Induction by endogenous noradrenaline of an α_1 -adrenoceptor-mediated positive inotropic effect in rabbit papillary muscles

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1 The possible involvement of α_1 -adrenoceptors in the inotropic and electrophysiological responses to endogenous noradrenaline released by tyramine was examined in rabbit papillary muscles.

2 A concentration-dependent positive inotropic effect was produced by tyramine. This effect of tyramine was not observed in muscles from rabbits pretreated with reserpine.

3 The positive inotropic effect of tyramine was greatly inhibited by propranolol, but not altered by prazosin. However, when β -adrenoceptors were blocked by pretreatment with propranolol, tyramine still produced a positive inotropic effect, an effect which was antagonized by prazosin.

4 Tyramine caused a decrease in action potential duration (APD) and an increase in action potential amplitude in a concentration-dependent manner. Isoprenaline also produced the same electrophysiological effects. These electrophysiological effects of both agents were inhibited by propranolol.

5 When β -adrenoceptors were blocked by propranolol, the observed prazosin-sensitive positive inotropic effect of tyramine was not accompanied by any change in APD. In contrast, APD was markedly prolonged by α_1 -adrenoceptor stimulation with phenylephrine in the presence of propranolol, in association with the positive inotropic effect.

6 It is concluded that in rabbit papillary muscles, endogenous noradrenaline causes a positive inotropic effect predominantly mediated by β -adrenoceptors, but can still evoke a positive inotropic effect through α_1 -adrenoceptors when β -adrenoceptor stimulation is eliminated. This suggests that the α_1 -adrenoceptor-mediated positive inotropic mechanism(s) may be masked by simultaneous activation of β -adrenoceptors. In addition, this study indicates that APD prolongation is not involved in the α_1 -adrenoceptor-mediated inotropic responses to endogenous noradrenaline.

Keywords: Tyramine; endogenous noradrenaline; α_1 -adrenoceptors; β -adrenoceptors; positive inotropic effect; action potential

Introduction

It is generally accepted that stimulation of myocardial α_1 -adrenoceptors results in a positive inotropic effect in various mammalian species (for reviews, see Scholz, 1980; Schümann, 1980; Benfey, 1982). The question has remained unsettled as to whether myocardial α_1 -adrenoceptors have a functional or physiological role in the regulation of myocardial contractility. Earlier studies have usually failed to demonstrate that the positive inotropic response to noradrenaline is antagonized by the α -adrenoceptor antagonists such as phentolamine in rabbit papillary muscles (Schümann *et al.*, 1974; Endoh *et al.*, 1976), in human atria (Schümann *et al.*, 1978; Wagner *et al.*, 1980) and in human papillary muscles (Jakob *et al.*, 1988). These authors have concluded that noradrenaline, in contrast to adrenaline and phenylephrine, does not evoke a significant α_1 -adrenoceptor-mediated inotropic response. However, Osnes and his collaborators (Aass *et al.*, 1983; Skomedal *et al.*, 1988) have found that when β -adrenoceptors are extensively blocked, noradrenaline is capable of evoking a positive inotropic effect through α_1 -adrenoceptors in rat and rabbit papillary muscles. Thus, demonstration of the α_1 -adrenoceptor-mediated positive inotropic response to noradrenaline appears to require extensive β -adrenoceptor blockade. It was therefore assumed that the expression of the inotropic response to α_1 -adrenoceptor stimulation might be restricted by simultaneous stimulation of β -adrenoceptors when both adrenoceptors are activated (Skomedal *et al.*, 1988; 1990).

The positive inotropic effect of α_1 -adrenoceptor stimulation is associated with sarcolemmal Ca^{2+} influx and a resultant elevation of peak Ca^{2+} transients (Endoh & Blinks, 1988).

Recently, stimulation of myocardial α_1 -adrenoceptors has been shown to cause an inhibition of the transient outward current (Apkon & Nerbonne, 1988; Fedida *et al.*, 1989; Tohse *et al.*, 1990). Inhibition of this current could result in prolongation of APD, leading to an elevation of $[\text{Ca}^{2+}]_i$ by increased Ca^{2+} influx during depolarization. Whereas the mechanism of the β -adrenoceptor-mediated positive inotropic effect is related to an increase in Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Tsien, 1977; Kameyama *et al.*, 1985) and to an increase in the accumulation rate of Ca^{2+} into the sarcoplasmic reticulum (Tada & Katz, 1982), it is known that activation of myocardial β -adrenoceptors causes a marked decrease in APD (Kass & Wiegers, 1982; Nakaya *et al.*, 1990) possibly due to activation of the delayed rectifier potassium current and/or the chloride current (Walsh & Kass, 1988; Harvey & Hume, 1989). Thus, it is possible that this opposite action on APD to α_1 -adrenoceptor stimulation might mask the positive inotropic effect mediated by α_1 -adrenoceptors during simultaneous stimulation of α_1 - and β -adrenoceptors by noradrenaline.

The present study was undertaken to determine whether endogenous noradrenaline can reveal a positive inotropic effect through α_1 -adrenoceptor stimulation in rabbit papillary muscles. For this purpose, we used tyramine which releases endogenous noradrenaline by stoichiometric displacement after being taken up into nerve endings (Trendelenburg, 1972). The results indicate that endogenous noradrenaline released by tyramine produced a positive inotropic response mediated by α_1 -adrenoceptors, only when the effect was unopposed by β -adrenoceptor stimulation, as seen with exogenous noradrenaline (Aass *et al.*, 1983; Skomedal *et al.*, 1988). Electrophysiological experiments were further carried out to address the possibility that the opposite effect of β -adrenoceptor stimulation on APD might contribute to the restricted expression of the α_1 -adrenoceptor-mediated ino-

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tropic response during simultaneous adrenoceptor activation by endogenous noradrenaline. For comparison, we also characterized the inotropic and electrophysiological effects of phenylephrine (in the presence of propranolol) and isoprenaline as representative exogenous stimulation of α_1 - and β -adrenoceptors, respectively.

Methods

Measurement of inotropic responses

New Zealand White rabbits of either sex weighing 2–3 kg were killed by a blow on the head. The hearts were quickly removed and transferred to a dissection bath filled with oxygenated warm Krebs-Henseleit solution. The composition of the solution was (mM): NaCl 119, CaCl_2 2.5, KCl 4.8, MgSO_4 1.2, KH_2PO_4 1.2, NaHCO_3 24.9 and glucose 10.0. The right ventricular papillary muscles were carefully dissected from the heart. The muscle was mounted under 0.5 g of resting tension in a water-jacketed organ bath containing 10 ml of Krebs-Henseleit solution. The solution in the bath was bubbled with 95% O_2 and 5% CO_2 and its temperature was maintained at $30 \pm 1^\circ\text{C}$. The muscle was stimulated by rectangular pulses of 5 ms duration and 1.5 times the threshold voltage of 0.5 Hz frequency. The pulses were delivered by a pair of spiral platinum electrodes connected to an electronic stimulator (Sanei-Sokki, 3F46, Tokyo, Japan) through an isolation unit (Sanei-Sokki, 5361). Isometric tension developed in the preparation was measured with a force transducer (Nihon Kohden, TB-612T, Tokyo, Japan) and recorded on a pen recorder (Nihon Kohden, RJG-3026) through a preamplifier (Nihon Kohden, RP-5). The preparations were allowed to equilibrate for at least 60 min before the experiments were begun.

The concentration-response curves for the positive inotropic effects of isoprenaline, phenylephrine and tyramine were determined in a cumulative manner by increasing the concentration of the tested agonists in steps of 0.5 log units (van Rossum, 1963). When propranolol and prazosin were used, they were added to the bath 60 min before the administration of the tested agents.

In some experiments, the papillary muscles were obtained from the rabbits pretreated with reserpine. The animals received reserpine, 1 mg kg^{-1} i.p., for 3 days and were used on the 4th day. Reserpine was dissolved in distilled water containing 10% ascorbic acid.

Measurement of electrophysiological responses

Rabbit right ventricular papillary muscles were prepared as described above. The muscle was transferred to a tissue bath of 5 ml in volume and superfused at a rate of 10 ml min^{-1} with Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2 . The temperature of perfusate was kept constant at $30 \pm 1^\circ\text{C}$.

One end of the muscle was hooked to the lever arm of a force transducer (Nihon Kohden, TB-651T) mounted on a micromanipulator and the other was pinned to the bottom of the tissue chamber. Resting tension of the preparation was increased to 200 mg gradually. The muscle was stimulated by rectangular pulses of 0.5 Hz frequency, 1 ms duration and 1.5 times threshold voltage, delivered through platinum field electrodes connected to an electronic stimulator (Nihon Kohden, SEN3101) and an isolation unit (Nihon Kohden, 22101J). Transmembrane potentials were recorded using glass microelectrodes filled with 3 M KCl (tip resistance, 10–30 M Ω). The microelectrode was coupled via an Ag/AgCl junction to a high impedance capacitance neutralizing amplifier (Nihon Kohden, MEZ8301). An agar bridge containing 3 M KCl was used as a reference electrode. An electronic differentiator whose output was linear from 50–1000 V s^{-1} was used for measuring the maximum rate of

rise ($\dot{V}_{\text{max}}$) of action potentials. These amplified signals were displayed on an oscilloscope (Nihon Kohden, VC-10), photographed on 35 mm film and recorded on a chart recorder (Watanabe Sokki, WR3101, Tokyo, Japan). Further details of the experimental procedure have been described elsewhere (Hattori *et al.*, 1990).

The preparations were allowed to equilibrate for at least 90 min before impalement by the microelectrode. We included in the study only the experiments in which a stable impalement was maintained throughout the protocol.

Drugs

The following compounds were used: (–)-isoprenaline HCl, (–)-phenylephrine HCl and (±)-propranolol HCl (Sigma, St. Louis, MO, U.S.A.); tyramine HCl (Wako, Osaka, Japan); prazosin HCl (Taito-Pfizer, Tokyo, Japan); and reserpine (Aldrich, Milwaukee, WI, U.S.A.). All drugs except reserpine (see above) were dissolved in distilled water and further dilutions were made with Krebs-Henseleit solution. Ascorbic acid (0.1 mM) was added to the solution of isoprenaline to retard its auto-oxidation.

Statistics

All values are presented as means $\pm$ s.e.mean. Analysis by Student's *t* test was performed for paired and unpaired observations. When appropriate, statistically significant differences were assessed by analysis of variance with repeated measurements. A *P* value less than 0.05 was considered an indication of statistical significance.

Results

Inotropic and electrophysiological effects of isoprenaline and phenylephrine

Figure 1 shows concentration-response curves for the positive inotropic effects of isoprenaline and phenylephrine in rabbit right ventricular papillary muscles electrically driven at 0.5 Hz. The inotropic response to phenylephrine was evaluated in the presence of $1 \mu\text{M}$ propranolol to eliminate its weak β -adrenoceptor agonistic action. Propranolol ($1 \mu\text{M}$)

Figure 1 Concentration-response curves for the positive inotropic effects of isoprenaline (○) and phenylephrine (●). Propranolol at $1 \mu\text{M}$ was added 60 min before the addition of phenylephrine. Points (mean $\pm$ s.e.mean, $n = 9$ –10) represent the net increases in force of contraction expressed as a percentage of basal values recorded before the addition of the agonists. The force of contraction was measured when it reached the maximum in response to the respective concentration of the agonists. Basal force of contraction was $441 \pm 53 \text{ mg}$ ($n = 19$).

itself did not affect force of contraction. The positive inotropic effect produced by phenylephrine after β -adrenoceptor blockade with $1 \mu\text{M}$ propranolol was exclusively mediated by α_1 -adrenoceptors, since its concentration-response curve was displaced rightward by prazosin in an apparently competitive manner (data not shown). The maximum increases in force of contraction induced by β -adrenoceptor stimulation (isoprenaline) and by α_1 -adrenoceptor stimulation (phenylephrine) were $211 \pm 21\%$ ($n = 9$) and $109 \pm 10\%$ ($n = 10$), respectively. Thus, the maximum positive inotropic effect of α_1 -adrenoceptor stimulation was about 52% of that of β -adrenoceptor stimulation.

Representative results showing the effects of isoprenaline and phenylephrine on action potential configuration and force of contraction are depicted in Figure 2. The positive inotropic effect of 1 nM isoprenaline was accompanied with a decrease in APD. On the other hand, APD was prolonged in association with the positive inotropic effect of $1 \mu\text{M}$ phenylephrine in the presence of $1 \mu\text{M}$ propranolol. At a concentration of $1 \mu\text{M}$, propranolol had no significant effect on action potential parameters.

As shown in Figure 3, isoprenaline caused concentration-dependent decreases in APD_{20} , APD_{50} as well as APD_{90} . In addition, isoprenaline produced an increase in action potential amplitude (APA). At a concentration of 1 nM isoprenaline significantly increased APA from 113.7 ± 1.6 to $116.4 \pm 1.5 \text{ mV}$ ($n = 7$, $P < 0.01$). These electrophysiological effects of isoprenaline were blocked by $1 \mu\text{M}$ propranolol (data not shown). Neither resting membrane potential nor V_{max} was altered by isoprenaline at concentrations up to 10 nM .

In contrast to isoprenaline, phenylephrine in the presence of propranolol increased APD_{20} , APD_{50} and APD_{90} in a concentration-dependent manner (Figure 3). It also caused a significant increase in APA: APA was increased from 112.4 ± 1.8 to $114.0 \pm 1.8 \text{ mV}$ ($n = 5$, $P < 0.05$) by $5 \mu\text{M}$ phenylephrine. The increasing effects of phenylephrine on APDs and APA were antagonized by $1 \mu\text{M}$ prazosin (data not shown). Resting membrane potential and V_{max} remained unchanged with phenylephrine.

Inotropic and electrophysiological effects of tyramine

Tyramine produced an increase in force of contraction in a concentration-dependent manner (Figure 4). The maximum increase by tyramine was about 37% of that by isoprenaline. The positive inotropic response to tyramine was abolished in papillary muscles obtained from the reserpine-treated rabbits: tyramine ($100 \mu\text{M}$) did not induce significant change in force of contraction in reserpinized muscles ($1.4 \pm 2.0\%$ of basal

force of contraction, $n = 5$). This finding indicates that the inotropic response to tyramine is attributable to endogenous noradrenaline released from reserpine-sensitive pools. The concentration-response curve for the positive inotropic effect of tyramine was not affected by pretreatment with $1 \mu\text{M}$ prazosin (Figure 4). On the other hand, pretreatment with $1 \mu\text{M}$ propranolol markedly inhibited the positive inotropic

Figure 3 Concentration-dependent effects of isoprenaline and phenylephrine of APD_{20} , APD_{50} and APD_{90} . Phenylephrine was added in the presence of $1 \mu\text{M}$ propranolol. The APD changes induced by the agonists were measured 10 min after the addition. Columns represent means $\pm$ s.e. mean of 5–7 experiments. Basal APD_{20} , APD_{50} and APD_{90} were 98 ± 6 , 159 ± 8 and $208 \pm 9 \text{ ms}$ ($n = 13$), respectively. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs the corresponding values before the addition of the agonists.

Figure 2 Typical effects of 1 nM isoprenaline (a) and $1 \mu\text{M}$ phenylephrine (b) on action potential and force of contraction. The effect of phenylephrine was measured in the presence of $1 \mu\text{M}$ propranolol. Superimposed records before and 10 min after the addition of isoprenaline (a) and phenylephrine (b) are shown. Arrows indicate traces after application of the agonists.

Figure 4 Concentration-response curves for the positive inotropic effect of tyramine in the absence (○) and presence of 1 μ M prazosin (●), 1 μ M propranolol (▲) and 1 μ M prazosin and 1 μ M propranolol (■). Points (means $\pm$ s.e.mean, $n = 6-7$) represent the net increase in force of contraction expressed as a percentage of basal values recorded before the addition of tyramine. The force of contraction was measured when it reached the maximum in response to the respective concentration of tyramine. Basal force of contraction was 730 ± 83 mg ($n = 26$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs the corresponding values with tyramine alone. †† $P < 0.01$ vs the values obtained in the presence of propranolol.

response to tyramine (Figure 4). Thus, the increase in force of contraction in response to endogenous noradrenaline released by tyramine appears to be mediated predominantly by β -adrenoceptors. However, tyramine at higher concentrations ($\geq 100 \mu$ M) produced a substantial increase in force of contraction even in the presence of propranolol. Further addition of prazosin to propranolol completely eliminated the positive inotropic effect of tyramine at concentrations of 100 and 300 μ M (Figure 4).

The concentration-dependent effects of tyramine on action potential parameters are summarized in Figure 5. Tyramine produced concentration-dependent decreases in APD_{20} , APD_{50} and APD_{90} . APA was increased by tyramine in a concentration-dependent fashion. These electrophysiological effects of tyramine were similar to those of isoprenaline. Resting membrane potential was significantly decreased by tyramine at a high concentration (100 μ M). This appears to be due to excess stimulation of β -adrenoceptors, because we have previously demonstrated that isoprenaline at higher concentrations (≥ 30 nM) produces a membrane depolarization in guinea-pig papillary muscles (Nakaya *et al.*, 1990). Tyramine did not produce a significant change in $\dot{V}_{max}$.

As expected, the tyramine-induced decreases in APDs were significantly inhibited by pretreatment with 1 μ M propranolol (Figures 6 and 7). In the presence of propranolol, tyramine still produced a positive inotropic effect, an effect which was completely abolished by further pretreatment with prazosin (Figure 6). However, any increase in APDs was not observed with tyramine in the presence of propranolol (Figure 7).

Discussion

From our study the results indicate that the positive inotropic effect of endogenous noradrenaline released by tyramine in rabbit papillary muscles is predominantly mediated by β -adrenoceptors. Support for this is provided by the findings that the concentration-response curve for the positive inotropic effect of tyramine was displaced downward and to the right by propranolol but remained unchanged with prazosin. However, when β -adrenoceptors were adequately blocked by propranolol, tyramine produced a prazosin-sensitive positive inotropic effect. In recent work, we have demonstrated that 5-hydroxytryptamine is capable not only of inducing a release of noradrenaline from nerve endings but also of blocking β -adrenoceptors in rabbit papillary muscles, resulting in an appearance of the α_1 -adrenoceptor-mediated positive inotropic effect in response to released noradrenaline (Hattori *et al.*, 1992). Thus, endogenous noradrenaline

Figure 5 Concentration-response curves for the effects of tyramine on action potential parameters. Tyramine was added to the organ bath in a cumulative way. The effects of tyramine were measured 10 min after its addition. Points are means $\pm$ s.e.mean of 5 experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs the corresponding control values.

Figure 6 Typical effects of tyramine on action potential and force of contraction in the absence (a) and presence of $1 \mu\text{M}$ propranolol (b) and $1 \mu\text{M}$ propranolol plus $1 \mu\text{M}$ prazosin (c). Propranolol and prazosin were added to the organ bath 30 min before application of tyramine. Superimposed records before and 10 min after the addition of $100 \mu\text{M}$ tyramine are shown. Arrows indicate traces after application of tyramine.

Figure 7 Influence of propranolol on the effect of tyramine on APD_{20} , APD_{50} and APD_{90} . The APD changes induced by $100 \mu\text{M}$ tyramine were measured 10 min after its administration. Propranolol at $1 \mu\text{M}$ was added to the organ bath 30 min before tyramine. Columns represent means $\pm$ s.e. mean of 5–7 experiments. Basal APD_{20} , APD_{50} and APD_{90} were 97 ± 5 , 156 ± 6 and 205 ± 6 ms ($n = 12$), respectively. ** $P < 0.01$; *** $P < 0.001$ vs the corresponding values with tyramine alone.

appears to cause an increase in force of contraction through activation of α_1 -adrenoceptors only when β -adrenoceptor stimulation is eliminated. Our results obtained with tyramine and 5-hydroxytryptamine are consistent with and extend earlier findings that exogenous noradrenaline can exhibit an α_1 -adrenoceptor-mediated positive inotropic effect after extensive blockade of β -adrenoceptors (Aass *et al.*, 1983; Skomedal *et al.*, 1988). The potencies of noradrenaline are reported to be equivalent in inhibiting the α -adrenoceptor

ligand [^3H]-dihydroergocryptine binding and the β -adrenoceptor ligand (–)-[^3H]-alprenolol binding to cardiac membranes (Alexander *et al.*, 1975; Williams & Lefkowitz, 1978). Furthermore, the number of α_1 -adrenoceptors is quite similar to that of β -adrenoceptors in rabbit heart (Mukherjee *et al.*, 1983). Therefore, we assume that, when β -adrenoceptors are activated concomitantly with α_1 -adrenoceptors, the mechanism(s) underlying the positive inotropic response to α_1 -adrenoceptors may be counteracted by simultaneous stimulation of β -adrenoceptors.

Tyramine caused a concentration-dependent decrease in APD. The same effect could be obtained with isoprenaline and could be suppressed with propranolol. Thus, the APD shortening in response to endogenous noradrenaline released by tyramine is likely to be due to mediation through β -adrenoceptors. In contrast to β -adrenoceptor stimulation, activation of α_1 -adrenoceptors is known to prolong APD in various cardiac tissues (Pappano, 1971; Giotti *et al.*, 1973; Miura & Inui, 1984; Dukes & Vaughan-Williams, 1984; Tohse *et al.*, 1987). We also found that α_1 -adrenoceptor stimulation with phenylephrine (in the presence of propranolol) produced significant APD prolongation in rabbit papillary muscles. The opposing effects of α_1 - and β -adrenoceptor stimulation on APD appear to involve different ionic mechanisms. Activation of the delayed rectifier potassium current and/or the chloride current contributes to the β -adrenoceptor-mediated APD shortening (Walsh & Kass, 1988; Harvey & Hume, 1989). Suppression of the transient outward current is related to the α_1 -adrenoceptor-mediated APD prolongation (Apkon & Nerbonne, 1988; Fedida *et al.*, 1989; Tohse *et al.*, 1990). However, when β -adrenoceptors are activated concomitantly with α_1 -adrenoceptors, the β -adrenoceptor-mediated APD shortening might apparently disturb the α_1 -adrenoceptor-mediated APD prolongation, and the balance between these opposing actions could ultimately determine APD. Since prolongation of APD would lead to an elevation of $[\text{Ca}^{2+}]_i$ by increasing Ca^{2+} influx during the plateau phase, it was suggested that the α_1 -adrenoceptor-mediated APD prolongation may be important and responsible for an increase in force of contraction (Miura & Inui, 1984; Dukes & Vaughan-Williams, 1984). If the APD prolongation is primarily involved in the positive inotropic response mediated by α_1 -adrenoceptors, the APD shortening elicited by β -adrenoceptor stimulation might mask full expression of the α_1 -adrenoceptor-mediated positive inotropic response during simultaneous activation of both adrenoceptors.

After β -adrenoceptor blockade with propranolol, endogenous noradrenaline released by tyramine evoked a prazosin-sensitive positive inotropic effect, i.e. an α_1 -adrenoceptor-mediated one. However, the α_1 -adrenoceptor-mediated positive inotropic effect was not accompanied by APD prolongation. Even in the presence of propranolol, tyramine still exhibited a small decrease in APD (see Figures 6 and 7). It is possible that

a substance other than noradrenaline might be released from cardiac nerve endings in response to tyramine and could be involved in the propranolol-resistant APD shortening. Nevertheless, it should be emphasized that tyramine can evoke a positive inotropic effect through α_1 -adrenoceptors without producing APD prolongation. Alternatively, the α_1 -adrenoceptor-mediated positive inotropic effect induced by endogenous noradrenaline seems to be independent of changes in APD. Furthermore, these results exclude the possibility that the β -adrenoceptor-mediated APD shortening might be involved in the restricted expression of the α_1 -adrenoceptor-mediated positive inotropic response when both adrenoceptors are simultaneously activated.

It has been postulated that the mechanisms underlying the positive inotropic effect of α_1 -adrenoceptor stimulation in rabbit papillary muscles are related to at least two Ca^{2+} -mediated processes: an increase in the intracellular Ca^{2+} transient and an increase in Ca^{2+} sensitivity of contractile proteins (Endoh & Blinks, 1988). The amplitude of the intracellular Ca^{2+} transient could be increased by the increased Ca^{2+} influx as a result of APD prolongation when α_1 -adrenoceptors are stimulated with phenylephrine. However, the increase in the amplitude of the Ca^{2+} transient produced by α_1 -adrenoceptor stimulation is much smaller than that by increased $[\text{Ca}^{2+}]_o$, when compared at the same level of inotropic responses (Endoh & Blinks, 1988). In this context, it seems very likely that the increase in Ca^{2+} sensitivity of contractile proteins may largely account for the α_1 -adrenoceptor-mediated positive inotropic response to endogenous noradrenaline, since we found that endogenous noradrenaline evoked this inotropic response with little influence on APD. Recent study on skinned rat ventricular myocytes has also demonstrated that α_1 -adrenoceptor stimulation leads to an increase in responsiveness of the myofilaments to Ca^{2+} (Puceat *et al.*, 1990). On the other hand, stimulation of β -adrenoceptors results in a decrease in the Ca^{2+} sensitivity of contractile proteins (Endoh & Blinks, 1988; Puceat *et al.*, 1990). It therefore is likely that the decrease in myofibrillar Ca^{2+} sensitivity caused by β -adrenoceptor stimulation could mask the α_1 -adrenoceptor-mediated positive inotropic response. This may explain the reason why β -adrenoceptors must be extensively blocked in order to reveal the α_1 -adrenoceptor-mediated inotropic response.

Recently, Guse *et al.* (1991) have reported that stimulation of β -adrenoceptors attenuates the α_1 -adrenoceptor-mediated formation of inositol phosphates in cultured rat ventricular myocytes. If there is a close association of phosphoinositide hydrolysis and its resultant products with the α_1 -adrenoceptor-mediated positive inotropic effect, the inhibitory effect of β -adrenoceptor stimulation on the α_1 -adrenoceptor-mediated phosphoinositide response might be responsible for the restricted positive inotropic response to α_1 -adrenoceptor stimulation. It has been suggested that the positive inotropic effect of α_1 -adrenoceptor stimulation may be mediated by inositol 1,4,5-trisphosphate through mobilization of intracellular Ca^{2+} and/or by 1,2-diacylglycerol through activation of protein kinase C (Otani *et al.*, 1988). However, the role of inositol 1,4,5-trisphosphate as an intracellular Ca^{2+} mobilizing agent in cardiac muscles is still unclear (Movsesian *et al.*, 1985; Nosek *et al.*, 1986; Ehrlich & Watras, 1988). Also, direct activation of protein kinase C with tumour-promoting phorbol esters rather causes a negative inotropic action (Yuan *et al.*, 1987; Kushida *et al.*, 1988). Finally, the relation between the acceleration of phosphoinositide hydrolysis and the positive inotropic effect in response to α_1 -adrenoceptor stimulation is still the subject of controversy (Endou *et al.*, 1991; Endoh *et al.*, 1991).

In conclusion, endogenous noradrenaline elicits an α_1 -adrenoceptor-mediated positive inotropic effect in rabbit papillary muscles that is unmasked by extensive blockade of β -adrenoceptors. The α_1 -adrenoceptor-mediated positive inotropic mechanism(s) may be masked when β -adrenoceptors are simultaneously activated. The present data also suggest that the APD prolongation which is usually induced by α_1 -adrenoceptor agonists such as phenylephrine does not appear to be a primary mechanism of the α_1 -adrenoceptor-mediated positive inotropic effect in rabbit papillary muscles.

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