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Short communication

Involvement of α_{1B}-adrenoceptors in the positive inotropic effect of endogenous noradrenaline in rabbit myocardium

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Abstract

We studied the α_1-adrenoceptor subtypes mediating the positive inotropic effects of phenylephrine and noradrenaline as well as endogenous noradrenaline released by tyramine in rabbit papillary muscle. In the presence of propranolol, both phenylephrine and tyramine produced a positive inotropic effect in a concentration-dependent manner. WB4101 (*N*-[2-(2,6-dimethoxyphenoxy]ethyl]-2,3-dihydro-1,4-benzodioxin-2-methanamine) and chlorethylclonidine each antagonized the positive inotropic effect of phenylephrine. On the other hand, only chlorethylclonidine significantly blocked the positive inotropic effect of tyramine. However, the presence of both antagonists was needed to block the positive inotropic effect elicited by the exogenous addition of the low concentration of noradrenaline. These data suggest that after extensive blockade of β-adrenoceptors the positive inotropic effects of phenylephrine and exogenous noradrenaline result from stimulation of the α_{1A}- and α_{1B}-adrenoceptor subtypes, whereas that of endogenous noradrenaline is mediated via the α_{1B}-adrenoceptor subtype. This could be explained by assuming that the α_{1B}-adrenoceptor subtype population may be located on a space confronting more closely to the sympathetic nerve endings than the α_{1A}-adrenoceptor subtype population. © 1997 Elsevier Science B.V.

Keywords: α_1-Adrenoceptor, subtype; Endogenous noradrenaline; WB4101; Chlorethylclonidine; Positive inotropic action; Myocardium, rabbit

1. Introduction

It is well established that stimulation of myocardial α_1-adrenoceptors results in a positive inotropic effect in most mammalian species (Terzic et al., 1993). Several lines of evidence suggest two possible mechanisms underlying the positive inotropic response to α_1-adrenoceptor stimulation: an increase in myofibrillar Ca^{2+} sensitivity and an elevation of the intracellular Ca^{2+} level due to increased transsarcolemmal Ca^{2+} influx (Endoh and Blinks, 1988; Pucéat et al., 1990; Fedida and Bouchard, 1992). It seems most likely that the increase in Ca^{2+} influx is attributed to prolongation of the action potential duration which results from inhibition of the transient outward current (Fedida et al., 1993).

It is now clarified that there are at least three subtypes of α_1-adrenoceptors (i.e., α_{1A}, α_{1B} and α_{1D}) and that each of these receptor subtypes is involved in mediating different functional responses to α_1-adrenoceptor agonists (Hieble et al., 1995). On the basis of this concept, we have considered that the two mechanisms by which α_1-adrenoceptor agonists produce a positive inotropic effect may be mediated by the different subtypes of α_1-adrenoceptors (Nagashima et al., 1996). Thus, using a pharmacological approach with *N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-1,4-benzodioxin-2-methanamine (WB4101), which has a relatively high affinity for the α_{1A}-adrenoceptor subtype (Minneman, 1988), and chlorethylclonidine, which selectively and irreversibly inactivates the α_{1B}-adrenoceptor subtype (Minneman, 1988), we have demonstrated that the α_{1A}- and α_{1B}-adrenoceptor subtypes are both coupled to positive inotropy but the inotropic effect associated with action potential prolongation as a result of transient outward inhibition is mediated exclusively by the α_{1A}-adrenoceptor subtype (Nagashima et al., 1996).

In rabbit papillary muscle, the positive inotropic effect of α_1-adrenoceptor stimulation appears to be in large part the result of an increase in myofibrillar Ca^{2+} sensitivity (Endoh and Blinks, 1988). This is consistent with our previous finding that endogenous noradrenaline released by tyramine can evoke an α_1-adrenoceptor-mediated positive inotropic effect which is not accompanied by action

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potential prolongation (Hattori et al., 1993). However, when phenylephrine in the presence of β-adrenoceptor blockade is used as an agonist of α_1-adrenoceptors, prolongation of the action potential duration may also contribute to the establishment of the positive inotropic response (Nagashima et al., 1996). If the two inotropic mechanisms are indeed mediated through different α_1-adrenoceptor subtypes as we have assumed (Nagashima et al., 1996), each of the positive inotropic effects elicited by endogenous noradrenaline and phenylephrine may be differently modulated by the α_{1A}- and α_{1B}-adrenoceptor subtype specific antagonists. To test this possibility, we investigated the effects of WB4101 and chlorethylclonidine on the positive inotropic responses to tyramine and phenylephrine in the presence of propranolol in rabbit papillary muscle. The effects of these antagonists on the inotropic response to exogenous noradrenaline were also examined.

2. Materials and methods

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 (in mM): NaCl 119, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 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 ± 1°C. The muscle was stimulated by rectangular pulses of 0.5 Hz in frequency, 5 ms in duration and 1.5 times the threshold voltage, 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 TB612T, Tokyo, Japan) and recorded on a thermal array recorder (Nihon Kohden RTA-1200) through a preamplifier (Nihon Kohden RP-5). The preparations were allowed to equilibrate for at least 60 min before the experiments were begun.

Concentration–response curves for the positive inotropic effects of phenylephrine and tyramine were determined in a cumulative manner by increasing the concentration of the agonists in steps of 0.5 log units. Propranolol at a concentration of 1 μM was present for more than 60 min before the addition of the agonists and thereafter throughout the experiments. When WB4101 and chlorethylclonidine were used, they were added to the bath 60 min before the construction of concentration–response curves. The EC_{50} value, i.e., the concentration required to produce 50% of the maximal response induced by the agonist, was determined from log-probit plots of the individual response vs. concentration and was expressed as the negative logarithm (pD_2 value).

The following compounds were used: *l*-phenylephrine hydrochloride and *d,l*-propranolol hydrochloride (Sigma, St. Louis, MO, USA); tyramine hydrochloride and *l*-noradrenaline bitartrate (Wako, Osaka, Japan); WB4101 hydrochloride and chlorethylclonidine dihydrochloride (Research Biochemicals International, Natick, MA, USA); and prazosin hydrochloride (Pfizer, Tokyo, Japan). All drugs were dissolved in distilled water and further dilutions were made with Krebs-Henseleit solution.

The data are expressed as means ± S.E.M. Statistical analysis was performed using Student's *t*-test for paired and unpaired observations. Differences were considered to be statistically significant when $P < 0.05$.

3. Results

In the presence of 1 μM propranolol, phenylephrine caused a concentration-dependent increase in force of contraction (Fig. 1A). The pD_2 value for phenylephrine was 6.39 ± 0.09 and the maximum increase was 105 ± 7% of basal force of contraction ($n = 10$). Pretreatment with 10 nM WB4101 shifted the concentration–response curves for phenylephrine to the right without significantly affecting the maximum inotropic effect. The pD_2 value for phenylephrine significantly decreased to 5.88 ± 0.12 ($n = 5$, $P < 0.01$) in the presence of WB4101. Pretreatment with 1 μM chlorethylclonidine caused a larger rightward shift in the concentration–response curve for phenylephrine than 10 nM WB4101 pretreatment; the pD_2 value in the presence of chlorethylclonidine was 5.09 ± 0.15 ($n = 5$, $P < 0.001$ vs. control, $P < 0.01$ vs. WB4101). Furthermore, chloreth-

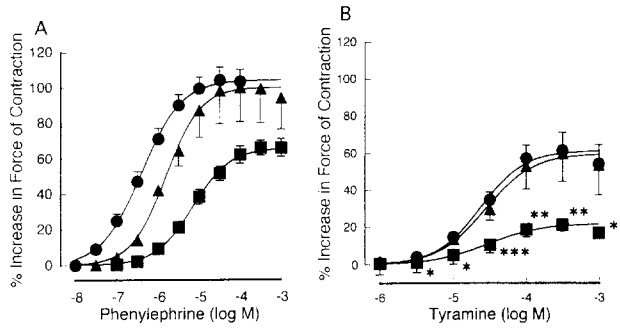

Fig. 1. Concentration–response curves for the positive inotropic effects of phenylephrine (A) and tyramine (B) in the absence (●) and presence of 10 nM WB4101 (▲) and 1 μM chlorethylclonidine (■). Points are means ± S.E. of 5–10 experiments. The values of contractile tension recorded immediately before the addition of the agonists were: (A) 417 ± 36 mg for untreated tissues, 416 ± 96 mg for WB4101-treated tissues and 542 ± 106 mg for chlorethylclonidine-treated tissues; (B) 356 ± 44 mg for untreated tissues, 435 ± 98 mg for WB4101-treated tissues and 699 ± 187 mg for chlorethylclonidine-treated tissues. All experiments were performed in the presence of 1 μM propranolol. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the corresponding values with tyramine alone.

ylclonidine significantly diminished the maximum inotropic effect of phenylephrine (66 ± 5%, $P < 0.01$).

Tyramine causes a positive inotropic response by releasing endogenous noradrenaline from reserpine-sensitive pools, because pretreatment of the animals with reserpine (1 mg/kg i.p., 3 days) could abolish the inotropic response of the papillary muscles to tyramine (Hattori et al., 1993). The positive inotropic effect of tyramine observed in the presence of 1 μM propranolol was completely eliminated by 1 μM prazosin, indicating that endogenous noradrenaline can induce an α_1-adrenoceptor-mediated positive inotropic effect after extensive blockade of β-adrenoceptors. Fig. 1B shows that tyramine produced a concentration-dependent increase in force of contraction in the presence of propranolol. The pD_2 value for tyramine was 4.57 ± 0.06 and the maximum increase was 63 ± 9% ($n = 7$). Thus, the maximum positive inotropic effect of tyramine was about 60% of that of phenylephrine. The concentration–response curve for tyramine was not affected by pretreatment with 10 nM WB4101. On the other hand, pretreatment with 1 μM chlorethylclonidine significantly inhibited the positive inotropic effect of tyramine. Even after treatment of the muscles with chlorethylclonidine, WB4101 did not antagonize the positive inotropic effect of tyramine; in chlorethylclonidine-treated muscles, 100 μM tyramine increased force of contraction by 22 ± 6% ($n = 3$) in the presence of WB4101, a value which was the same as that obtained in its absence (19 ± 2%, $n = 6$).

Representative inotropic effects of 500 nM noradrenaline added exogenously in the presence of 1 μM propranolol are depicted in Fig. 2A. The positive inotropic effect of 500 nM noradrenaline was attenuated by pretreatment with 1 μM chlorethylclonidine. However, noradrenaline still produced a substantial increase in force of contraction. Further application of 10 nM WB4101 nearly completely eliminated the positive inotropic effect of noradrenaline. The inhibition of the positive inotropic effect of noradrenaline by the further addition of WB4101 was indeed due to the effect of WB4101, but not to long exposure to chlorethylclonidine, because we found that the degree of inhibition by chlorethylclonidine was the same regardless of whether the exposure time was 60 or 120 min. The results of these experiments are summarized in Fig. 2B.

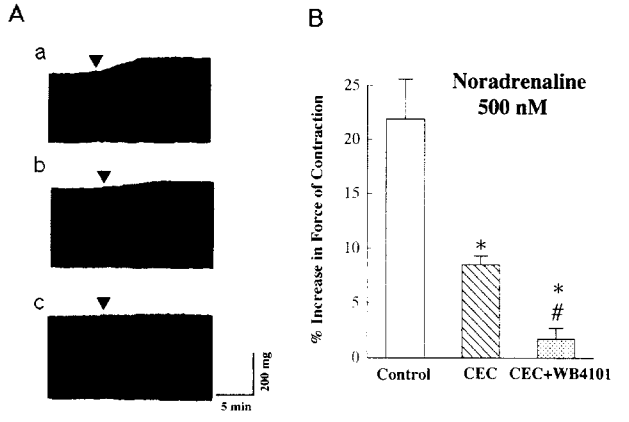

Fig. 2. Influences of 1 μM chlorethylclonidine (CEC) and 10 nM WB4101 on the positive inotropic effect of 500 nM noradrenaline. Propranolol (1 μM) was present throughout. (A) Typical effects of noradrenaline in the absence (a), and presence of chlorethylclonidine (b) and chlorethylclonidine and WB4101 (c). Noradrenaline was added at the arrowheads. Recordings were from the same preparation. (B) Bar graph summarizing the data obtained in panel A. Bars are means ± S.E. of four experiments. The values of contractile tension recorded immediately before the addition of noradrenaline were 329 ± 70 mg for untreated tissues, 359 ± 98 mg for tissues treated with chlorethylclonidine and 341 ± 114 mg for tissues treated with chlorethylclonidine and WB4101. * $P < 0.05$ compared with noradrenaline alone. # $P < 0.05$ compared with the value obtained in the presence of chlorethylclonidine.

4. Discussion

Early radioligand binding studies have indicated the simultaneous presence of the α_{1A}- and α_{1B}-adrenoceptor subtypes in rabbit ventricular myocardium (Takanashi et al., 1991; Endoh et al., 1992). We have also shown that this tissue contains the α_{1A}- and α_{1B}-adrenoceptor subtypes in a ratio of about 30:70 (Hattori et al., 1996; Nagashima et al., 1996). In the present study, WB4101 and chlorethylclonidine each antagonized the positive inotropic effect of phenylephrine in rabbit papillary muscle. Based on the different sensitivities of the α_{1A}- and α_{1B}-adrenoceptor subtypes to these antagonists, the present data suggest that both of the α_1-adrenoceptor subtypes are involved in the positive inotropic effect of phenylephrine in this preparation. We have recently demonstrated that chlorethylclonidine at the concentration used in this study (1 μM) is ineffective in the transient outward current inhibition and action potential prolongation induced by phenylephrine (Nagashima et al., 1996). These electrophysiological effects could be suppressed by WB4101 (Nagashima et al., 1996). Thus, the positive inotropism that is correlated with the action potential prolongation resulting from the transient outward current reduction appears to be mediated by the α_{1A}-adrenoceptor subtype. Consistent with this idea, Williamson et al. (1996) have reported that 4-aminopyridine, an inhibitor of the transient outward current, has no effect on the positive inotropic action of phenylephrine in the presence of WB4101. The inhibition of the inward rectifier K^+ current may also have an important role in the inotropic responses to α_1-adrenoceptor stimulation (Fedida et al., 1993), although the α_1-adrenoceptor subtype involved in this current inhibition remains unclear. On the other hand, the chlorethylclonidine-sensitive α_1-adrenoceptor subtype, i.e., α_{1B}-adrenoceptor subtypes, may mediate the positive inotropism associated with increased myofibrillar Ca^{2+} sensitivity. In rabbit ventricular myocardium, a major part of the positive inotropic effect of α_1-adrenoceptor stimulation is considered to be attributable to an increase in myofibrillar Ca^{2+} sensitivity (Endoh and Blinks, 1988).

We have previously found in rabbit papillary muscle that endogenous noradrenaline released by tyramine can

produce an α_1-adrenoceptor-mediated positive inotropic effect without any action potential change (Hattori et al., 1993). We interpret this to mean that the α_1-adrenoceptor-mediated positive inotropic effect of endogenous noradrenaline is entirely due to an increase in myofibrillar Ca^{2+} sensitivity. The present study showed that the positive inotropic effect of tyramine was antagonized by chlorethylclonidine but not by WB4101, suggesting that endogenous noradrenaline produces a positive inotropic effect exclusively mediated by the α_{1B}-adrenoceptor subtype when β-adrenoceptors are adequately blocked by propranolol. Therefore, our current data with tyramine together with those of our previous study (Hattori et al., 1993) lend further support to the hypothesis that the positive inotropic effect induced by activation of the α_{1B}-adrenoceptor subtype may involve an increase in myofibrillar sensitivity to Ca^{2+}.

Exogenous noradrenaline clearly exerts its inotropic effect primarily through β-adrenoceptors. However, it is also capable of producing an α_1-adrenoceptor-mediated positive inotropic effect in rabbit papillary muscle, an effect which is detectable when β-adrenoceptors are extensively blocked (Aass et al., 1983). In contrast to our previous data with endogenous noradrenaline (Hattori et al., 1993), earlier studies have demonstrated that exogenous noradrenaline causes an increase in action potential duration via α_1-adrenoceptors in rabbit papillary muscle (Dukes and Vaughan Williams, 1984). It therefore is likely that the α_1-adrenoceptor-mediated component in the inotropic response to exogenous noradrenaline may be initiated in part by activation of the α_{1A}-adrenoceptor subtype. This was supported by the present finding that the positive inotropic effect of the low concentration of noradrenaline observed in the presence of propranolol was nearly completely eliminated by the addition of WB4101 together with chlorethylclonidine. The different involvement of α_1-adrenoceptor subtypes in the effects of endogenous and exogenous noradrenaline could be explained by assuming that noradrenaline, when released from the sympathetic nerve endings, may be more easily accessible to the α_{1B}-adrenoceptor subtype than to the α_{1A}-adrenoceptor subtype. Thus, the α_{1B}-adrenoceptor subtype population may be located on a space confronting more closely to the sympathetic nerve endings than the α_{1A}-adrenoceptor subtype population. However, experimental evidence to support this assumption awaits further study.

In conclusion, the present data indicate that the α_{1A}- and α_{1B}-adrenoceptor subtypes are involved in the positive inotropic responses to phenylephrine and exogenous noradrenaline, whereas only the α_{1B}-adrenoceptor subtype contributes to the α_1-adrenoceptor-mediated inotropic component caused by endogenous noradrenaline. This could indicate that in rabbit myocardium the α_{1B}-adrenoceptor subtype may be possibly located on a space confronting more closely to the sympathetic nerve endings and thus noradrenaline released as neurotransmitter may be more accessible to the α_{1B}-adrenoceptor subtype than to the α_{1A}-adrenoceptor subtype, although a more complete experimental design is required.

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