INTERACTIONS OF PHENYLEPHRINE AND THEOPHYLLINE IN CONTRACTILITY AND EXCITABILITY OF ISOLATED RABBIT LEFT ATRIA

Hiroshi HAMAKAWA, Takeshi SHIMIZU and Noboru TODA
Research Laboratories, Toyo Jozo Co., Ltd., Ohito, Shizuoka and
Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku,
Kyoto, Japan
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Abstract—Rabbit left atria were driven electrically at frequencies from 6 to 240/min or higher. The contractile tension-frequency curve was significantly moved upward by phenylephrine ($10^{-6}$ and $10^{-5}$ M). The positive inotropic effect was not influenced by propranolol but was markedly attenuated by phentolamine. Theophylline in a concentration ($10^{-4}$ M) sufficient to potentiate the effect of isoproterenol did not alter the positive inotropic effect of phenylephrine. Cardiac excitability studied in preparations driven at high frequencies was reduced by phenylephrine, the effect being inhibited by phentolamine and potentiated by propranolol. The excitability was enhanced by isoproterenol. Theophylline potentiated the effect of isoproterenol but inhibited the effect of phenylephrine in a high concentration ($10^{-4}$ M). It may be concluded that an enhancement of the contractile force by stimulation of myocardial alpha-receptors is not due to increased formation of cyclic AMP. Theophylline does not appear to change the effect of alpha-receptor stimulation on cardiac excitability but rather to unmask the effect of beta-receptor stimulation when high concentrations of phenylephrine are applied.

It has been demonstrated that stimulation of myocardial adrenergic alpha-receptors causes an increase in the contractile force (1-3) and a prolongation of the functional refractory period in isolated guinea pig, rat and rabbit atria (4, 5). Increase in the myocardial contractility induced by stimulation of beta-receptors is considered to result from an increased conversion of ATP to cyclic AMP (6, 7) which is expected to elicit in turn an increase in the Ca++ permeability of cellular and subcellular membranes (8). Rall and West (9) suggested that stimulation of the myocardial contractility and potentiation of the positive inotropic response to catecholamines by theophylline may be due to accumulation of cyclic AMP in the tissue, resulting from an inhibition of phosphodiesterase activity (10). The rate of formation of cyclic AMP in homogenates of the rabbit and chicken heart is increased by noradrenaline but not by phenylephrine (11, 12).

The aim of the present study was to investigate the effect of phenylephrine on the contractile tension-frequency relationship and on excitability of the atrial myocardium to electrical stimulation applied at high frequencies and interactions of phenylephrine and theophylline in isolated rabbit left atria.
METHODS

Eighty-five albino rabbits of both sexes, weighing 1.8 to 2.3 kg, were used. Under ether anesthesia the animals were sacrificed by exsanguination from common carotid arteries. The entire heart was removed and the ventricles discarded. In warm, oxygenated nutrient solution the left atrium was separated from the right along the interatrial septum as described in an earlier report (13). The isolated specimen was fixed horizontally between hooks under a resting tension of 300 to 450 mg in a muscle bath of 50 ml capacity containing the nutrient solution. The solution was maintained at 30±0.5°C and aerated with a mixture of 95% O₂ and 5% CO₂. Hooks anchoring an appendage of the left atrium were connected to the arm of a force-displacement transducer (Nihonkoden Kogyo Co.). The preparation was stimulated electrically through a pair of hooks fixing the cut end of the atrium. The composition of the nutrient solution was as follows (mM): Na⁺, 162.1; K⁺, 5.4; Ca²⁺, 2.2; Cl⁻, 157.0; HCO₃⁻, 14.9; dextrose, 5.6. The pH of the solution was 7.2 to 7.4. Preparations driven at a frequency of 60/min were equilibrated for 90 to 120 min before measurements were begun.

The preparations were driven electrically by a train of 3 msec rectangular pulses of supramaximal intensity (three times threshold intensity) at a frequency of 60/min, except when tension-frequency relationship was obtained. Stimulus pulses were provided by a Nihonkoden type MSE-3R electronic stimulator.

Contractile tension of the left atrium was displayed on a two-channel penwriter (Nihonkoden Kogyo Co.). The tension-frequency curve was obtained by raising the driving frequency stepwise from 6 to 240/min or higher until the preparation failed to respond to all the stimuli (14). Constant frequencies of stimulation were maintained until the steady state contractile tension was attained. The tension-frequency curve was also obtained after 10 min exposure to phenylephrine and isoproterenol in control solutions and solutions containing phentolamine, propranolol or theophylline. Preparations had been exposed for 20 min to phentolamine, propranolol and theophylline before the tension-frequency curve was obtained. The results shown in the text, figures and tables are expressed as mean values±standard errors of the means. Comparisons of the results were made using the Student's t test.

Drugs used were 1-phenylephrine hydrochloride, 1-isoproterenol hydrochloride, phentolamine mesylate (Ciba-Geigy), dl-propranolol hydrochloride and theophylline. The drugs were added directly to the nutrient solution of the muscle bath.

RESULTS

Contractility

The contractile force of electrically-driven left atria varied with frequency of stimulation: the developed tension increased with increasing frequencies between 6 and 120/min and decreased with increasing frequencies higher than 180/min. The application of phenylephrine in concentrations of 10⁻⁸ and 10⁻⁶ M shifted the tension-frequency curve upward in a dose-dependent manner (Fig. 1). The positive inotropic effect of phenylephrine
was markedly reduced by treatment with 10^{-6} M phentolamine (Fig. 1) but was not altered by 10^{-6} M propranolol.

The application of theophylline in a concentration of 10^{-4} M slightly enhanced the
atrial contractility. Treatment of atria with theophylline produced no significant changes in the positive inotropic effect of phenylephrine (10⁻⁶ and 10⁻⁵ M) (Fig. 2) but markedly potentiated the effect of isoproterenol (2×10⁻⁹ and 10⁻⁸ M) (Fig. 3). Fig. 4 illustrates...
the effect of theophylline on the increase in the developed tension induced by phenylephrine

**TABLE 1.** Modification by phentolamine and propranolol of the effect of phenylephrine on cardiac excitability.

| Procedure           | N  | R₁ (°/min) | R₂ (°/min) |
|---------------------|----|------------|------------|
| Control             | 45 | 176±7      | 337±9      |
| Phenylephrine 10⁻⁶ M| 45 | 157±5      | 288±8      |
| Phenylephrine 10⁻³ M| 45 | 157±6      | 252±8      |
| Control             | 12 | 150±11     | 315±8      |
| Phentolamine 10⁻⁴ M | 12 | 155±9      | 313±9      |
| Phenylephrine 10⁻⁴ M| 12 | 148±11     | 318±11     |
| Phenylephrine 10⁻³ M| 12 | 133±9      | 288±15     |
| Propranolol 10⁻⁶ M  | 13 | 168±12     | 360±15     |
| Phenylephrine 10⁻⁶ M| 13 | 166±12     | 253±12     |
| Phenylephrine 10⁻³ M| 13 | 136±11     | 196±9      |

N, number of preparations used. R₁ and R₂, see text. a, significant difference from values obtained prior to phenylephrine, P<0.01.

**TABLE 2.** Modification by theophylline of the effect of phenylephrine and isoproterenol on cardiac excitability.

| Procedure           | N  | R₁ (°/min) | R₂ (°/min) |
|---------------------|----|------------|------------|
| Control             | 8  | 191±11     | 330±16     |
| Theophylline 10⁻⁴ M | 8  | 203±11     | 383±25     |
| Phenylephrine 10⁻⁴ M| 8  | 203±10     | 293±14     |
| Phenylephrine 10⁻³ M| 8  | 221±11     | 345±35     |
| Theophylline 10⁻⁴ M | 7  | 150±17     | 351±16     |
| + Propranolol 10⁻⁴ M| 7  | 167±18     | 291±16     |
| Phenylephrine 10⁻⁴ M| 7  | 154±18     | 240±13     |
| Phenylephrine 10⁻³ M| 7  | 133±13     | 189±9      |
| Control             | 12 | 163±16     | 330±16     |
| Isoproterenol 2×10⁻⁴ M| 12 | 150±18     | 390±17     |
| Isoproterenol 10⁻⁴ M| 12 | 161±18     | 423±12     |
| Control             | 12 | 164±14     | 330±17     |
| Theophylline 10⁻⁴ M | 12 | 173±28     | 348±14     |
| Isoproterenol 2×10⁻⁴ M| 12 | 188±14     | 415±12     |
| Isoproterenol 10⁻⁴ M| 12 | 240±18     | 442±15     |

N, number of preparations used. R₁ and R₂, see text. a, significant difference from values obtained prior to phenylephrine or isoproterenol, P<0.01. b, P<0.05.

**Excitability at high driving frequencies**

Left atrial preparations were driven by electrical pulses at high frequencies. Maxi-
mum driving frequency at which the amplitude of two successive contractions was the same will be termed "R1," and the maximum frequency at which alternating contractions were produced in which the amplitude of the small contractions were not less than one-third the amplitude of the large contractions will be termed "R2" in the remainder of this report (14).

Modifications of values of R1 and R2 by phenylephrine and interactions between the amine and adrenergic receptor blocking agents are summarized in Table 1. The application of phenylephrine (10^-6 and 10^-5 M) significantly decreased the R2 in a dose-dependent manner. The R2 was significantly decreased by exposure of atria to 10^-6 M propranolol (P<0.05). The effect of phenylephrine was not inhibited but rather potentiated by propranolol. The application of phentolamine did not significantly alter the R1 and R2 but inhibited the effect of phenylephrine. Alterations by theophylline of the effect of phenylephrine and isoproterenol on the R1 and R2 are illustrated in Table 2. With theophylline a tendency of the R1 and R2 to increase was observed. The effect of 10^-6 M phenylephrine was not influenced but the effect at 10^-5 M was antagonized by theophylline. This antagonistic effect was abolished by treatment with 10^-5 M propranolol. The application of isoproterenol (2 x 10^-9 and 10^-8 M) significantly increased the R2, the effect being potentiated by 10^-4 M theophylline.

### DISCUSSION

The present study revealed that phenylephrine, a relatively pure adrenergic alpha-receptor stimulant, caused an enhancement of the contractile force of rabbit left atria, which was not antagonized by propranolol but was by phentolamine. This is consistent with results observed already in the guinea pig (3), rat (1, 15) and rabbit heart (5, 11). These findings support the view that adrenergic alpha-receptors mediate the contractile force of the heart (2). Changes by phenylephrine in the contractile tension-frequency relationship were qualitatively similar to those by isoproterenol and noradrenaline (14), the inotropic effect of these amines being suppressed by propranolol. It has been suggested that the positive inotropic effect of catecholamines is mediated by adenylyl cyclase which catalyses the conversion of ATP to cyclic AMP. This assumption is supported by the data that theophylline, a known phosphodiesterase inhibitor, potentiates the inotropic effect of catecholamines (9). On the other hand, it is demonstrated that the contraction of vascular smooth muscles in response to catecholamines, possibly mediated by adrenergic alpha-receptors, is potentiated by treatment with theophylline and cyclic AMP (16) which increase the tissue content of cyclic AMP. In the present study, however, the effect of phenylephrine was not significantly influenced by treatment with theophylline. Benfey and Carolin (11) and Benfey (12) reported that the activity of adenylyl cyclase and the formation of cyclic AMP were not changed by phenylephrine. Thus, it may be concluded that an enhancement of the contractile force by stimulation of myocardial alpha-receptors is not due to an increase in the formation of cyclic AMP.

When the refractory period is taken as a parameter of study, stimulation of adrenergic
beta-receptors is antagonistic to that of alpha-receptors (4, 5); however, as already shown in the case of contractile force, stimulation of beta-receptors is synergistic with that of alpha-receptors. As indicated in an earlier report (14), values of R₁ and R₂ do not represent directly the refractory period of isolated atria but may provide parameters indicating changes in cardiac excitability including the refractory period. The value of R₂ was significantly decreased by phenylephrine, which would be expected to derive mainly from a prolongation of the effective refractory period, whereas the value was increased by isoproterenol. Tendency of the R₁ and R₂ to increase was observed in preparations treated with theophylline. The application of theophylline in a concentration sufficient to potentiate the effect of isoproterenol did not influence the effect of phenylephrine at low concentrations but inhibited the effect of high concentrations of the amine. The theophylline-induced inhibition of the phenylephrine effect was diminished by treatment with propranolol. These results appear to indicate that theophylline does not change the effect of alpha-receptor stimulation (i.e. decrease in R₁) but to unmask the effect of beta-receptor stimulation when high concentrations of phenylephrine are applied. Changes in cardiac excitability by adrenergic beta stimulants may be the result of an increased formation of cyclic AMP, whereas those by alpha-receptor stimulation do not seem to relate with the nucleotide.

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