EVIDENCE THAT THE P_1-PURINOCEPTOR IN THE MOUSE ISOLATED VAS DEFERENS IS AN A_1-SUBTYPE

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Abstract—The effects of adenosine,5'-N-ethylcarboxamidoadenosine (NECA), 2-chloroadenosine (2-CA), N^6-phenylisopropyladenosine (L-PIA and D-PIA) and N^6-cyclohexyladenosine (CHA) were examined on the mouse isolated vas deferens. All the compounds in a concentration-dependent manner inhibited electrically induced contractions. IC_{50} (μM) of adenosine and its analogues were 13.68 ± 5.97 for Ado, 0.736 ± 0.087 for 2-CA, 0.034 ± 0.009 for CHA, 0.056 ± 0.008 for L-PIA, 0.099 ± 0.028 for NECA, and 1.444 ± 0.183 for D-PIA. The P_1-purinoceptor antagonist, 8-PT (10μM), caused a rightward shift of all the adenosine and its analogues concentration-response curve. Dipyridamole, an adenosine uptake inhibitor (0.5μM) potentiated the relaxation to adenosine thus causing a leftward shift of adenosine concentration-response curve. Dipyridamole had no effect on the relaxation induced by the analogues. The order of the potency for the adenosine and its analogues on the mouse isolated vas deferens was: CHA> L-PIA> NECA> 2-CA> D-PIA> Ado. This study proposes that adenosine and its analogues mediate their inhibitory effects on the mouse isolated vas deferens via A_1 adenosine receptors. Acta Medica Iranica 33 (3&4): 64-68; 1995

Key words: adenosine; purinoceptor; mouse vas deferens

INTRODUCTION

The purine nucleoside adenosine has a well-characterized role as a neuromodulator in the peripheral and central nervous system, and has been shown to inhibit the release of many neurotransmitters (1). Adenosine reduces the release of [^{3}H] noradrenaline from several sympathetically innervated tissues, that is, the heart, ventricle, salivary gland, and vas deferens of the rat (2); the adipose tissue of the canine inhibits nerve-mediated contractions of the rabbit vas deferens and rat isolated uterus (3,4,5,6).

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The effects of adenosine are mediated by specific receptors called P_1-purinoceptors. Two major classes of these receptors (A_1 and A_2) can be clearly distinguished in pharmacological and binding studies by agonist potency orders and selective antagonists. For A_1-receptors, N6 -substituted analogues of adenosine such as L-N6-phenylisopropyladenosine (L-PIA) and N6- cyclohexyladenosine (CHA) are more potent than 5'-substituted analogues such as 5'-N-ethylcarboxamidoadenosine (NECA). For A_2 - receptors, NECA is more potent than CHA and L -PIA (7).

In general, the order of potency at A_1 -receptors is CHA> L-PIA> NECA and at A_2-receptors, NECA> L-PIA> CHA. In addition, at the A_1-site, a high degree of stereoselectivity for L-PIA over its isomer (D-PIA) is displayed (7).

In the present study, the P_1-purinoceptors which mediate the inhibition by adenosine of nerve-mediated contractions of the mouse vas deferens have been investigated by use of the agonists and antagonists CHA, L-PIA, 2-chloroadenosine (2-CA) and NECA, and the antagonist, 8-Phenyltheophylline (8-PT).

MATERIALS AND METHODS

Preparation

Adult male albino mice (30-40g) were sacrificed by cervical dislocation. Vasa deferentia were dissected, freed from connective tissue and transferred to water-jacketed organ chambers containing 20 ml of a Krebs, solution (mM): NaCl, 118. 10; KCl, 4.75; $CaCl_2$, 2.54; KH_2PO_4, 1.2; $NaHCO_3$, 25.00 and glucose, 11.10 (8) which was maintained at 37° C and gassed with 95% O_2 and 5% CO_2.

Vasa deferentia were inserted into platinum ring electrodes and were attached to an auxotonic strain gauge transducer under a resting tension of 0.5g (8). At an equilibration period of 60 min, tissues were washed at 10 min intervals and the, were subjected to electrical field

stimulation (0.1 Hz, 3 ms duration, 100 V) (8), with a Palmer Bioscience stimulator 200. Contractile responses to electrical stimulation were recorded on a physiograph (Type DMP-4B) pen recorder.

Effects of adenosine and its analogues on neurotransmission

Adenosine and its analogues were administered cumulatively to the preparations every 10 min. Adenosine at concentrations of 0.03-200 μM, NECA at the concentrations of 0.001-3μM, 2-CA at concentrations of 0.01-30μM, CHA at concentrations of 0.001-0.1μM, L-PIA at concentrations of 0.001 1μM and D-PIA at the concentrations of 0.01-100μM were administered to determine their effects on the contractile response to electrical stimulation. Concentration-response curves to adenosine and its analogues with or without 8-PT (10μM) and dipyridamole (0.5μM) were obtained from each preparation. A half-hour equilibration period was allowed for 8-PT or dipyridamole (9). Stock solutions of drugs were made up as follows: Adenosine, NECA, CHA, L-PIA, D-PIA, and 2-CA and were dissolved in small amount of 0.2N HCl and were diluted further with deionized distilled water to the desired concentration. The pH range were from 3.5 to 9.5 (10). 8-PT was dissolved in small amount of ethylenediamine and was diluted further with deionized distilled water to desired concentration. Dipyridamole was dissolved in 96% ethanol. Solvents had no effect on the tissue response.

Drugs

Drugs used in this study were adenosine, 2-chloroadenosine, cyclohexyladenosine, 5'-N-ethylcarboxamidoadenosine, (-)-N6-phenylisopropyladenosine, (+)-N6-phenylisopropyladenosine, 8-phenyltheophylline and dipyridamole (all purchased from Sigma Chemical Co.).

Statistical analysis

All values were expressed as mean ± standard error. Curves were compared by the analysis of variance for randomized blocks and potentiates. Individual values were compared by the Student's unpaired t-test. A p-value of 0.05 or less was considered statistically significant. All calculations were done by computer, using SPSS software.

RESULTS

Potency series

CHA, L-PIA, NECA, 2-CA, D-PIA, and adenosine all caused concentration-dependent inhibition of contractile response to electrical stimulation. CHA (selective for A_1 receptors, $IC_{50} = 0.034 \pm 0.009\mu M$) was 2.9-fold more potent than NECA (selective for A_2 receptors, $IC_{50}=0.099\pm0.028\mu M$) in inhibition of neurogenic contractions of the mouse vas deference ($P < 0.02$). Adenosine was the least potent with an IC_{50} of $13.68 \pm 5.97\ \mu M$. The rank order of potency based on the IC_{50} values was: CHA > L-PIA > NECA > 2-CA > D-PIA > Adenosine (Table 1).

Effect of 8-phenyltheophylline

8-PT (10 μM), a potent adenosine receptor antagonist, shifted each concentration-response curve to the right. 8-PT significantly antagonized adenosine and its analogues effects (Figs. 1-6 and Table 1).

Effects of dipyridamole

Dipyridamole (0.5 μM), an adenosine uptake inhibitor, significantly potentiated the relaxations induced by adenosine, thus causing a leftward shift in the concentration-response curve (Fig. 1). Dipyridamole (0.5 μM) had not significant effect on concentration-response curves for the other analogues (Figs. 2-6).

DISCUSSION

The results presented here show that adenosine-induced relaxation of the mouse isolated vas deferens is mediated via a receptor which resembles the A_1 subtype. Compounds which are substituted at the N^6 amino position on the purine ring (CHA and L-PIA) were more potent than compounds which are substituted at the C^5 position of the ribose ring (NECA) at inhibition of contractile response of mouse isolated vas deferens to electrical stimulation. This agonist profile is almost identical with those of Blakeley et al (11). In addition, the present study revealed that the adenosine receptors in the mouse vas deferens show marked stereoselectivity for PIA. This stereoselectivity has been claimed to indicate the presence of A_1 -receptors, since L-PIA which acts on an A_1 -receptor, is significantly more potent than D-PIA (12,13). Also, the 5'-substituted adenosine analogue, NECA, has been extensively used to define tissue responses mediated by A_2-receptor activation. This analogue is however nonselective in its interaction with adenosine receptor, and is approximately equipotent ([Ki] ≈ 10 nM) at both A_1 and A_2 receptors. Ascribing effects elicited by NECA to A_2- receptor-mediated processes can only be validated if such effects are not seen with equivalent doses / concentrations of A_1 selective ligands such as CHA. Therefore, because such an effect has been seen by CHA and L-PIA, it is further

Table 1. IC_{50} s for adenosine and analogues on the vas deferens of the mouse in the presence of 8-phenyltheophylline (8-PT) (10μM) or dipyridamole (Dipy) (0.5μM) (Values in mean ± s.e.).

Control	NECA	L-PIA	D-PIA	CHA	2-CA	Ado
IC_{50} (μM)	0.099±0.028 (5)	0.056±0.008 (5)	1.444±0.183 (6)	0.034±0.009 (5)	0.736±0.087 (6)	13.68±5.97 (6)
pD2	7.004	7.252	5.84	7.47	6.133	4.864
Relative activity	138.182	244.29	9.47	402.35	18.59	1
In the presence of 8-PT (10μM)						
IC_{50} (μM)	4.47±1.99 (5)	1.4±0.314 (5)	16.6±11.5 (5)	1.08±0.458 (5)	14.6±5.56 (5)	21.3±3.12 (6)
pD_2	5.35	5.85	4.78	5.97	4.84	4.67
Relative antagonism	45.15	25	11.5	31.76	19.84	1.56
In the presence of Dipy (0.5μM)						
IC50(μM)	0.1±0.022 (4)	0.046±0.014 (4)	1.803±0.462 (6)	1.21±0.452 (6)	2.6±0.452 (6)	2.6±0.731 (6)
pD_2	7	7.34	5.744	5.92	5.92	5.58
Relative potentiation	0.99	1.22	0.8	0.61	0.61	5.26

Ado=Adenosine
L-PIA=L-N6-phenylisopropyladenosine
D-PIA=D-N6-phenylisopropyladenosine
NECA=5'-N Ethylcarboxamidoadenosine
CHA=N6-Cyclohexyladenosine
8-PT=8-phenyltheophylline
2-CA=2-Chloroadenosine
pD_2=negative log of IC_{50}
Relative activity=ratio of analogue IC_{50} in absence and presence of dipyridamole

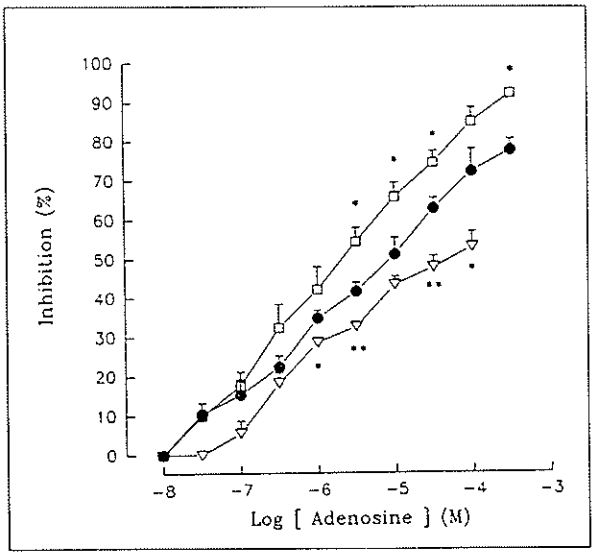

Fig. 1. Effect of adenosine on the electrically-induced contraction of mouse isolated vas deferens in the absence (●) and presence of 8-PT (▽, 10μM) or dipyridamole (□, 0.5μM) (n=6). 8-PT (10μM) significantly antagonized adenosine effects (*p < 0.05; ** p < 0.01). Dipyridamole (0.5μM) significantly potentiated adenosine effects (*p < 0.05).

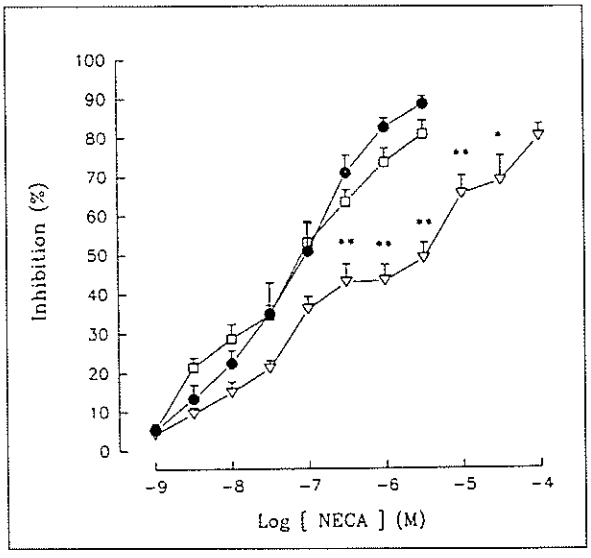

Fig. 2. Effect of NECA on the electrically-induced contraction of mouse vas deferens in the absence (●) and presence of 8-pt (▽, 10 μM) or dipyridamole (□, 0.5 μM) (n=5 for control and 8-PT; n=6 for dipyridamole). 8-PT (10 μM) significantly antagonized NECA effects (**p < 0.01). Dipyridamole (0.5 μM) did not significantly potentiate NECA effects.

evidence that A_1- subtype is more probable than A_2-subtype. The adenosine-induced relaxation of the guinea-pig taenia coli is mediated via a receptor which closely resembles the A_2-receptor as defined in other systems (9).

The inhibitory effects of adenosine and its analogues on the rabbit vas deferens are mediated via both A_1 and A_2 adenosine receptors (5). The rat vas deferens contain both prejunctional A_1-receptors and postjunctional A_2-receptors (7).

Methylxanthines have been shown to antagonize competitively both A_1 and A_2 adenosine receptors (13,14). Such findings agree with the ability of 8-

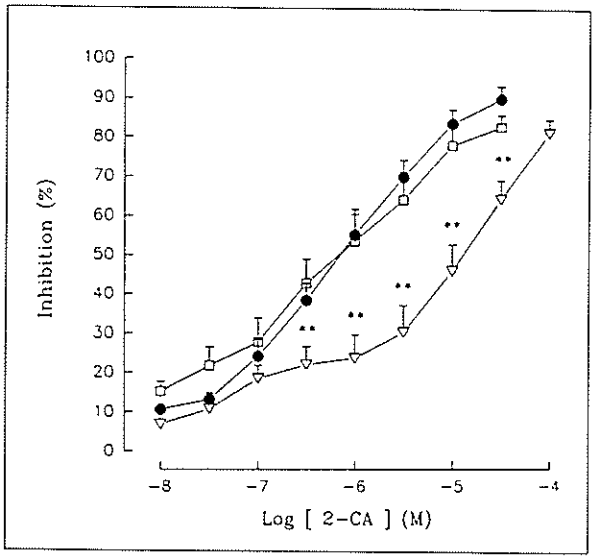

Fig. 3. Effect of 2-CA on the electrically-induced contraction of mouse isolated vas deferens in the absence (●) or presence of 8-PT (▽, 10 μM) and dipyridamole (□, 0.5 μM) (n=6 for control and dipyridamole, n=5 for 8-PT). 8-PT (10 μM) significantly antagonized 2-CA effects (**p < 0.01). Dipyridamole (0.5 μM) did not significantly potentiate 2-CA effects.

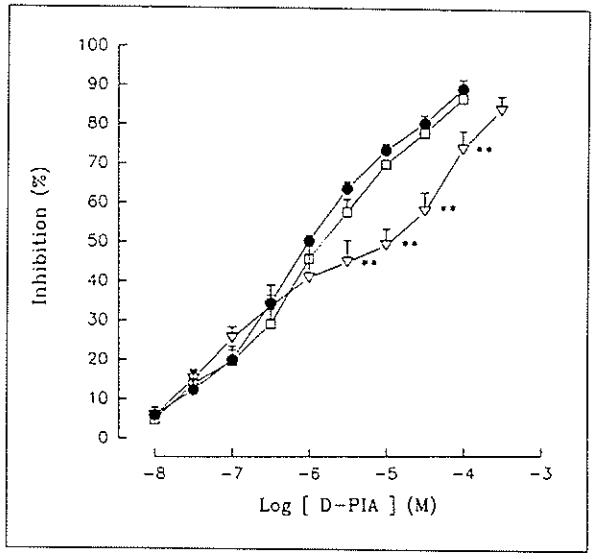

Fig. 5. Effect of D-PIA on the electrically-induced contraction of mouse isolated vas deferens in the absence (●) and presence of 8-PT (▽, 10 μM) or dipyridamole (□, 0.5 μM) (n=5 for control and 8-PT, n=6 for dipyridamole). 8-PT (10 μM) significantly antagonized D-PIA effects (**p < 0.01). Dipyridamole (0.5 μM) did not significantly potentiate D-PIA effects.

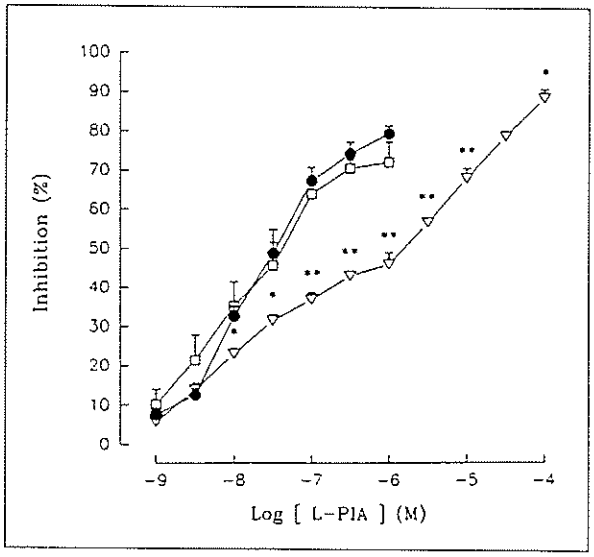

Fig. 4. Effect of L-PIA on the electrically-induced contraction of mouse isolated vas deferens in the absence (●) and presence of 8-PT (▽, 10 μM) or dipyridamole (□, 0.5 μM) (n=5 for control and 8-PT, n=6 for dipyridamole). 8-PT (10 μM) significantly antagonized L-PIA effects (*p<0.05;**p <0.01). Dipyridamole (0.5 μM) did not significantly potentiate L-PIA effects.

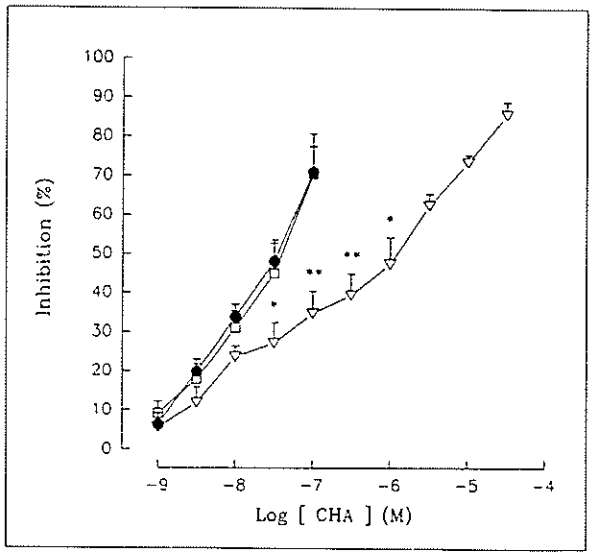

Fig. 6. Effect of CHA on the electrically-induced contraction of mouse isolated vas deferens in the absence (●) and presence of 8-PT (▽, 10 μM) or dipyridamole (□, 0.5 μM) (n=5 per group). 8-PT (10 μM) significantly antagonized CHA effects (*p < 0.05; **p < 0.01). Dipyridamole (0.5 μM) did not significantly potentiate CHA effects.

phenyltheophylline, a potent P_1 - purinoceptor antagonist (15), to antagonize the responses to adenosine and to all of its analogues, in the mouse vas deferens.

Dipyridamole enhances the adenosine-induced

relaxation of the guinea-pig taenia coli by preventing adenosine uptake (16). However in this work dipyridamole did not significantly alter the responses to adenosine analogues. This is not surprising, because adenosine analogues do not share the adenosine uptake mechanism sensitive to dipyridamole (17).

Our results showed that the P_1-purinoceptor in the mouse vas deferens is of the A_1-subtype. Such proposal was made on the rank order of agonist potency of adenosine analogues inducing inhibition and stereo-selectivity displayed for PIA. However the interpretation of effects of adenosine analogues on electrically evoked contractions is difficult, since adenosine analogues can act at prejunctional receptors as well as on postjunctional receptors (7). Furthermore, involvement of newer P_1 - purinoceptor subtypes, such as those recently elucidated by other investigators (6) may render the correct decision making difficult.

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