BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; 16 (3): 243—246

Influences of phenylephrine on delayed afterdepolarization and triggered activity in sheep Purkinje fibers[1]

SHI Wei-Bin, XU You-Qiu, CHEN Xi-Luo, YU Pei-De
(Department of Physiology, Shanghai Second Medical University, Shanghai 200025, China)

AIM: To compare the effects of phenylephrine (Phe) on DAD and TA in the absence or presence of propranolol (Pro). **METHODS**: Using intracellular microelectrode methods to record transmembrane potentials in sheep Purkinje fibers. **RESULTS**: Perfused Phe 1 $\mu mol \cdot L^{-1}$ for 60 min, in the absence of Pro, Phe increased the amplitude of DAD from 7.5±1.2 to 9.0±2.0 mV ($n=8$, $P<0.05$), nonsignificantly changed the duration of DAD and induced TA in 3/11 preparations; but in the presence of Pro 0.5 $\mu mol \cdot L^{-1}$ blocking β-adrenoceptors, the amplitude of DAD increased from 7.2±1.8 to 8.3±2.1 mV at the first 20 min ($n=8$, $P<0.01$), and then decreased to 6.3±1.6 mV, the duration of DAD prolonged from 192±17 to 280±27 ms ($n=8$, $P<0.01$ *vs* control), meanwhile Phe could suppress the TA induced by acetylstrophanthidin and this effect was blocked by prazosin. **CONCLUSION**: When Pro is efficiently used in abolishing arrhythmias evoked by catecholmines, apart from directly blocking β-adrenoceptors, relatively aggravating excitation of α-adrenoceptors to suppress DAD and TA is one of the important reasons.

KEY WORDS phenylephrine; adrenergic alpha receptor agonists; acetylstrophanthidin; Purkinje fibers; electrophysiology

Delayed afterdepolarization (DAD) and triggered activity (TA) were the the electrophysiological basis of the cardiac arrhythmia induced by digitalis intoxication. Catecholamine could strengthen these abnormal electric activities via the activation of β-adrenoceptor. But the effect of the excitation of α-adrenoceptor on DAD and TA is still in debate. Kimura found that phenylephrine (Phe) had a strengthening effect on DAD[1,2], while Hewett did not[3]. In contrast, a suppressive effect of Phe on transient inward current was reported[4]. Phe 1 $\mu mol \cdot L^{-1}$ excited both α- and β-adrenoceptors[5]. The present study observed the effect of Phe on DAD and TA in the absence or presence of β-adrenoceptor blocker. The aim is to find out whether the β-adrenoceptor blocker has any influence on when Phe affected DAD and TA, and the possible mechanism of Phe effect.

MATERIALS AND METHODS

Adult sheeps (♂ or ♀), weighing 30±5 kg ($n=40$), were killed by cutting the carotid arteries. The Purkinje fibers (5—10 mm long, 0.5—1.0 mm wide) were mounted into the tissue bath 1.5 mL. The preparations were perfused with Tyrode solution gassed with 95 % O_2 + 5 % CO_2 at a 5 $mL \cdot min^{-1}$, at 36±0.5 C, pH 7.4.

Extracellular stimuli were given at 1 Hz, 1 ms square wave, and 150 % of the diastolic threshold. Intracellular glass microelectrode filled with KCl 3 $mol \cdot L^{-1}$ was penetrated into the Purkinje cells and the transmembrane potentials were recorded. After 20 min equilibration period, acetylstrophanthidin (Ace) was added into perfusate to 0.2±0.01 $\mu mol \cdot L^{-1}$ ($n=18$). (The thicker the specimen in diameter was, the higher concentration of Ace should be). A stable DAD with medium amplitude was induced in 1 h and was

[1]Project supported by the National Natural Science Foundation of China, № 39070399.
Received 1994-03-17 Accepted 1994-11-21

kept stable for another 2 h[6]. Phe was added 30 min after the appearance of DAD.

Measorement of amplitude and duration of DAD The maximal diastolic potential of 2 adjacent action potentials was connected as baseline, and the amplitude of DAD was denoted from the distance between the baseline and the peak of DAD. In the presence of Phe, the DAD became flat, and for avoiding the measuring error, the duration or width of DAD was measured at 50 % amplitude of DAD. The measuring method was shown in Fig 1.

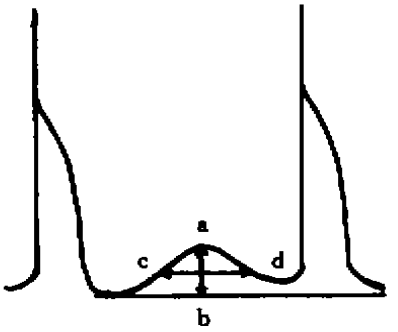

Fig 1. Measurement of amplitude (a—b) and duration (c—d) of DAD.

All drugs were products of Sigma Co.

Data were presented as $\bar{x} \pm s$, and analyzed with a paired t test.

RESULTS

Influence of Phe on DAD induced by Ace When the DAD was induced by Ace 0.2 μmol·L^{-1} and maintained in a stable level, Phe 1 μmol·L^{-1} was added into the perfusate for 60 min. The amplitude of DAD increased from control value 7.5±1.3 to 9.0±2.0 mV (n=8, P<0.05). The change of the duration of DAD was negligible, from control value 213±25 to 216±18 ms (P>0.05). In 3/11 preparations, TA was induced by augmented DAD, and lasted till the preparation lost its excitability if Phe was not washed out (Fig 2a).

Influence of β-adrenocepter blocker on DAD Perfusing the preparation with propranolol 0.5 μmol·L^{-1} for 100 min when the DAD was in a stable level, the amplitude changed from 7.2±1.5 to 7.6±1.9 mV and the duration lengthened changed from 212±18 to 224±27 ms (n=8, P>0.05).

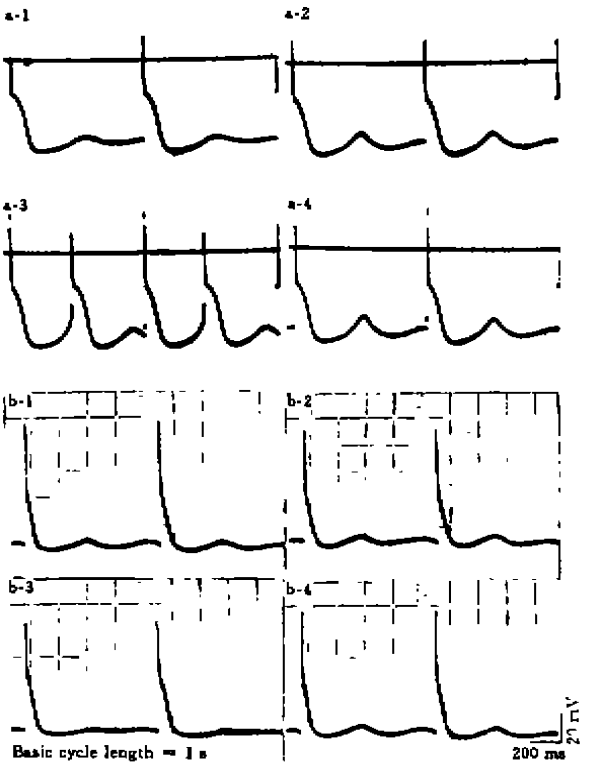

Fig 2. Effects of phenylephrine (Phe) 1 μmol·L^{-1} on amplitude and duration of DAD. a-1. control. Ace 0.2 μmol·L^{-1}; a-2. 45 min after Phe 1 μmol·L^{-1}; a-3. 48 min after Phe 1 μmol·L^{-1} triggered activity (TA) was induced; a-4. washout Phe for 15 min, TA disappeared. b-1. control, Ace 0.2 μmol·L^{-1} + propranolol 0.5 μmol·L^{-1}; b-2. & b-3. 20 & 60 min after Phe 1 μmol·L^{-1}, respectively; b-4. washout Phe, 30 min.

Influences of Phe on DAD In the presence of Ace 0.2 μmol·L^{-1} and propranolol 0.5 μmol·L^{-1}, the influences of Phe 1 μmol·L^{-1} on DAD was a biphasic one. At the first 20 min, the amplitude of DAD increased from 7.2±1.8 to 8.3±2.1 mV (n=8, P<0.01) and the duration of DAD prolonged from 192

±17 to 224±34 ms ($P<0.05$). Afterwards, the amplitude of DAD decreased gradually to 6.3±1.6 mV ($P<0.01$ *vs* control) and the duration prolonged continuously to 280±27 ms ($P<0.01$ *vs* control) at 60 min. These effects were reversable when Phe was washed out, and DAD returned to the control level (Fig 2b).

Influence of Phe on TA In the presence of propranolol 0.5 μmol·L^{-1}, Ace 0.32 μmol·L^{-1} induced TA in sheep Purkinje fibers[6]. Adding Phe 1 μmol·L^{-1} decreased the frequency of TA or even eliminated it ($n=6$). When the α_1-adrenoceptor blocker prazosin 0.5 μmol·L^{-1} was added for 15 min, the effect of Phe was blocked, the TA appeared again ($n=3$) (Fig 3).

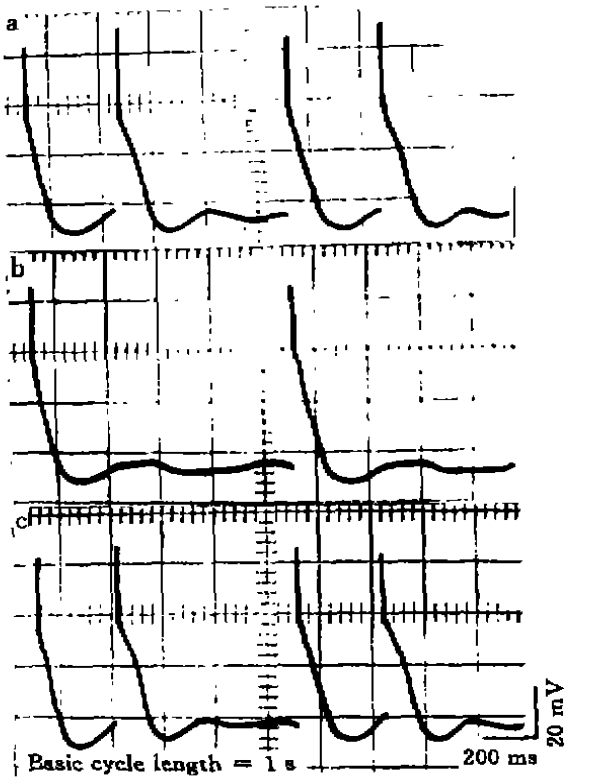

Fig 3. Effects of phenylephrine (Phe) 1 μmol·L^{-1} on triggered activity (TA) induced by Ace 0.32 μmol·L^{-1}. a. control, Ace 0.32 μmol·L^{-1} + proprandol 0.5 μmol·L^{-1}, TA was induced; b. after Phe 1 μmol·L^{-1}, 22 min, TA was abolished; c. effects of Phe was blocked by prazoxin 0.5 μmol·L^{-1} for 15 min, TA appeared again.

DISCUSSION

β-Adrenoceptor agonist as isoprenaline strengthened DAD by increasing its amplitude and shortening its durtion, when the augmented DAD reached threshold, TA would be induced[6]. Phe 1 μmol·L^{-1} excited both α- and β-adrenoceptors[5], its effect on DAD was somewhat different from that of beta agonist. It increased the amplitude of DAD gradually and showed almost no effect on its duration for 60 min. In the presence of β-blocker, the effect of Phe on DAD amplitude was biphasic, a slight excitatory effect first followed by a stronger inhibitory effect. Since the sustained effect of Phe is to reduce the amplitude of DAD and to prolong the duration of DAD (make it become flat), the DAD becomes too difficult to reach the threshold potential, so it will be helpful to eliminate the triggered activity. The effects of Phe were blocked by prazosin, it indicates that effects were via the excitation of α_1-adrenoceptors. The sustained inhibitory effect of Phe on DAD could be due to the decreasing of intracellular calcium concentraiton by the regulaiton of IP_3 and DAG[7] to activate protein kinase C (PKC)[8], which perhaps increases the activity of sodium-potassium pump and calcium pump[9].

Consequently, the class I antiarrhythmic agents as propranolol not only block excitatory effect of the β-adrenoceptors, but also relatively aggravate stimulation of α-adrenoceptors, which will be helpful to suppress ectopic rhythm. This might be another important reason why β-blockers are efficiently used in abolishing arrhthmias evoked by excitation of sympathetic or augmentation of catecholmines.

Since the influences of Phe on DAD is a slight and slow process, it needs a long observation time. If the DAD induced in the experiment is not stable or only keeps a short time, it will be difficult to observe the whole biphasic process, that may be the source of argument in the previous study[1-4]. However, the species difference could not be excluded[7].

REFERENCES

1 Kimura S, Cameron JS, Kozlovskis PL, Bassett AL, Myerburg RJ. Delayed afterdepolarizations and triggered activity induced in feline Purkinje fibers by α-adrenergic stimulation in the presence of elevated calcium levels. Circulation 1984; **70**: 1074-82.

2 Kimura S, Bassett AL, Kohya T, Kozlovskis PL, Myerburg RJ. Automaticity, triggered activity, and responses to adrenergic stimulation in cat subendocardial Purkinje fibers after healing of myocardial infarction. Circulation 1987; **75**: 651-60.

3 Hewett KW, Rosen MR. Alpha and beta adrenergic interactions with ouabain-induced delayed afterdepolarizations. J Pharmacol Exp Ther 1984; **229**: 188-92.

4 Ferrier GR, Carmeliet E. Effects of α-adrenergic agents on transient inward current in rabbit Purkinje fibers. J Mol Cell Cardiol 1990; **22**: 191-200.

5 Schumann HJ, Endo M, Wagner J. Positive inotropic effects of phenylephrine in the isolated rabbit papillary muscle are mediated both by α and β-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol 1974; **284**: 133-48.

6 Shi WB, Xu YQ, Zhang HD. Effects of isoprenaline and carbachol on delayed afterdepolarization in myocardium. Acta Physiol Sin 1989; **41**: 361-66.

7 David Fedida, Andrew P Braun, Giles WR. α_1-Adrenoceptors in myocardium: functional aspects and transmembrane signaling mechanims. Pharmcol Rev 1993; **78**: 469-87.

8 Capogrossi MC, Kaku T, Filburn CR, Pelto DJ, Hansford RG, Spurgeon HA, *et al*. Phorbol ester and diocranoylglycerol stimulate membrane association of protein kinase C and have a negative inotropic effect mediated by changes in cytosolic Ca^{2+} in adult rat cardiac myocytes. Circ Res 1990; **66**: 1143-55.

9 Moscucci A, Sharma VK, Sheu SS. Cellular machanisms of phorbol ester induced reduction of contractile force in rat papillary muscle. Circulation 1988; **78**: 1-142.

243-246

12

苯福林对羊浦肯野纤维延迟后除极与触发活动的影响

R965

施渭彬，徐有秋，陈希洛，俞培德
（上海第二医科大学生理教研室，上海200025，中国）

目的：比较普萘洛尔(Pro)药物应用与否时，苯福林(Phe)对 DAD 与 TA 的不同影响。**方法**：绵羊浦肯野纤维细胞内微电极记录跨膜电位法。**结果**：Phe 1 μmol·L^{-1} 灌流60 min，无 Pro 时，使 DAD 幅值由7.5±1.2增加到9.0±2.0 mV（n=8，P<0.05），DAD 时程变化不显著，3/11可诱发 TA；Pro 0.5 μmol·L^{-1}阻断 β 受体时，前20 min 使 DAD 幅值由7.2±1.8增加到8.3±2.1 mV（n=8，P<0.01），而后减少至6.3±1.6 mV，DAD 时程从灌流前192±17延长至280±27 ms（n=8，P<0.01）；同时对乙酰毒毛旋花子苷元诱发的触发活动有抑制，并可被哌唑嗪所阻断。**结论**：在 Pro 有效消除儿茶酚胺致使心律失常发生中，除阻断 β 受体直接作用外，相应加强 α 受体激动对 DAD 与 TA 的抑制亦是其中重要原因之一。

关键词 苯福林；肾上腺素α受体激动剂；乙酰毒毛旋花子苷元；浦肯野纤维；电生理学