricles, which was mediated through $\beta$-adrenergic mechanisms. A part of the effect of phenylephrine, which was sensitive to phentolamine, might be different from $\beta$-adrenergic effects, but it is
difficult to regard it simply as an \( \alpha \)-adrenergic effect. Several investigators classified such positive inotropism by \( \alpha \)-agonists as "\( \alpha \)-adrenergic effects" and emphasized their pharmacological significance in cardiac tissues of some species (1–3). However, we feel that such a classification may not be adequate at least in the case of chick ventricles.

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