BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Mar; 16 (2): 163—168

Effects of N^6-cyclopentyladenosine on afterdepolarizations and triggered activity induced by isoproterenol in guinea pig papillary muscle[1]

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AIM: To investigate the effects of N^6-cyclopentyladenosine (CPA, selective adenosine A_1 receptor agonist) on afterdepolarizations and triggered activity induced by isoproterenol (Iso) in guinea pig papillary muscle. **METHODS:** The stable and reproducible early afterdepolarization (EAD) and delayed afterdepolarizaiton (DAD) of guinea pig papillary muslce were induced by Iso 50 nmol·L^{-1}. The parameters of EAD and DAD were recorded using intracellular microelectrodes. RESULTS: CPA markedly attenuated the development of EAD, DAD, and triggered activity (TA) induced by Iso in guinea pig papillary muscle. The inhibitory effects of CPA on Iso-induced EAD and DAD were antagonized by 8-phenyltheophylline (8-PT) and glibenclamide (Gli). **CONCLUSION:** ATP-sensitive K^+ channels were involved in Iso-induced EAD and DAD, and in the inhibitory effects of CPA on EAD and DAD.

KEY WORDS adenosine; catecholamines; theophylline; glyburide; papillary muscles; electrophysiology

Triggered activity (TA) caused by either early afterdepolarizations (EAD) or delayed afterdepolarizations (DAD) has been emphasized as an important cellular mechanism for the genesis of arrhythmias in human[1] and dog[2]. DAD have been well characterized and attributed to an oscillatory membrane current occurring near the very end of repolarization or after full repolarization[3,4]. EAD is a depolarizing after-potential that occurs during phase 2 or phase 3 of repolarization and has been induced in isolated cardiac tissues under a variety of conditions[5].

TA can be induced in isolated ventricular myocytes exposed to catecholamines[6,7]. Adenosine effectively terminates isoproterenol (Iso)-induced ventricular tachycardias in patients with heart disease[8]. We hypothesized that effects of adenosine on Iso-induced ventricular tachycardias were mediated by the inhibitory effects of adenosine on TA caused by either EAD and DAD. The purpose of this study was to observe the effects of N^6-cyclopentyladenosine (CPA, selective A_1 adenosine receptor agonist) on afterdepolarizations and TA induced by Iso.

MATERIALS AND METHODS

Papillary muscle Guinea pigs of either sex weighing 0.38 ± *s* 0.05 kg were decapitated and the hearts were superfused with cold Tyrode's solution. Isolated papillary muscle of right ventricle was mounted on a perforated silicon rubber block in a tissue bath and perfused at a rate of 8 mL·min^{-1} with Tyrode's solution (NaCl 130, KCl 4.5, NaH_2PO_4 1.8, $MgCl_2$ 0.5, $CaCl_2$ 1.8, $NaHCO_3$ 18, glucose 5.5 mmol·L^{-1}) gassed with 100 % O_2 was maintained at 35±1 ℃.

The preparation was stimulated through a bipolar electrode at a control basic cycle length (BCL) of 500 ms (5 ms rectangular pulse and two times threshold intensity) from the stimulator (SEN-3201). Transmembrane potentials were led to the microelectrode amplifier (MEZ-8201) by a standard intracellular glass

[1] Project supported by the Natural Science Foundation of Hebei Province, № 393125.
Received 1994-01-31 Accepted 1994-08-15

electrode (filled with KCl 3 mol·L^{-1}) with tip resistance of 10—30 MΩ. The amplified signal was fed to the microcomputer and monitored with a storage oscilloscope (VC — 11). Microcomputer collected the transmembrane potential signals and analyzed the parameters of DAD, EAD, and action potentials (AP). Iso was used to induce EAD, DAD, and TA.

Experimental protocol Experiments were divided into four parts: 1) Effects of Iso on the transmembrane potentials: After a stabilization period of 1 h, the preparation was perfused with Tyrode's solution containing Iso (10—50 nmol·L^{-1}) at a BCL of 2000 ms. The variables of AP measured included maximal diastolic potential (MDP), amplitude of AP (APA), duration of 90 % repolarization (APD_{90}), and maximal rate of depolarization (V_{max}). 2) Characteristics of EAD and DAD induced by Iso: In this part of experiment, the preparations were divided into 2 groups at random. a) Effects of Iso on characteristics of EAD and DAD. Under the BCL of 2000 ms, amplitudes of DAD and EAD, and duration of triggered bursts (TBD) were measured when the preparation was perfused with Tyrode's solution containing Iso (10—50 nmol·L^{-1}). b) Effects of BCL on characteristics of EAD. As BCL was changed from 500 to 3000 ms, TBD and the amplitude of EAD induced by Iso (50 nmol·L^{-1}) were observed. 3) Effects of CPA on the afterdepolarization and triggered activity induced by Iso: The preparation was perfused with Tyrode's solution containing CPA (1, 5, and 20 nmol·L^{-1}) for 10 min and then perfused with Tyrode's solution containing CPA (1, 5, and 20 nmol·L^{-1}) and Iso (50 nmol·L^{-1}). This part of experiment was undertaken to evaluate the inhibitory effect of CPA on afterdepolarization and triggered activity induced by Iso. 4) Effects of 8-PT and Gli on the actions of CPA. After 8-PT 0.1 μmol·L^{-1} or Gli 10 μmol·L^{-1} was perfused with Tyrode's solution for 10 min, the protocol of the third part was undertaken again.

Using a program designed by our department, the following parameters of EAD and DAD were defined automatically by an on-line microcomputer analyzing system: 1) amplitude of DAD: the difference between the membrane potential at which DAD begins and the peak of DAD; 2) amplitude of EAD: the difference between the membrane potential at which EAD begin and the peak of EAD[5]; 3) duration of triggered burst (TBD): total duration from the upstroke of AP to the time when complete repolarization is attained[9]; 4) take-off potential of the first triggered burst (TOP): the lowest point in the repolarization phase when the first triggered burst starts[9].

The solvent and resources of CPA, 8-PT, and Gli have previously been described[10]. Iso was diluted in Tyrode's solution.

The changes in parameters of AP expressed as $\bar{x} \pm s$ were analyzed using t test. Differences among groups were compared using F test.

RESULTS

Effects of Iso on transmembrane potential Guinea pig papillary muscle superfused with the Tyrode's solution had a resting membrane potential of −90.2±1.4 mV. Upon stimulation (BCL 2000 ms), transmembrane APs were elicited. The APA was 116±5.4 mV. APD_{50} and APD_{90} were 286±11 and 361±9 ms, respectively.

Under BCL of 2000 ms, Iso induced the decreases of MDP and APA, and the prolon-

Tab 1. Effects of Iso on transmembrane potentials of guinea pig papillary muscle. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Iso/nmol·L^{-1}	MDP/mV	APA/mV	V_{max}/V·s^{-1}	APD_{50}/ms	APD_{90}/ms
0	90.2±1.4	116±5.4	347±19	286±11	361±9
10	89.4±2.1^a	109±4.2^a	345±12^a	310±13^b	382±10^b
20	88.3±1.5^a	105±3.2^b	346±14^a	355±10^c	404±12^c
40	86.2±1.9^b	100±2.1^c	339±10^a	397±15^c	450±15^c
50	81.2±2.3^c	97±5.3^c	335±15^a	414±12^c	476±11^c

gations of APD_{50} and APD_{90} in a concentration-dependent manner. V_{max} showed no change (Tab 1).

Afterdepolarization and triggered activity induced by Iso In the presence of Iso, EAD and DAD were elicited, which sometimes developed into triggered activity (Fig 1, 2).

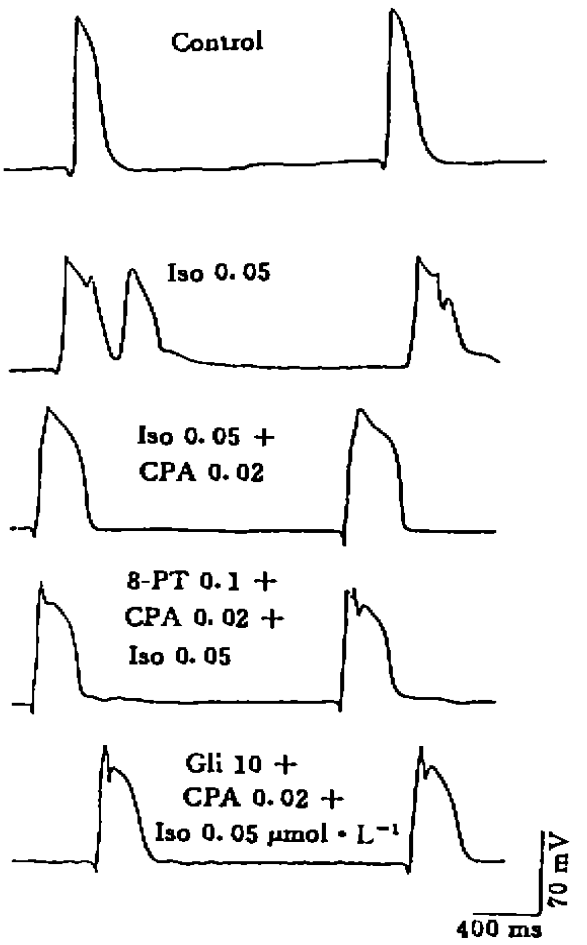

Fig 1. Effects of CPA on EAD and TA induced by Iso and antagonism of CPA effects by 8-PT and Gli (basic cycle length 2000 ms).

Under BCL of 2000 ms, the amplitude of DAD and EAD, and TBD was correlated positively with the concentration of Iso (Fig 3).

The occurrence of such EAD and their parameters were also influenced by the BCL

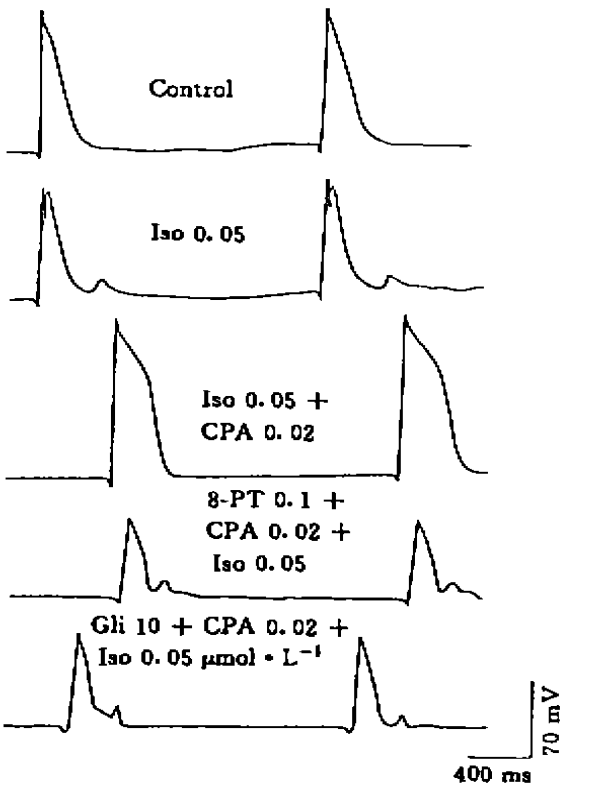

Fig 2. Effects of CPA on DAD induced by Iso and antagonism of CPA effects by 8-PT and Gli (basic cycle length 2000 ms).

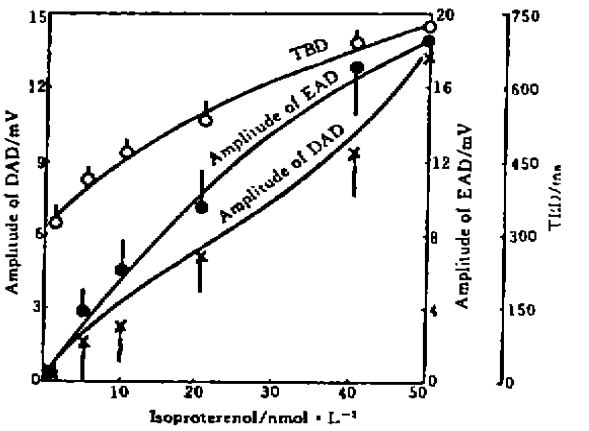

Fig 3. Effect of Iso on amplitude of DAD, amplitude of EAD and TBD at 2 s of basic cycle length. $n=10$.

when Iso 50 nmol·L^{-1} was perfused. At short BCL (<500 ms) EAD did not occur. As BCL

was increased over 500 ms, EAD began to appear and the amplitudes of EAD and TBD were gradually increased (Fig 4). However, the amplitude of DAD was correlated negatively with the BCL.

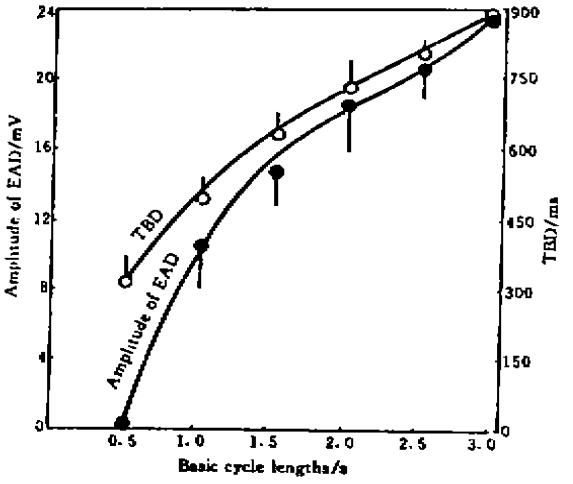

Fig 4. Effect of basic cycle length on amplitude of EAD and TBD induced by Iso 50 nmol·L^{-1}. $n=10$.

Effects of CPA on afterdepolarization and triggered activity induced by Iso At CPA 0.001 μmol·L^{-1}, amplitude of DAD and parameters of EAD were not influenced. When the concentration of CPA was increased, the parameters became decreasing gradually. When the concentration of CPA was increased to 0.02 μmol·L^{-1}, the occurrence of DAD and EAD were completely inhibited (Tab 2 & 3, Fig 1 & 2).

Tab 3. Effects of CPA on DAD induced by Iso and antagonism of CPA effects by 8-PT and Gli (basic cycle length 2000 ms). $n=10$. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* Iso 0.05 μmol·L^{-1}; $^dP<0.01$ *vs* Iso 0.05+CPA 0.02 μmol·L^{-1}.

Drug/ μmol·L^{-1}	Amplitude of DAD/mV
Iso 0.05	13.1±1.8
CPA treatment before Iso	
0.001	11.9±1.0^a
0.005	2.7±0.6^c
0.02	0^c
Iso (0.05)+CPA (0.02)+	
8-PT 0.1	12.6±1.4^{ad}
Gli 10	10.6±1.1^{ad}

Effects of 8-PT and Gli on actions of CPA The inhibitory effects of CPA on Iso-induced DAD and EAD were antagonized by 8-PT and Gli (Tab 2 & 3, Fig 1 & 2).

Tab 2. Effects of N^6-cyclopentyladenosine (CPA), 8-phenyltheophylline (8-PT), and glibenclamide (Gli) on early afterdepolarizations (EAD) and triggered activity (TA) induced by isoproterenol (Iso) in guinea pig papillary muscle. TBD: total duration from upstroke of AP to the time when complete repolarization was attained; TOP: the lowerest point in the repolarization phase when the first EAD starts. $n=10$. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* Iso; $^aP<0.05$, $^dP<0.01$, *vs* Iso (0.05 μmol·L^{-1})+CPA (0.02 μmol·L^{-1}).

Drug/ μmol·L^{-1}	Incidence of EAD/%	Amplitude of EAD/mV	TBD/ms	TOP/mV	Incidence of TA/%
Iso 0.05	90	18.4±2.7	729±34	−20±7	60
CPA treatment before Iso					
0.001	80^a	17.3±4.2^a	693±37^a	−21±8^a	70^a
0.005	30^b	7.7±1.4^b	406±42^c	−43±10^b	0^c
0.02	0^b	0^c	303±29^c	−88±3^c	0^c
Iso (0.05)+CPA (0.02)+					
8-PT 0.1	80^{ad}	17.7±3.4^{ad}	707±39^{ad}	−23±6^{ad}	70^{ad}
Gli 10	50^{bc}	14.2±7.2^{ac}	564±47^{bd}	−30±7^{ad}	30^{bc}

DISCUSSION

In this study, the stable and reproducible EAD and DAD were induced by Iso. The parameters of EAD and DAD were correlated positively with the concentration of Iso. Our results also showed that lengthening of the BCL might enhance TBD and the amplitude of EAD, and reduce the amplitude of DAD.

A selective A_1 adenosine receptor agonist, CPA markedly attenuated the development of EAD, DAD and TA induced by Iso. The inhibitory effects of CPA on Iso-induced EAD and DAD were significantly antagonized by 8-PT and Gli.

Although the mechanisms responsible for generating the afterdepolarization are largely unknown, EAD, DAD, and TA induced by Iso, are thought to be caused by intracellular calcium overload resulting from elevation of intracellular cAMP[11]. Adenosine inhibits cAMP production via a common intracellular pathway, after binding to its extracellular receptors (adenosine-A_1 receptors)[12]. It is thought that coupling of the GTP-dependent regulating protein (Gi) with adenosine-A_1 receptors results in a high-affinity state of the receptor for its agonist[8]. After the agonist binds to the cell receptor, intracellular GTP binds to Gi. This process leads to the dissociation of Gi from the receptor and an inhibition of the catalytic subunit of adenylate cyclase, thereby preventing cAMP formation[13], intracellular calcium overload, afterdepolarization, and triggered activity[8]. CPA may also reduce the intracellular accumulation of cAMP caused by Iso[14]. Thus, the inhibitory effects of CPA on Iso-induced EAD and DAD were mediated by preventing cAMP formation. However, in our study, the inhibitory effects of CPA on Iso-induced EAD and DAD were significantly antagonized by Gli, a drug known to inhibit ATP-sensitive K^+ channels. ATP-sensitive K^+ channels may be coupled to adenosine receptor via GTP-binding proteins[15]. It is implicated that ATP-sensitive K^+ channels were involved in Iso-induced EAD and DAD, and in the inhibitory effects of CPA on EAD and DAD induced by Iso.

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环戊腺苷对异丙肾上腺素诱发的豚鼠乳头状肌后除极和触发活动的影响

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目的：观察选择性 A_1 受体激动剂环戊腺苷(CPA)对异丙肾上腺素(Iso)诱发的豚鼠乳头状肌后除极和触发活动的影响. **方法**：应用 Iso 50 nmol·L^{-1} 诱发稳定而可重复的早发和迟发后除极(EAD 和 DAD)，用玻璃微电极技术记录 EAD 和 DAD 诸参数. **结果**：CPA 能明显地缓解 Iso 诱发的豚鼠乳状肌早发后除极、迟发后除极和触发活动. 8-苯茶碱和格列苯脲能拮抗 CPA 对 Iso 诱发早发和迟发后除极的抑制作用. **结论**：ATP 敏感性钾通道参与了 Iso 诱发的早发和迟发后除极以及 CPA 对 Iso 诱发的两种后除极的抑制作用.

关键词 腺苷；异丙肾上腺素；茶碱；格列苯脲；乳头状肌；电生理学