European Journal of Pharmacology 419 (2001) 249–252
www.elsevier.nl/locate/ejphar

Short communication

Pertussis toxin-sensitive and -insensitive mechanisms of α_1-adrenoceptor-mediated inotropic responses in rat heart

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Received 26 March 2001; accepted 10 April 2001

Abstract

In rat left ventricular papillary muscle, phenylephrine, an α1-adrenoceptor agonist, induced a triphasic inotropic response; an initial transient, small, positive inotropic effect followed by a transient chloroethylclonidine-sensitive negative inotropic effect and a sustained 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane (WB4101)-sensitive positive inotropic effect. Treatment with pertussis toxin for 2 days significantly inhibited only the transient negative inotropic effect without changing the sustained positive inotropic effect. This treatment also prevented the acetylcholine (1 μM)-induced negative inotropic effect. Further, phenylephrine-induced transient negative inotropic effect was attenuated in the presence of ouabain. These results suggest that pertussis toxin-sensitive or-insensitive G-protein may be responsible for α1-adrenoceptor subtype-mediated negative inotropic effect or positive inotropic effect, respectively, in which the transient negative inotropic effect was produced via the stimulation of Na^+, K^+ pump, presumably through pertussis toxin-sensitive G-protein-dependent pathway.

Keywords: α_1-Adrenoceptor; Cardiac contractility; Pertussis toxin; Na^+, K^+ pump

1. Introduction

Recent molecular pharmacological studies have identified three α_1-adrenoceptor subtypes designated as α_{1A}, α_{1B} and α_{1D}. In the adult rat myocardium, α_{1A} and α_{1B} exist as dominant subtypes and regulate cardiac functions including contractility (Varma and Deng, 2000). Our previous studies have shown that α_1-adrenoceptor stimulation with phenylephrine in rat ventricular papillary muscle produced a triphasic inotropic response (an initial transient small positive inotropic effect followed by a transient negative inotropic effect and a sustained positive inotropic effect) associated with phosphoinositide hydrolysis (Otani et al., 1992). Regarding to these components, positive inotropic effect occurs through the stimulation of Na^+/H^+ exchange (Otani et al., 1992; Gambassi et al., 1998), while the mechanism that induces transient negative inotropic effect remains unknown.

α_1-Adrenoceptor signalling is known to involve pertussis toxin- insensitive G-protein (Gq). However, an accumulation of evidence indicates that pertussis toxin-sensitive G-protein is also involved in α_1-adrenoceptor-mediated cellular responses such as vasoconstriction and arachidonic acid release (Nishio et al., 1996; Gurdal et al., 1997). As in these studies, different G-proteins may be involved in the diverse inotropic responses by phenylephrine in cardiac muscle.

In the present study, we evaluated the effects of pertussis toxin on α_1-adrenoceptor subtypes (α_{1A} or α_{1B})-mediated inotropic responses in the rat heart, with special attention to the mechanism of the transient negative inotropic effect.

2. Materials and methods

2.1. Preparation and stimulation of rat papillary muscles

All animals were handled in accordance with "Rules of the Animal Experimentation of Committee, Kansai Medical University".

Male Sprague–Dawley rats (250–300 g) were anaesthetized with sodium pentobarbitone and the hearts were quickly removed. Isolated left ventricular papillary muscle was suspended in an organ bath containing a Tyrode solution of the following composition (mM): NaCl 122.5,

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)00979-7

KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 24 and glucose 10 (pH 7.4), aerated with 95% O_2 and 5% CO_2 at 32°C. The muscle was initially loaded with 500 mg and driven electrically by a rectangular pulse (1 Hz, 10-ms duration and 2–4 V). The isometric tension was measured by a force displacement transducer (Shinko-Tsusin, Japan, UL-2) and recorded through an amplifier (Shinko-Tsusin, DS-601B). After a 50-min equilibration, the preparation was treated with phenylephrine or acetylcholine. When phenylephrine was used, 0.3 μM propranolol was always present to inhibit β-adrenoceptor stimulation. Pertussis toxin treatment was carried out by intravenous injection (25 μg/kg) 2 days before sacrifice. 2-(2,6-Dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane (WB4101) or chloroethylchlonidine was used as an α_{1A}-antagonist or as an irreversible α_{1B}-adrenoceptor inhibitor, respectively. In treatment with chloroethylchlonidine, muscles were exposed to this agent for 30 min and then kept in a drug-free buffer for 20 min before α_1-adrenoceptor stimulation.

2.2. Materials

Phenylephrine, propranolol, acetylcholine, pertussis toxin and ouabain were obtained from Sigma (St Louis, MO, USA). WB4101 and chloroethylclonidine 2 HCl were from Research Biochemicals International (Natick, MA, USA).

2.3. Statistical analysis

Student's *t*-test was used for statistical analysis. The differences between mean values with P values less than 0.05 were considered significant.

3. Results

Phenylephrine (10 μM), in the presence of 0.3 μM propranolol, elicited an initial transient small positive inotropic effect followed by a transient negative inotropic effect and a sustained positive inotropic effect (Fig. 1A). As shown in Fig. 1B, the transient negative inotropic effect or the sustained positive inotropic effect was selectively inhibited by chloroethylchlonidine (10 μM) or WB4101 (10 nM), respectively. The initial transient positive inotropic effect tended to be diminished or augmented by WB4101 or chloroethylchlonidine, respectively. These inhibitors had no effects on the basal contractility (data not shown).

Next, phenylephrine stimulation was carried out in pertussis toxin-treated papillary muscle. Pertussis toxin treatment tended to augment the initial transient positive inotropic effect, but significantly inhibited the transient negative inotropic effect. The sustained positive inotropic effect was unaffected by this pretreatment (Fig. 1C, left). Further, we tested the effect of pertussis toxin on mus-

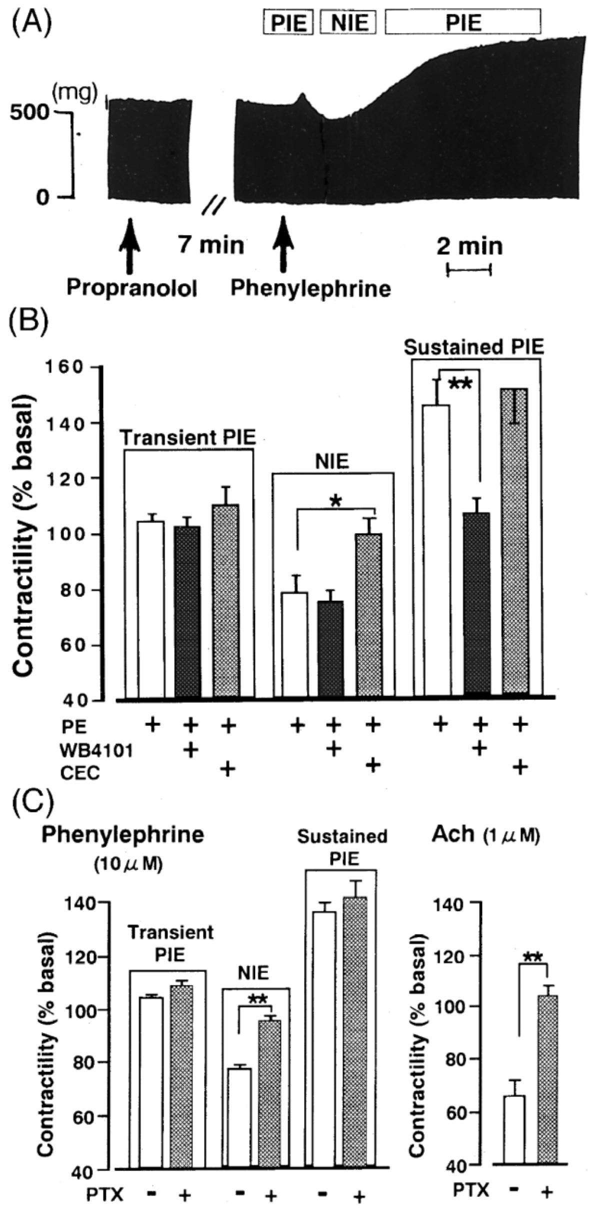

Fig. 1. (A) Representative trace of the effect of phenylephrine on contractile force. Stimulation of rat left ventricular papillary muscle with 10 μM phenylephrine produced a triphasic inotropic response. NIE, negative inotropic effect; PIE, positive inotropic effect. (B) Effects of pretreatment with WB4101 (10 nM) or chloroethylclonidine (CEC, 10 μM) on phenylephrine (PE, 10 μM)-induced triphasic inotropic response. (C) Effects of pertussis toxin (PTX) treatment on phenylephrine- or acetylcholine-induced inotropic responses. Rat ventricular papillary muscles pretreated with or without pertussis toxin were exposed to10 μM phenylephrine (left panel) or 1 μM acetylcholine (Ach, right panel). In panels (B) and (C), the changes in the contractile force were expressed as a percentage of the values obtained just before the addition of each agonist. Each value represents the mean ± S.E. of six to eight preparations. Significant difference ($^*P<0.05$, $^{**}P<0.01$) between two groups.

carinic response, since muscarinic receptor couples to K^+ channel via pertussis toxin-sensitive G-protein (Mcmorn et al., 1993; Ito et al., 1995) (Fig. 1C, right). Acetylcholine (1 μM) produced a negative inotropic effect, although the potency (33% decrease) was somewhat greater than that (25%) reported in rat ventricular myocytes (Mcmorn et al., 1993). This negative inotropic effect was completely blocked by pertussis toxin treatment, confirming the functional inactivation of pertussis toxin-sensitive G protein by the present procedure.

Previously, α_1-adrenoceptor stimulation has been demonstrated to stimulate Na^+, K^+ pump activity via pertussis toxin-sensitive pathway (Shah et al., 1988; Lee et al., 1991). Therefore, we examined the effect of the Na^+, K^+ pump inhibition on α_1-adrenoceptor-mediated negative inotropic effect. The application of 200 μM ouabain produced a substantial increase in the basal contractility that reached a plateau after 5 min. The transient negative inotropic effect by 10 μM phenylephrine was obviously attenuated under a ouabain-treated condition (Fig. 2A). As shown in Fig. 2B,C, ouabain (10–200 μM) concentration-dependently attenuated the phenylephrine-induced negative inotropic effect, although it increased the basal contractilities.

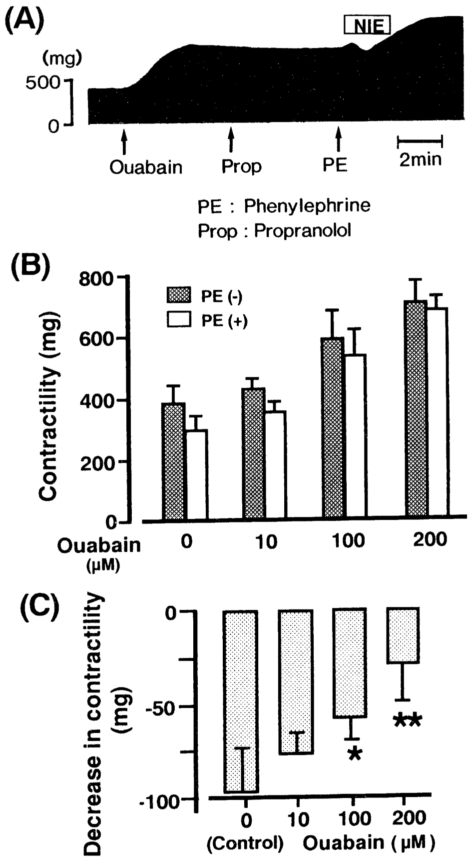

Fig. 2. (A) Representative inotropic response induced by 10 μM phenylephrine in the presence of 200 μM ouabain. (B) Concentration-dependent effects of ouabain on the contractility in the presence or absence of phenylephrine (PE, 10 μM). (C) Phenylephrine (10 μM)-induced decreases in contractility (NIE, negative inotropic effect) in the presence of ouabain (10 to 200 μM). Each value represents the mean ± S.E. of five preparations. $^*P < 0.05$, $^{**}P < 0.01$ compared to control.

4. Discussion

The present study evaluated the effects of inhibitors of α_1-adrenoceptor subtypes and pertussis toxin to determine whether different signalling pathways underlie the α_1-adrenoceptor stimulation-mediated diverse inotropic responses. Our results suggest that pertussis toxin-sensitive or -insensitive G-protein is involved in phenylephrine-induced negative inotropic effect or positive inotropic effect, respectively. Further, based on the inhibitory effects on negative inotropic effect of chloroethylchlonidine and pertussis toxin, it is assumed that α_{1B}-adrenoceptor couples with pertussis toxin-sensitive G-protein to exert negative inotropic effect. Tendency of augmentation of the initial transient positive inotropic effect by chloroethylchlonidine or pertussis toxin may be caused by the elimination of underlying negative inotropic effect. Such an α_{1B}/pertussis toxin-sensitive signalling pathway has also been proposed in other α_1-adrenoceptor-mediated effects such as vasoconstricton and arachidonic acid release (Nishio et al., 1996; Gurdal et al., 1997). There is, however, controversial evidence that the negative inotropic effect of phenylephrine was not inhibited by chloroethylchlonidine (1 μM) in the rat cardiac muscle (Nagashima et al., 1997). This discrepancy may be caused by a difference between the concentrations of chloroethylchlonidine (10 and 1 μM) used in these studies.

Next, we envisaged the involvement of Na^+, K^+ pump as a possible mechanism by which pertussis toxin-sensitive G-protein mediates negative inotropic effect, since α_1-adrenoceptor-mediated stimulation of this pump reportedly induces negative inotropic effect by diminishing intracellular Na^+ activity and subsequent modification of Na^+/Ca^{2+} exchange (Williamson et al., 1993; Jo et al., 2000). Indeed, phenylephrine-induced negative inotropic effect was attenuated under the Na^+, K^+ pump-inhibited condition, as shown in Fig. 2. The molecular linkage between pertussis toxin-sensitive G-protein and Na^+, K^+ pump was not determined in this study. However, from previous evidence showing pertussis toxin-sensitive stimulation of Na^+, K^+ pump by phenylephrine (Shah et al., 1988), it is possible to conclude that the transient negative inotropic effect was produced through the stimulation of Na^+, K^+ pump via α_{1B}/pertussis toxin-sensitive G-protein-dependent pathway. Further, as the other candidate for the mechanism of phenylephrine-induced negative inotropic effect, hyperpolarization of membrane potential have been proposed in rat ventricular papillary muscle (Nagashima et al., 1997). This possibility appears to be compatible with our hypothesis since stimulation of Na^+, K^+ pump could cause a negative shift of membrane potential.

The present data also suggest that α_{1A}-adrenoceptor mediates phenylephrine-induced sustained positive inotropic effect through coupling to pertussis toxin-insensitive G-protein (Gq). This Gq-dependent signalling stimulates Na^+/H^+ exchange to exert positive inotropic effect, presumably via protein kinase C activation (Otani et al., 1992; Snabaitis et al.,2000). In contrast to the initial transient positive inotropic effect, this sustained positive inotropic effect was almost unaffected by the pretreatment with chloroethylchlonidine or pertussis toxin, which was employed to eliminate the underlying negative inotropic effect.

This paper reports the first evidence that phenylephrine-induced negative inotropic effect is sensitive to both pertussis toxin and chloroethylchlonidine. Receptor subtype specific coupling to pertussis toxin-sensitive or insensitive G-protein appears to be involved in α_1-adrenoceptor-mediated inhibitory or stimulatory regulation of cardiac contractility, presumably via stimulation of Na^+, K^+ pump or Na^+/H^+ exchange, respectively.

Acknowledgements

This work was supported by a grant from Kansai Medical University.

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