J. Pharm. Pharmacol. 1985, 37: 278–280
Communicated July 30, 1984

Comparison of the potency of 8-phenyltheophylline as an antagonist at A_1 and A_2 adenosine receptors in atria and aorta from the guinea-pig

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The potency of 8-phenyltheophylline as an antagonist at A_1 adenosine receptors in guinea-pig atria and at A_2 adenosine receptors in the guinea-pig aorta has been investigated. 8-Phenyltheophylline was an apparently competitive antagonist of the negative chronotropic effect of adenosine, 2-chloroadenosine, L-N^6-phenyl-isopropyl adenosine (L-PIA) and 5'-*N*-ethylcarboxamide adenosine (NECA) on atria and of the relaxant effect of adenosine, 2-chloroadenosine and NECA on the aorta. The pA_2 values for 8-phenyltheophylline ranged from 6·4 to 6·6 and were not significantly different, irrespective of the agonist or tissue used. These results indicate that 8-phenyltheophylline is a relatively potent antagonist at adenosine receptors but does not exhibit selectivity for either of the putative sub-types in isolated tissues.

It has been proposed that cell membrane adenosine receptors can be divided into two types which have been designated A_1 and A_2 (Van Calker et al 1979). This sub-division is based on the relative potency or affinity of a series of adenosine analogues (Bruns et al 1980). At the A_1 receptor the order of affinity is L-N^6-phenylisopropyl adenosine L-PIA) ≥ 2-chloroadenosine > 5'-*N*-ethylcarboxamide adenosine (NECA), whereas at the A_2 receptor it is NECA > 2-chloroadenosine > L-PIA. In addition, the A_1 receptor exhibits marked stereoselectivity for the L-isomer of PIA whereas there is only a small difference in potency between the L- and the D-isomers of PIA at the A_2 receptor.

If two types of adenosine receptors exist, then it may be possible to distinguish between them by the relative potency of an adenosine antagonist. In the present study, we have examined the potency of 8-phenyltheophylline as a purine receptor antagonist on guinea-pig atria which appears to possess an A_1 receptor (Evans et al 1982; Collis 1983) and on the guinea-pig aorta which appears to possess an A_2 receptor (Collis & Brown 1983). 8-phenyltheophylline was selected for use in this study because it is a potent adenosine receptor antagonist with minimal effects on cyclic (c)AMP phosphodiesterase (Smellie et al 1979).

Methods

Rings of guinea-pig thoracic aorta or guinea-pig atrial pairs were mounted in organ baths containing Krebs solution (37 °C) for isometric recording (Collis 1983; Collis & Brown 1983). Atenolol (ICI, 10^{-6}M) was routinely present in the Krebs solution used for the atria. The tissues were placed under a resting tension of 1g and allowed to equilibrate for 1 h.

The aortic rings were sub-maximally contracted by noradrenaline (2×10^{-6}M, noradrenaline bitartrate, Sigma). Purines were added to the bath contents on a 3 min cumulative dosing cycle. The relaxations evoked by the purines were expressed as the % reduction in the amplitude of the noradrenaline induced contraction.

The atrial pairs were allowed to beat spontaneously. Purines were added to the bath contents on a 5 min cumulative dosing cycle. The decrease in the rate of contraction evoked by the purines was expressed as the % reduction from the control rate.

8-Phenyltheophylline (Cal-Biochem) was dissolved at 10^{-2}M in 80% methanol containing 0·2M NaOH, and aqueous dilutions made from this solution. Control dose–response curves were made to acetylcholine chloride (Sigma, atria only), adenosine (Sigma), 2-chloroadenosine (Sigma). L-N^6-phenylisopropyl adenosine (L-N^6-(*R*-phenylisopropyl)-adenosine, Boehringer Mannheim, atria only) and 5'-*N*-ethylcarboxamide adenosine (prepared at ICI) in the presence of the appropriate amount of solvent for 8-phenyltheophylline. The tissues were then incubated with 8-phenyltheophylline (10^{-6}, 3×10^{-6}, 10^{-5}M) for 30 min and the agonist dose–response curve repeated. When NECA was used as the agonist on the aorta, control dose–response curves and those in the presence of 8-phenyltheophylline were made in paired preparations from the same animal. This was necessary since previous studies have indicated that the dose–response curve to NECA is not reproducible in this preparation (Collis & Brown 1983). When adenosine was used as the agonist on the atrium or aorta and when 2-chloroadenosine was used on the aorta, dipyridamole (10^{-5}M, Boehringer Ingelheim) was present to prevent transport of the agonist into the tissue (Collis 1983; Collis & Brown 1983).

The magnitude of the 8-phenyltheophylline induced right-wards shift of the agonist dose–response curve was assessed by the dose-ratio at the ED50% level. Dose-ratios were analysed as described by Arunlakshana & Schild (1959) and pA_2 values derived by linear regres-

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sion. Significant differences were calculated by Students' *t*-test and by analysis of variance.

Results

Adenosine and its analogues relaxed the aorta and inhibited the rate of atrial contraction in a dose-related manner. The maximal relaxation of the aorta evoked by the purine was 60–70% of the noradrenaline evoked contraction. High concentrations of the purines caused the cessation of atrial contraction.

The order of potency of the purines differed in the two tissues. On the atria the order was (L)-PIA ≥ 2 chloroadenosine > NECA > adenosine, whereas on the aorta it was NECA > adenosine > 2-chloroadenosine.

8-Phenyltheophylline (10^{-6}, 3×10^{-6}, 10^{-5}M) caused parallel right-wards shifts of the dose–response curves to purines in both tissues. Maximal responses were not altered by 8-phenyltheophylline. The slopes of the Arunlakshana & Schild (1959) plots for 8-phenyltheophylline as an antagonist of the effects of purines in both tissues were not significantly different from unity (Table 1). The pA_2 values for 8-phenyltheophylline were not significantly different, irrespective of the agonist or tissue used (Table 1).

Table 1. pA_2 values for 8-phenyltheophylline as an antagonist of the responses evoked by purines on the guinea-pig atria and aorta.

Tissue and Agonist (−log ED50)	pA_2	Slope[2]	r	n
Atria				
Adenosine[1] (6·11 ± 0.08)	6·39 ± 0·09	−1·16 ± 0·22	0·85	13
2-chloroadenosine (6·72 ± 0·07)	6·53 ± 0·06	−0·95 ± 0·15	0·88	14
L-PIA (6·79 ± 0·05)	6·47 ± 0·08	−0·94 ± 0·21	0·82	12
NECA (6·58 ± 0·11)	6·43 ± 0·07	−1·12 ± 0·18	0·9	11
Aorta				
Adenosine[1] (5·58 ± 0·04)	6·41 ± 0·07	−0·95 ± 0·18	0·8	18
2-chloroadenosine[1] (5·19 ± 0·07)	6·43 ± 0·1	−0·98 ± 0·28	0·67	18
NECA (6·15 ± 0·05)	6·6 ± 0·08	−0·92 ± 0·19	0·8	15

[1] In presence of dipyridamole (10^{-5}M).
[2] Slope of log. (dose-ratio-1) against -log molar concentration of 8-phenyltheophylline. L-PIA = L-N^6-phenylisopropyl adenosine, NECA = 5′-*N*-ethyl carboxamide adenosine.

The negative chronotropic effect of acetylcholine on the atria was not significantly altered by 8-phenyltheophylline (10^{-5}M, n = 4). Previous studies (Collis & Brown 1983) have shown that the aortic relaxant effect of the non-specific vasodilator sodium nitrite is not altered by 10^{-5}M 8-phenyltheophylline.

Discussion

The results of this study indicate that 8-phenyltheophylline is a competitive antagonist at adenosine receptors in the guinea-pig aorta and atria. This alkylxanthine, however, does not exhibit selective antagonism of the adenosine receptors in either tissue. This lack of selectivity is independent of the adenosine analogue that is used as the agonist.

The apparent lack of selectivity of 8-phenyltheophylline for putative A_1 or A_2 receptors in the atria and aorta is in agreement with the results of a recent biochemical study. Fredholm & Persson (1982) reported that 8-phenyltheophylline was an equi-effective antagonist of the inhibitory effect of 2-chloroadenosine on c AMP levels in adipocytes and of the stimulatory effect of NECA in rat hippocampal cells.

Two other groups have studied the antagonistic potency of 8-phenyltheophylline in isolated tissue preparations. Edvinsson & Fredholm (1983) reported a pA_2 value of 6·5 for 8-phenyltheophylline as an antagonist of NECA in cat cerebral arteries. This value is in close agreement with those reported in the present study. Griffith et al (1981) found 8-phenyltheophylline to be a competitive antagonist of adenosine evoked responses in the guinea-pig atrium (pA_2 = 6·24) and ileum (pA_2 = 6·12). The small difference between the pA_2 values for 8-phenyltheophylline derived in the present study and in that by Griffith et al (1981) may be due to the omission of an inhibitor of nucleoside transport in the earlier study. Clanachan & Muller (1980) have demonstrated that failure to either inhibit nucleoside transport, or to use agonists that are not substrates for the transport system, decreases the apparent potency of adenosine receptor antagonists. In the present study the nucleoside transport system was inhibited by dipyridamole when adenosine was used as an agonist. It was also blocked when 2-chloroadenosine was used on the aorta in order to prevent the action of this purine at intracellular sites (Collis & Brown 1983). When NECA was used on either tissue and when L-PIA was used on the atria, dipyridamole was not included since these analogues are not substrates for the nucleoside transport system (Collis 1983; Collis & Brown 1983; Plagemann & Wohlheuter 1984). L-PIA could not be used in the experiments on the aorta because of its low potency in this tissue (Collis & Brown 1983).

In conclusion, although previous studies have shown that adenosine analogues exhibit a different order of potency at adenosine receptors in the guinea-pig aorta and atria, the adenosine antagonist 8-phenyltheophylline does not exhibit selectivity. The concept that there are two types of adenosine receptor cannot be fully accepted until selective antagonists for these putative receptors are discovered.

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J. Pharm. Pharmacol. 1985, 37: 280–283
Communicated September 13, 1984

The absorption of β-adrenoceptor antagonists in rat in-situ small intestine; the effect of lipophilicity

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Intestinal absorption characteristics of eleven β-adrenoceptor antagonists were measured by monitoring their disappearance from in-situ intestinal loops in the anaesthetized rat. All have basic pK_a values of around 9·5 (with the exception of sotalol) but show a wide range of lipophilic character (octanol–water log P values from −0·79 to 3·65). The results show two types of absorption behaviour, indicating different mechanisms for 'hydrophilic' and 'lipophilic' β-adrenoceptor antagonists. The four most hydrophilic molecules (sotalol, atenolol, nadolol and practolol) show virtually identical absorption rate constants. Absorption is slow and relative rates in jejunum (mean pH 6·5) and ileum (mean pH 7·3) are not consistent with pH-partition (jejunum ≥ ileum). The more lipophilic members of the series (pindolol, timolol, metoprolol, oxprenolol, alprenolol and propranolol) are all absorbed much more rapidly. Absorption rate constant rises rapidly with log P and the expected pH effects are seen (ileum > jejunum). Acebutolol shows anomalously slow absorption for its log P value.

The β-adrenoceptor antagonists ('β-blockers') show a wide range of lipophilic character and have been classified in two groups as 'hydrophilic' and 'lipophilic' (Cruickshank 1980). All are absorbed from the intestinal tract in man and are active after oral administration, but there is evidence of marked quantitative differences in absorption properties. In general, absorption of the 'hydrophilic' compounds is slow and incomplete, and that of the 'lipophilic' compounds rapid and complete, but little detailed information on absorption characteristics is available. In this paper, we report absorption measurements in an in-situ anaesthetized rat gut preparation on eleven β-adrenoceptor antagonists which are, or have been, in clinical usage. Attempts are made to relate the measured absorption properties to octanol–water log P values and to what is known of their absorption in man.

Methods

(i) Physicochemical parameters

Octanol–water log P (log partition coefficient) values were obtained from the literature (Cruickshank 1980). Values of log D (log distribution coefficient) at the pH of the jejunum and ileum were calculated from log P, pH and pK_a.

(ii) Drug disappearance from intestinal lumen

The experimental technique was a modification of that reported by Dolusio et al (1969), using rate of drug disappearance from ligated in-situ segments of small intestine as a measure of absorption. Male albino rats (Alderley Park strain), 210–250 g, were fasted 18 h before use. Anaesthesia was induced and maintained with halothane (Fluothane, ICI). Following laparotomy, two 7·5 cm segments of small intestine were identified and loosely ligated, one in the jejunum approximately 2 cm distal to the ligament of Treitz and the other in the ileum approximately 2 cm proximal to the ileocaecal junction. Each segment was rinsed gently with isotonic sodium chloride (37 °C) to remove traces of food. Drug solution (0·5 mg ml^{-1} drug in 0·154 M sodium chloride), adjusted to pH 7 and warmed to 37 °C, was then introduced via a blunt needle, the ligatures being tightened to contain the drug solution. Drug remaining in the segment was recovered immediately after introduction (zero time) or at 20, 40 or 60 min after introduction, by excising the segment, with ligatures intact, cutting it open and washing out the contents with 0·154 M sodium chloride (1 ml). Recovered drug solution and washings were pooled and the pH measured. The solution was diluted to known volume and analysed for drug content by HPLC. A reverse phase column was used (5μ Hypersil C18, 0·5 × 10 cm), with a mobile phase of methanol–water–ammonia or methanol–water–trifluoracetic acid–sodium lauryl sulphate. An internal standard with a retention time similar to the drug was used in each case. Detection was by ultraviolet absorbance.

For most of the drugs, absorption measurements were made at zero and 20 min (rapidly absorbed molecules) or at zero and 60 min (slowly absorbed molecules), although time-course experiments were

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