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Alpha$_{1A}$- and alpha$_{1B}$- adrenoceptor-mediated positive chronotropic effects on isolated rat atrium[1]

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ABSTRACT The positive chronotropic effect of α_1-adrenoceptor subtypes was studied on the isolated right atrium of adult male Wistar rats. In the presence of β-blocker propranolol, chloroethylclonidine (Chl) (an irreversible α_{1B} inactivator) reduced the positive chronotropic effect of phenylephrine (Phe). When the basal rate was lowered by cholinergic stimulation. Chl showed less effect on the increase of spontaneous beat rate. H-7, a protein kinase C (PKC) inhibitor, lowered the chronotropic effect to Phe under physiological condition, but did not affect the Phe-induced positive chronotropic effect when basal rate was lessened by carbachol. These suggested that the chronotropic effect was mainly mediated by α_{1B} receptors, and partially related to the activation of PKC by second messengers under physiological conditions. The compensatory effect of the α_1-adrenoceptors was mainly mediated by α_{1A} receptors, which is scarcely involved in the activation of PKC.

KEY WORDS alpha adrenergic receptors; heart rate; heart atrium; phenylephrine; 1-(5-isoquinolinylfonyl)-2-methyl-piperazine; chloroethylclonidine; myocardial contraction

Positive chronotropic effect and inotropic effect can be produced by stimulation of α_1-adrenoceptors[1-4], which have been classified into at least 2 subtypes, α_{1A} and α_{1B} receptors[5]. The subtypes have different distributions and densities in various tissues[6,7]. But their physiological functions on cardiovascular system remain unclear. The present study was to investigate the effect of α_1-adrenoceptor subtypes on spontaneous beat rate of isolated atrium. The effect of H-7, a protein kinase C (PKC) inhibitor[7], on the positive chronotropic effect mediated by α_1-adrenoceptors was also examined.

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MATERIALS AND METHODS

Isolated atria Adult ♀ Wistar rats (238±21 g) were decapitated, the right heart atrium was placed in 15 ml Kreba's solution at 36℃, bubbled with 95% O_2 +5% CO_2. The atrium was suapended on a holder and pierced wihtout stretching by 2 metallic hooks connected to a press sensor (LW-1 type). The resting tension was adjusted to 0.3 g. The force of contraction and the beat rate were continuously recorded with a double-pen recorder (XWT-204 type).

After an equilibration of 1 h, in the presence of β-blocker, propranolol 1 μmol·L^{-1}, a concentration-response curve for (−)-phenylephrine (Phe) was obtained by increasing the concentration of Phe in steps of 0.5 lg units in a cumulative manner. After washing out for 1 h, the force of contraction and basal rate were restored to pretreatment levels. Then carbachol (0.3 μmol·L^{-1}) was added and the basal rate was reduced about 50%. The effect of Phe on spontaneous beat rate was determined again. The first curve represented the α_1-adrenoceptor-mediated chronotropic effect under physiological conditions, and the second curve, that under the condition of lowered basal rate. The atria were exposed to chloroethylclonidine (Chl) or H-7 30 min before Phe was added.

Preparation of solutions (−)-Phenylephrine hydrochloride (Phe, Sigma) was dissolved in 0.5% ascorbic acid solution. Chloroethylclonidine (Chl, Research Biochemical Inc.) was dissolved in Kreba's solution. 1-(5-Isoquinolinylfonyl)-2-methyl-piperazine (H-7, Sigma) was dissolved in Me_2SO. Propranolol (Pro, Sigma); carbachol (Sigma). The Krebs' solution contained NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, glucose 11.1, $CaCl_2$ 2.5, and EDTA-Na_2 0.001 (mmol·L^{-1}).

Statistical analysis The n was the number of

rats, significances were evaluated by *t* test.

RESULTS

Phe induced positive chronotropic effect In the presence of propranolol (1 μmol·L^{-1}), Phe produced a positive chronotropic effect and an inotropic effect on the isolated right atrium of rats by stimulating α_1- adrenoceptors. The maximal increase of spontaneous beat rate induced by Phe 1 —100 μmol·L^{-1} was 31±4 bpm (n=11). When cholinergic receptors of atrium were activated by carbachol 0.3 μmol·L^{-1}, the basal rate was reduced about 50%. The chronotropic effect of α_1-adrenoceptors was found to be strengthened. Phe (100 μmol·L^{-1}) caused quickening of spontaneous beat rate up to 92±10 bpm (n=8, Fig 1). These results represented the effect of α_1-adrenoceptors on beat rate under the normal and the inhibited cardiac state, respectively.

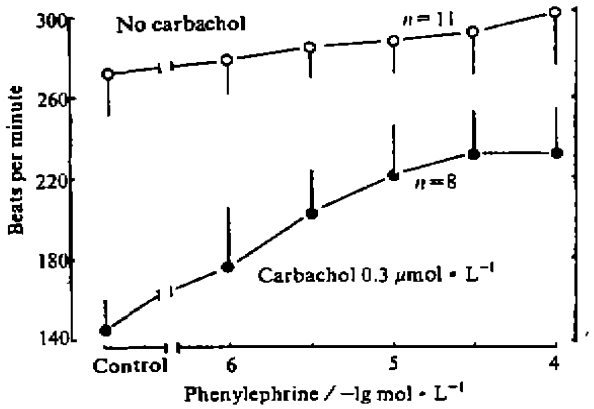

Fig 1. Effect of phenylephrine in the absence/presence of carbachol. $\bar{x}\pm s$.

Chronotropic effect mediated by α_1-adrenoceptor subtypes After incubation with Chl 100 μmol·L^{-1} for 30 min, the effect of α_{1A} receptors was examined. Phe 100 μmol·L^{-1} caused a maximal response of 10±2 bpm (n=9), which was smaller than 31±4 bpm (control), $P<0.05$ (Fig 2). In the presence of carbachol 0.3 μmol·L^{-1}, the maximal increase of beat rate of atrium induced by Phe (n=12) was 84±4 bpm, which was not significantly different from the control (92±10 bpm) (Fig 2).

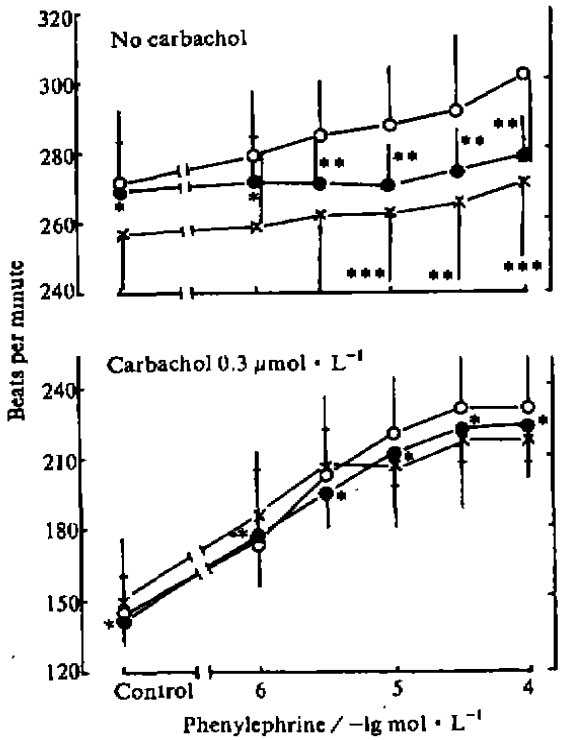

Fig 2. Effects of chloroethylclonidine (Chl, 100 μmol·L^{-1}) and H-7 10 μmol·L^{-1} on the phenylephrine-induced positive chronotropic response in the absence/presence of carbachol. (○) control, n=9—11. (●) Chl, n=8—12, (×) H-7, n=8—9. $\bar{x}\pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Effect of H-7 on α_1-adrenoceptor-mediated chronotropic response After the right atrium was exposed to H-7 10 μmol·L^{-1} for 30 min, the maximal increase of beat rate induced by Phe 100 μmol·L^{-1} was significantly reduced from 31±4 bpm (control) to 17±3 bpm (n=11), $P<0.05$ (Fig 2). In the presence of carbachol 0.3 μmol·L^{-1}, the maximal increase of beat rate 69±10 bpm was not significantly

different from the control value 92 ± 10 bpm ($P>0.05$, Fig 2).

DISCUSSION

By examing the physiological function of α_1-adrenoceptor subtypes, we found that α_{1A} and α_{1B} receptors both could mediate the positive chronotropic effect. The functional response mediated by α_{1A} receptors was only 32% of the whole α_1-adrenoceptors under normal physiological condition, while 68% was mediated by α_{1B} receptors. When the heart was inhibited by carbachol, however, α_{1A} receptors played an important role in raising the spontaneous beat rate, consisting 92% of the total α_1-adrenoceptors.

In the presence of H-7, an inhibitor of PKC, the chronotropic effect of α_1-adrenoceptors was inhibited 54% under physiological conditions. When the spontaneous beat rate was lowered by carbachol, H-7 showed a less effect on the positive chrono- tropic effect mediated by α_1-receptors *vs* control, its chronotropic effect was reduced 24.6% by H-7 10 μmol·L^{-1}. These results suggested that the chronotropic effect by α_1-receptors stimulation was partially related to the activation of PKC by a second messenger. Whereas, in the presence of carbachol, the compensatory effect of α_1-receptors was little involved in the activation of PKC. These indicated that there may be multiple mechanisms for the α_1-adrenoceptor-induced effect.

Previous studies showed that α_{1B} receptors caused functional response relating to PI turnover, whereas α_{1A} caused physiological response depending mainly on the activity of calcium channels[6]. From previous studies and our results, we deduced that under physiological conditions, the positive chronotropic effect of α_1-adrenoceptors is mainly mediated by α_{1B} subtypes, which is closely related to the second messengers. And the compensatory effect of α_{1A} receptors may be closely relevant to the calcium channels.

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大鼠离体右心房 α_{1A}和 α_{1B}肾上腺素受体介导的正性频率作用

R965.2

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摘要 氯乙基可乐定(Chl)明显抑制 Phe 引起的心率增加，ACh 抑制自发心率时，Chl 作用不明显. H-7部分抑制 α_1受体的正性频率作用，心脏抑制时则较少影响 α_1受体的代偿作用. 提示生理条件下，α_1受体激动引起正性频率作用主要通过 α_{1B}亚型并依赖于蛋白激酶 C (PKC)的激活. 心脏抑制时，α_{1A}亚型起到主要的心率代偿作用.

关键词 α肾上腺素受体；心率；心房；苯福林；1-(5-异喹啉磺酰基)-2-甲基哌嗪；氯乙基可乐定；心肌收缩