Presynaptic inhibition of neurotransmission in rat vas deferens by 2-(*p*-methoxyphenyl) adenosine, ethyl adenosine-5′-carboxylate and *N*-cyclopropyl adenosine-5′-carboxamide

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Adenosine inhibits adrenergic transmission presynaptically in a number of tissues (Paton 1979) including the rat vas deferens (Clanachan et al 1977). The structural requirements for presynaptic inhibitory activity in the rat vas deferens include a primary or secondary amine function at C_6 of the purine ring with little tolerance for major steric changes or substitutions on the ribose moiety (Paton et al 1978).

In this study we have examined the effects of a C_2 substituted analogue, i.e. 2-(*p*-methoxyphenyl) adenosine, and of the 5′-substituted adenosine analogues, ethyl adenosine-5′-carboxylate and *N*-cyclopropyl adenosine-5′-carboxamide on neurotransmission in the rat vas deferens. All three compounds were potent coronary vasodilators in animal studies (Hirata et al 1977; Stein & Prasad 1979).

Isometric recordings of longitudinal contractions of the mid-portion of the rat vas deferens were made at 37 °C as described previously (Clanachan et al 1977; Paton et al 1978; Muller & Paton 1979). Electric field stimulation of the muscle was continuously achieved by passing biphasic pulses of 0·1 Hz, 1 ms duration and supramaximal voltage between platinum electrodes. Adenosine analogues were added for 30 s before being washed out. The responses obtained after 30 s exposure to the analogues were expressed as a percentage of the preceding control responses. In other experiments responses were obtained every 5 min to 10^{-5} M (−)-noradrenaline, the adenosine analogues being added 60 s earlier. All experiments were performed on a paired basis comparing responses of the two vasa deferentia of the same animal. Results are expressed as

Table 1. Effect of theophylline (10^{-4} M) on inhibitory actions of adenosine analogues on neurotransmission in the rat vas deferens. Isolated rat vasa deferentia were continuously transmurally stimulated at 0·1 Hz. Adenosine and analogues were added in the concentrations indicated, during transmural stimulation and the inhibition of responses produced was determined. Group A (Control group) are tissues exposed to adenosine or analogues only. Group B are tissues that were continuously exposed to 10^{-4} M theophylline. *, $P < 0.05$, compared to responses of Group A. Mean ± s.e. mean of 6 observations.

Group	Adenosine analogue (M)	Inhibition of neurotransmission (%) by Adenosine	2-(*p*-Methoxyphenyl) adenosine	Ethyl adenosine-5-′carboxylate	*N*-Cyclopropyl adenosine-5′-carboxamide
A	1.25×10^{-8}	—	—	—	11·2 ± 3·6
B					0*
A	3.75×10^{-8}	—	—	—	34·5 ± 4·9
B					1·3 ± 1·3*
A	1.25×10^{-7}	—	—	—	56·8 ± 5·9
B					15·1 ± 5·7*
A	3.75×10^{-7}	4·3 ± 1·6	2·3 ± 1·1	2·1 ± 1·1	83·3 ± 6·4
B		0 *	0 *	0 *	31·8 ± 10·5
A	1.25×10^{-6}	19·4 ± 3·6	11·2 ± 3·3	5·7 ± 3·3	94·6 ± 3·2
B		0 *	1·3 ± 1·3*	1·3 ± 1·3*	50·3 ± 13·3*
A	3.75×10^{-6}	28·9 ± 3·3	20·1 ± 4·3	21·2 ± 2·7	99·8 ± 0·2
B		0 *	1·7 ± 1·8*	12·2 ± 1·1*	67·3 ± 10·5*
A	1.25×10^{-5}	33·3 ± 3·3	32·2 ± 5·4	37·2 ± 3·6	100
B		2·2 ± 2·2*	3·2 ± 2·6*	13·1 ± 5·0*	81·4 ± 7·7*
A	3.75×10^{-5}	43·4 ± 3·4	42·1 ± 4·9	54·0 ± 2·3	100
B		4·3 ± 2·6*	9·6 ± 3·4*	15·3 ± 5·4	91·3 ± 3·5*
A	1.25×10^{-4}	54·3 ± 4·1	57·4 ± 4·0	71·7 ± 4·5	100
B		8·7 ± 3·2*	24·0 ± 3·3*	32·6 ± 6·3*	97·8 ± 1·6

Table 2. Effect of dipyridamole (10^{-5} M) on inhibitory action of adenosine analogues on neurotransmission in the rat vas deferens. Details as in Table 1 except Group B are tissues which were exposed to 10^{-5} M dipyridamole for 30 min before the addition of adenosine or analogues. Mean ± s.e. mean of 7 observations.

Group	Adenosine analogue (M)	Inhibition of neurotransmission (%) by Adenosine	2-(*p*-Methoxyphenyl) adenosine	Ethyl adenosine-5'-carboxylate	*N*-Cyclopropyl adenosine-5'-carboxamide
A	3.75×10^{-9}	—	—	—	4·8 ± 1·9
B					4·9 ± 1·7
A	1.25×10^{-8}	—	—	—	13·2 ± 2·4
B					13·5 ± 4·3
A	3.75×10^{-8}	—	—	—	37·5 ± 7·6
B					38·9 ± 5·9
A	1.25×10^{-7}	—	4·1 ± 1·3	9·9 ± 1·4	77·3 ± 5·4
B			5·5 ± 1·2	10·6 ± 1·9	75·6 ± 3·6
A	3.75×10^{-7}	12·0 ± 3·1	24·7 ± 4·1	26·6 ± 5·2	100
B		22·2 ± 3·4*	24·9 ± 2·8	29·3 ± 3·2	83·4 ± 14·0
A	1.25×10^{-6}	25·0 ± 6·7	54·3 ± 4·1	64·3 ± 4·7	—
B		34·1 ± 4·9*	50·0 ± 3·6	63·0 ± 2·3	
A	3.75×10^{-6}	33·1 ± 7·4	79·0 ± 4·6	84·8 ± 3·2	—
B		48·5 ± 4·8*	75·8 ± 4·2	83·9 ± 2·9	
A	1.25×10^{-5}	44·2 ± 6·0	100	99·3 ± 0·7	—
B		61·9 ± 6·2*	100	100	

the mean inhibition of responses (%) ± standard error of the mean (s.e. mean). Significance levels were estimated using Student's paired *t*-test and the difference between groups was regarded as significant when $P < 0.05$.

Drugs and their sources were: *N*-cyclopropyl adenosine-5'-carboxamide*, ethyl adenosine-5'-carboxylate hydrochloride* (Abbott Laboratories); dipyridamole* (Boehringer Ingelheim (NZ) Ltd.); adenosine, theophylline (Sigma Chemical Co.); 2-(*p*-methoxyphenyl)-adenosine* (Takeda Chemical Industries Ltd.). Compounds marked with an asterisk were generously donated by the Companies indicated. Tissues were pretreated with theophylline or dipyridamole for 30 min before the effects of the adenosine analogues were studied. Dipyridamole was then washed out while theophylline was present throughout.

Responses of the rat vas deferens to transmural stimulation at 0·1 Hz were significantly inhibited by adenosine, 2-(*p*-methoxyphenyl)adenosine, *N*-cyclopropyl adenosine-5'-carboxamide and ethyl adenosine-5'-carboxylate, the most potent being *N*-cyclopropyl adenosine-5'-carboxamide followed by ethyl adenosine-5'-carboxylate (Tables 1 and 2). The inhibitory actions of all these compounds were significantly antagonized by 10^{-4} M theophylline (Table 1). The inhibitory action of adenosine was potentiated by 10^{-5} M dipyridamole, but dipyridamole failed to potentiate the actions of the other compounds (Table 2).

At 10^{-4} M, these compounds produces minimal (1·6–4·4%) inhibition of the response to 10^{-5} M (−)-noradrenaline, showing that they all inhibited responses to transmural stimulation by a predominantly presynaptic action.

Previous studies demonstrated that adenosine analogues with halogen substitutions at C_2 of the purine ring were potent presynaptic inhibitors or neurotransmission in the rat vas deferens and were not potentiated by dipyridamole (Muller & Paton 1979) while 5'-deoxyadenosine was also a presynaptic inhibitor in this tissue (Paton et al 1978). The present studies confirm that substitutions at the C_2 position on the purine ring and at the 5' position on the ribose moiety are compatible with presynaptic activity in the rat vas deferens. Similar structure–activity relationships have been reported for the depressant action of adenosine in rat cerebral cortical neurons (Phillis & Edstrom 1976). *N*-Cyclopropyl adenosine-5'-carboxamide is the most potent adenosine analogue in the rat vas deferens we have studied to date, being at least 100 times more potent than adenosine.

The finding that dipyridamole did not potentiate the inhibitory effects of the adenosine analogues suggests that they are not substrates for the adenosine uptake

system. Dipyridamole is a relatively potent inhibitor of adenosine uptake (Huang & Daly 1974).

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In vitro hepatic oxidative metabolism of antipyrine, phenytoin and phenylbutazone in uraemic rabbits

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Earlier (Van Peer et al 1978) we calculated from serum concentrations that in uraemic rabbits the intrinsic clearance of free drug, i.e. the inherent metabolizing ability of the liver (Rowland et al 1973; Wilkinson & Shand 1975), is not altered for antipyrine (phenazone), increased for phenytoin and decreased for phenylbutazone. Conflicting results on the biotransformation rate of drugs in renal failure have been reported from in vivo and in vitro experiments (Reidenberg 1977). We decided therefore to study in vitro the hepatic oxidative metabolism of antipyrine, phenytoin and phenylbutazone in acute uraemic rabbits. We also measured amidopyrine *N*-demethylase and aniline hydroloxylase, commonly used markers of the hepatic oxidative metabolism.

Female albino rabbits were used; five of the animals received 0·9% NaCl (saline) intravenously and five of the animals the nefrotoxic agent uranyl nitrate (2 mg kg^{-1}) (Van Peer et al 1978). Five days after the injection, when serum urea was markedly (700%) increased in the uranyl nitrate-treated rabbits, control and treated rabbits were killed after an overnight fast. The livers (which showed only slight cytolysis in the treated animals) were homogenized in 4 volumes of 1·15% KCl–0·01 M sodium phosphate buffer pH 7·4, and 9000 *g* fractio s and microsomes were prepared.

Microsomal protein (Lowry et al 1951), cytochrome P-450 (Schoene et al 1972), amidopyrine *N*-demethylase (Mazel 1971) and aniline hydroxylase (Mazel 1971) were measured as described in the literature.

For antipyrine hydroxylase, 2 ml incubation mixture contained antipyrine 20, glucose-6-phosphate 16, NADP 2, $MgCl_2$ 12 μmol, 0·1 M sodium phosphate buffer pH 7·4, 0·8 ml; 9000 *g* fraction or microsomal fraction 1 ml, and glucose-6-phosphate dehydrogenase 1.4 I.U. After incubation at 37°C for 60 min, the reaction was stopped by addition of 0·5 ml 20% trichloroacetic acid. The 4-hydroxyantipyrine formed was measured spectrophotometrically as described by Tabarelli-Poplawski & Uehleke (1977).

For phenytoin hydroxylase, 1 ml incubation mixture contained [^{3}H]phenytoin (specific activity 4 nCi $nmol^{-1}$)12, NADP 1, glucose-6-phosphate 8, $MgCl_2$ 6 nmol, 0·1 M sodium phosphate buffer pH 7·4 0·8 ml, 9000 *g* fraction or microsomal fraction 0·2 ml and glucose-6-phosphate dehydrogenase 0·7 I.U. After incubation of the mixture at 37° C for 20 min the unmetabolized phenytoin was extracted with 1-chlorobutane as described by Gerber et al (1971) and the radioactivity measured. The amount of phenytoin transformed during the incubation was calculated from the difference in extracted radioactivity between a sample kept at 0° C and the sample incubated at 37° C.

For phenylbutazone hydroxylase, 1 ml incubation mixture contained phenylbutazone 162, NADP 1, glucose-6-phosphate$_2$ 8, MgCl 6 μmol, 0·1 M sodium phosphate buffer pH 7·4 0·7 ml, and 9000 *g* fraction or microsomal fraction 0·3 ml and glucose-6-phosphate dehydrogenase 0·7 I.U. Incubation time was 45 min at 37° C. After the incubation, phenylbutazone was measured spectrophotometrically by the permanganate oxidation method of Jähnchen & Levy (1972) which also measures γ-hydroxyphenbutazone. The amount of phenylbutazone transformed during the incubation was calculated from the difference in extinction of a sample kept at 0° C and a sample after incubation at 37° C. In the same 9000 *g* fractions of control and uraemic rabbits the activity of phenylbutazone hydroxylase was measured before and after dialysis of the 9000 *g* fraction for 16 h at 4° C.

Table 1 shows that in the 9000 *g* fraction and in the microsomes the content of microsomal protein and

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