

## Effects of adenosine and its derivatives on cultured myocardial cells and detection of adenosine receptors in rat

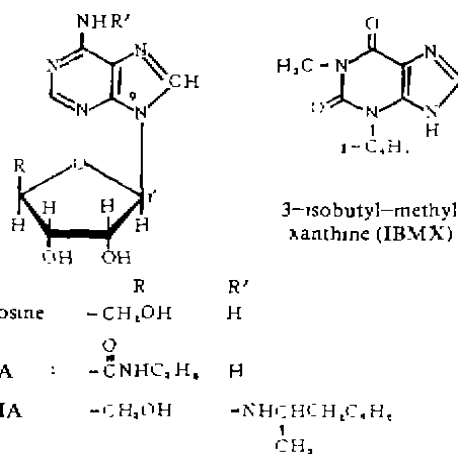
WEI Xiao-Dong<sup>1</sup>, CAI Shang-Da, QIU Yi-Guang, YOU Shi-Xiang (*Department of Biology, Sun Yat-Sen University of Medical Science, Guangzhou 510089, China*)

**ABSTRACT** Adenosine and its derivatives  $1 \text{ nmol} \cdot \text{L}^{-1} - 0.1 \text{ mmol} \cdot \text{L}^{-1}$  elicited negative chronotropic effect on cultured rat myocardial cells. The respective  $\text{IC}_{50}$  were:  $L\text{-PIA } 85 \pm 10 > \text{NECA } 160 \pm 64 > \text{adenosine } 361 \pm 41 \text{ nmol} \cdot \text{L}^{-1}$ . The negative chronotropic effect was antagonized by IBMX. [<sup>3</sup>H]Adenosine bound to myocardial cells of rat in a saturable way with a  $K_d$  of  $238 \text{ nmol} \cdot \text{L}^{-1}$  and a  $B_{\text{max}}$  of  $7.6 \text{ pmol}/10^6 \text{ cells}$ . The binding of [<sup>3</sup>H]adenosine was competitively antagonized by adenosine,  $L\text{-PIA}$ , NECA, and IBMX. These results indicated the existence of adenosine receptor in rat myocardial cells.

**KEY WORDS** adenosine;  $L\text{-N}^6\text{-phenylisopropyladenosine}$ ;  $5'\text{-N-ethylcarboxamidoadenosine}$ ; purinergic receptors; myocardium; radioligand assay; xanthines; cultured cells

Adenosine is a potent coronary vasodilator. In addition, it exerts negative chronotropic, inotropic, and dromotropic effects upon the intact heart of several species<sup>(1)</sup>. Clinically, this drug was used to terminate episodes of paroxysmal supraventricular tachycardia<sup>(2)</sup>, suggesting its potential clinical relevance which is possibly mediated through a direct action on the myocardium.

Guinea pig global heart experiment showed that the action of adenosine on the heart was mediated by A1 receptors<sup>(3)</sup>. In order to look upon the direct action of adenosine on myocardial cells, the present investigation was designed to determine the effects of adenosine on cultured myocardial cells.



### MATERIALS AND METHODS

Sprague-Dawley rats were used.  $L\text{-N}^6\text{-phenylisopropyladenosine}$  ( $L\text{-PIA}$ ),  $5'\text{-N-ethylcarboxamidoadenosine}$  (NECA), and 3-isobutyl-methylxanthine (IBMX) were purchased from Sigma Co and the other reagents were AR grades produced in Shanghai or Guangzhou. [<sup>3</sup>H]Adenosine ( $1110 \text{ TBq} \cdot \text{mol}^{-1}$ ) was produced by Shanghai Institute of Nuclear Research.

**Tissue culture and contractility experiment** The myocardial cells were cultured by using hearts of d 1-3 newborn Sprague-Dawley rats of either sex according to Marsh and Smith<sup>(4)</sup>. At least 80% of cells in the form of monolayer contracted spontaneously on culture d 5, then the cells were used in the contractility experiment. The contractile states of the myocardial cells were delineated at  $36.5 - 37.5^\circ\text{C}$  by a delineating system. Before delineation, the old culture media were displaced by fresh ones which were

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<sup>1</sup> Now in *Department of Biology, Guilin Medical College, Guilin 541001, China*

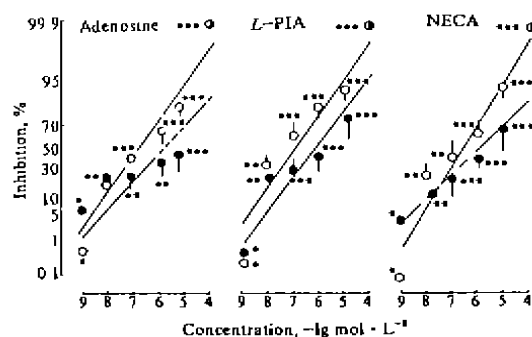
without fetal calf serum. The contractile state of the cells in the blank control group was delineated, 2 h and thereafter, while that of the cells in the water–contrast group was delineated at 1 min after the addition of 15  $\mu$ l distilled water into the culture. The delineation of the experimental group was carried out at 1 min and 10 min after the addition of 15  $\mu$ l drug solutions.

**Radioligand assay** This test included 3 kinds of assay: saturation binding assay, competitive binding assay, and binding kinetics assay. The suspensions used in all the 3 assays were prepared from the cultures of d 5<sup>(4)</sup>. At the end of incubation 5 ml of buffer (Tris-HCl 50 mmol  $\cdot$  L<sup>-1</sup>, pH 7.4, 4°C) were added into each tube in ice water. The mixtures were rapidly filtered through microfibre filters (49 type). The filters were washed twice with 5 ml wash buffer each and then dried at 70°C and counted in a LKB-1215 counter (counting efficiency 50%). Nonspecific bindings in these assays were determined in the mixtures containing adenosine 1 mmol  $\cdot$  L<sup>-1</sup>.

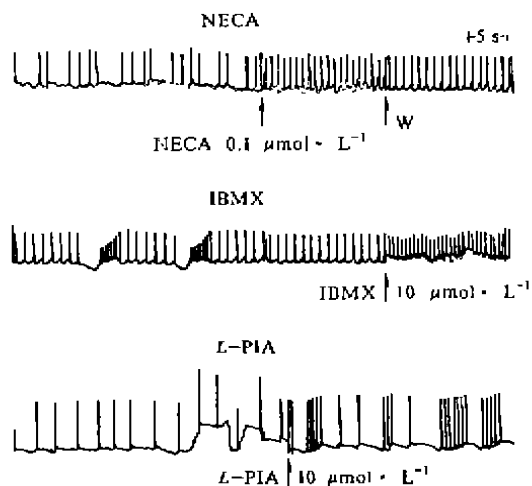
## RESULTS

**Contractility experiment** At concentrations greater than 10 nmol  $\cdot$  L<sup>-1</sup>, adenosine, *L*-PIA, and NECA elicited remarkable negative chronotropic effects on rat myocardial cells (Fig 1). The IC<sub>50</sub> were in the order of: *L*-PIA 85  $\pm$  10 > NECA 160  $\pm$  64 > adenosine 361  $\pm$  41 nmol  $\cdot$  L<sup>-1</sup>. The negative chronotropic effects of adenosine, *L*-PIA, and NECA were antagonized by IBMX. Thus in the presence of IBMX (10  $\mu$ mol  $\cdot$  L<sup>-1</sup>), the IC<sub>50</sub> of *L*-PIA, NECA, and adenosine were increased to 490  $\pm$  26 nmol  $\cdot$  L<sup>-1</sup>, 1.40  $\pm$  0.20  $\mu$ mol  $\cdot$  L<sup>-1</sup>, 8.0  $\pm$  1.6  $\mu$ mol  $\cdot$  L<sup>-1</sup> respectively. By contrast, the maximal chronotropic effects of *L*-PIA, NECA, and adenosine were not affected by IBMX. In addition, the 3 drugs at 0.1 mmol  $\cdot$  L<sup>-1</sup> stopped the contractibility of the cells com-

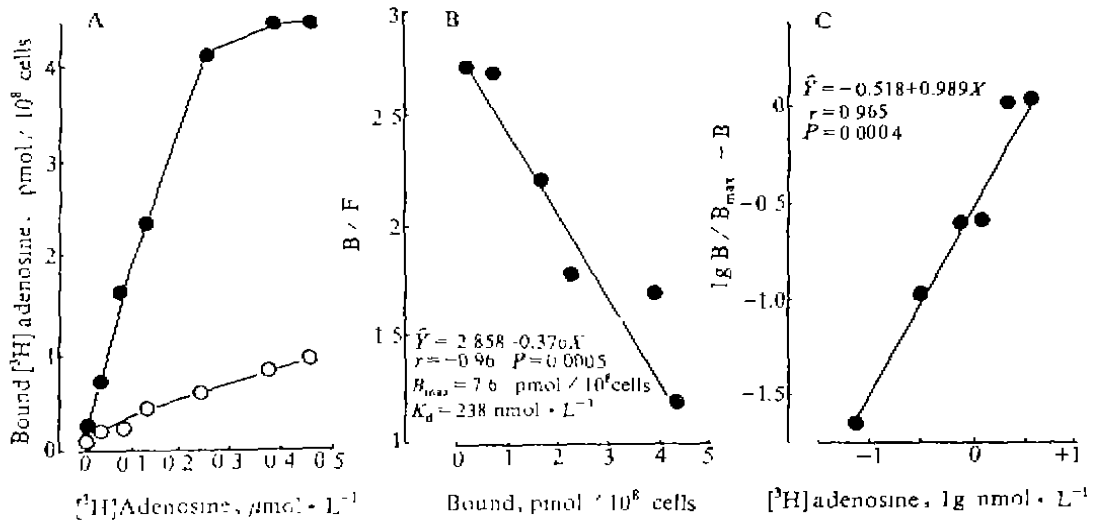
pletely, a negative inotropism which was not changed by IBMX either. NECA (0.1  $\mu$ mol  $\cdot$  L<sup>-1</sup>) and IBMX (10  $\mu$ mol  $\cdot$  L<sup>-1</sup>) caused occasional arrhythmia in myocardial cells; *L*-PIA 10  $\mu$ mol  $\cdot$  L<sup>-1</sup> turned the myocardial arrhythmia into a regular rhythm, but adenosine elicited no effect on the rhythm (Fig 2).



**Fig 1.** Negative chronotropic effects of adenosine, *L*-PIA, and NECA without (○) and with (●) IBMX on cultured myocardial cells of rat ( $n=6$ ). The inhibition % was obtained by:  $[1-(\text{bpm after medication} / \text{bpm before medication})] \times 100$ . \* $P > 0.05$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .



**Fig 2.** Effects of NECA, IBMX, and *L*-PIA on rhythmicity of primary cultured myocardial cells of rat (W: water).



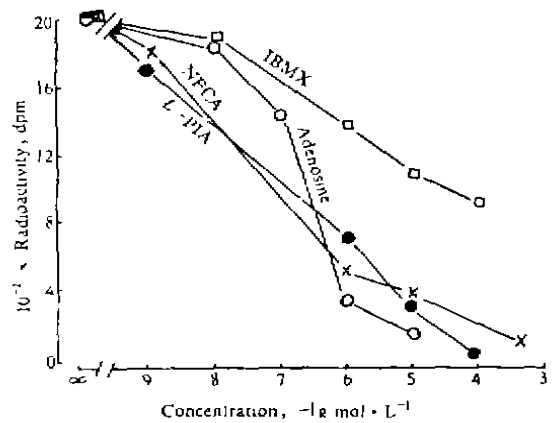
**Fig 3.** A) Saturation binding of [<sup>3</sup>H]adenosine to intact myocardial cells of rat. 10<sup>8</sup> cells were incubated with [<sup>3</sup>H]adenosine in 0.3 ml of assay mixture for 15 min at 37°C, (n=2). (○): nonspecific binding, (●): specific binding. B) Scatchard plot of the same data. C) Hill plot of the same data.

**Radioligand assays** The saturation assay demonstrated a saturable binding model of [<sup>3</sup>H]adenosine. The Scatchard analysis gave a linear plot ( $r=0.962$ ) and Hill transformation of the same data gave a  $n_H$  of 0.99, which indicated a homogeneous population of binding sites. The other parameters from this assay were given in Fig 3.

Data from competition assay indicated that L-PIA, NECA, adenosine, and IBMX competed for the binding site with [<sup>3</sup>H]adenosine. The potencies of competitive effects of the 3 drugs followed the same order as in the case of their negative chronotropic effects. Their  $K_i$  values calculated by logit transformation and Chen-Prusoff methods were L-PIA 62 nmol · L<sup>-1</sup> > NECA 141 nmol · L<sup>-1</sup> > adenosine 219 nmol · L<sup>-1</sup> > IBMX 20 μmol · L<sup>-1</sup> (Fig 4).

The 3 drugs showed reversible binding capacity from the binding kinetics experiments. The association of [<sup>3</sup>H]adenosine

reached equilibrium within 15 min and the dissociation completed in another 6 min. The  $K_d$  and  $T_{1/2}$  calculated by Tian and LO's method<sup>(5)</sup> were 125.5 nmol · L<sup>-1</sup> and 2.9 min respectively.



**Fig 4.** Competition for [<sup>3</sup>H]adenosine (25.24 nmol · L<sup>-1</sup>) binding to intact myocardial cells, for 15 min at 37°C, (n=3).

DISCUSSION

The results of this study have confirmed the previous findings that adenosine and its derivatives exert a negative chronotropic effect on the heart<sup>(1)</sup>. This action of adenosine analogues is a direct one in cultured myocardial cells *in vitro*.

From the radioligand binding studies, the binding of adenosine to myocardial cell was shown to be saturable, structure-specific, and of high affinity, suggesting the existence of adenosine receptors in rat myocardial cells. In conformance with the hypothesis suggested by Collis<sup>(3)</sup> and in view of the same order of potency of effects and the antagonism produced by an adenosine receptor blocker, the authors conclude that these physiologic effects are mediated by an adenosine receptor, the A<sub>1</sub> receptor.

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腺苷及其衍生物对培养的大鼠心肌细胞的影响及腺苷受体测定

韦小东<sup>1</sup>、蔡尚达、邱逸光、游仕湘  
(中山医科大学生物教研室, 广州 510089, 中国)

摘要 腺苷类物质对培养的鼠心肌细胞有直接负性心率作用, 此作用可被腺苷受体拮抗剂 IBMX 所拮抗. 放射配基-受体结合实验表明 [<sup>3</sup>H]腺苷与心肌细胞的结合具饱和性、高亲和性、结构特异性, 并可被腺苷衍生物 L-<sup>6</sup>N-苯异丙基腺苷、5'-乙基酰胺腺苷等竞争性拮抗, 这表明心肌腺苷作用可能是通过心肌细胞上的腺苷受体介导的.

关键词 腺苷; L-苯异丙基腺苷; 乙基酰胺腺苷; 嘌呤能受体; 心肌; 放射配位体测定; 黄嘌呤类; 培养细胞

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Please contact Prof SHIBUYA Takeshi,  
Department of Pharmacology,  
Tokyo Medical College,  
Tokyo 160, Japan.

Telephone: 813-3351-6141. Fax: 813-3352-0316.