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Hypothermia-induced supersensitivity to adenosine for responses mediated via A_1-receptors but not A_2-receptors

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1 Four isolated tissues were examined in which the responses to adenosine are mediated via either A_1- or A_2-receptors.

2 The responses examined were the inhibition of cholinergic transmission of field-stimulated guinea-pig ileum (A_1), inhibition of noradrenergic transmission of field-stimulated rat vas deferens (A_1), inhibition of developed tension of rat paced left atria (A_1) and relaxation of carbachol-contracted guinea-pig trachea (A_2).

3 Cumulative concentration-response curves for adenosine and 2-chloroadenosine were constructed at 37, 30 or 27°C.

4 When plotted as a percentage of the maximum response, the concentration-response curves were displaced to the left by cooling in the ileum, vas deferens and atria, indicative of supersensitivity.

5 This increase in sensitivity does not arise from inhibition of uptake or deamination by cooling, since it occurs equally for adenosine and 2-chloroadenosine, the latter being immune to these processes.

6 In contrast, the sensitivity of the trachea was not affected (2-chloroadenosine) or reduced (adenosine) by cooling.

7 Thus responses mediated via adenosine receptors of the A_1 subtype exhibit hypothermia-induced supersensitivity, whereas those mediated via A_2-receptors do not. This suggests a fundamental temperature-dependent difference between the two adenosine receptor subtypes.

Introduction

Temperature is known to influence the sensitivity of isolated tissues to the effects of agonist drugs. For example, lowering the temperature of isolated cardiac tissues increases the sensitivity to the β-adrenoceptor-mediated positive inotropic and chronotropic effects of sympathomimetic amines (Broadley & Williams, 1983). This phenomenon appears to be selective for the β_1-adrenoceptor (Williams & Broadley, 1982), and may thus indicate a fundamental temperature-dependent difference between the two β-adrenoceptor subtypes. Responses mediated via β-adrenoceptors are associated with stimulation of adenylate cyclase and accumulation of adenosine 3':5'-cyclic monophosphate (cyclic AMP, Wollenburger, 1975), which in parallel with the pharmacological response is enhanced by cooling (Duncan & Broadley, 1978; Reinhardt *et al.*, 1978).

Cell surface adenosine receptors of the P_1 type are also linked to adenylate cyclase and are classified into the A_1- and A_2-subtypes. The original basis for this classification was whether their stimulation initiated an inhibition (A_1) or an activation (A_2) of adenylate cyclase (van Calker *et al.*, 1979). These receptors may now be identified by radioligand binding techniques (Schwabe & Trost, 1980; Wu *et al.*, 1980). The receptors mediating pharmacological responses of isolated tissues may also be classified from the potency orders of adenosine analogues and their stereospecificity (Paton, 1981; 1983; Brown & Collis, 1982; Collis, 1983).

The present study examines whether the pharmacological responses to adenosine are also temperature-dependent and whether this differs for the A_1- or A_2-receptor. The responses of four isolated tissues to adenosine or 2-chloroadenosine were ex-

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amined; these were the negative inotropic response of paced left atria of the rat (A_1), inhibition of cholinergic transmitter release from transmurally stimulated guinea-pig ileum (A_1), inhibition of noradrenergic transmitter release from rat vas deferens (A_1) and relaxation of carbachol-contracted guinea-pig trachea (A_2).

Methods

Guinea-pigs of either sex (> 300 g) were killed by a blow to the head and male rats (150–250 g) were killed by CO_2 asphyxiation. The tissues were removed and set up in modified Krebs-bicarbonate solution (composition, mM in distilled water: NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22.0, D-glucose 11.2 and EDTA 0.04) gassed with 95% O_2:5% CO_2. Individual tissues were set up at one temperature, 37, 30 or 27°C.

Test preparations

Ileal segments from the guinea-pig were taken 10 cm above the ileocaecal junction and prepared for field stimulation as described previously (Muller & Paton, 1979). They were suspended in tissue baths (4 ml) under a resting tension of 0.5 g for 60 min. Field stimulation was effected by passing biphasic pulses of 1 ms duration at 0.2 Hz and supramaximal voltage delivered by a Grass stimulator (SD9), between platinum electrodes located at the top and bottom of the organ bath. After establishing twitch contractions of constant height, a cumulative concentration-response curve to adenosine or 2-chloroadenosine was obtained.

Vasa deferentia of rats were cleared of adhering tissue and the mid-portions consisting of equal lengths of testicular and urethral portions were suspended in tissue baths. A resting tension of 0.5 g was applied and field stimulation was by the same method as for the ileum with the same stimulation parameters. After establishing steady-state contractions, a cumulative concentration-response curve to adenosine or 2-chloroadenosine was obtained.

Left atria from rats were secured to the combined tissue holder and electrode, suspended in organ baths and a resting tension of 0.5 g applied. They were paced at 2 Hz with rectangular pulses (5 ms, threshold voltage + 30%) delivered by a Grass stimulator (SD9). After an equilibration period of 1 h with ocassional washing of the bath, cumulative concentration-response curves for the negative inotropic effects of 2-chloroadenosine were established.

Tracheal spirals from the guinea-pig were prepared by the method of Constantine (1965), and secured to tissue holders. They were suspended in tissue baths under a resting tension of 1.5 g. An equilibration period of 1.5 h was allowed during which several changes of bathing medium were made. The tissues were then contracted submaximally with carbachol (10^{-5} or 10^{-4}M) and when the tension had plateaued, a cumulative concentration-response curve to adenosine or 2-chloroadenosine was obtained. Under some conditions maximum responses were not obtained, but at the highest concentration used, a high concentration of isoprenaline (2×10^{-4}M) was added so that the responses to adenosine or 2-chloroadenosine could be plotted as a percentage of this maximum relaxation.

All tissues were attached to force-displacement transducers (Grass FTO3C) and tension recorded on a Grass Polygraph (model 7D). The bathing medium contained phentolamine (10^{-6}M) and propranolol (10^{-6}M) throughout to block α- and β- adrenoceptors respectively. Additionally, the medium for the atria and the vas deferens contained atropine (10^{-6}M) to block muscarinic receptors.

Protocol

A single cumulative concentration-curve was constructed for each preparation at 37, 30 or 27°C, after which the tissue was discarded. Concentration-response curves were constructed by half-log increments in concentration until the maximum response was obtained, when no change in tension occurred with further increases in concentration. However, in the case of the trachea, under some conditions a maximum response to adenosine or 2-chloroadenosine was not obtained. With the highest concentration in the bath a large concentration of isoprenaline (2×10^{-4}M) was added to produce a maximum relaxation.

Plotting dose-response curves

The tension at the resting level and at each concentration of adenosine or 2-chloroadenosine was measured. The change in tension induced by the agonists was then calculated and expressed as a percentage of the maximum inhibition on that tissue. In the case of the trachea, the maximum response to isoprenaline was used. Individual EC_{50} values were determined at the 50% of maximal response, expressed as the negative log of the molar concentration (pD_2) and the geometric mean (± s.e.mean) values calculated. Comparisons between pD_2 values at different temperatures were made by unpaired Student's t test and P values less than 0.05 were considered to be significant.

Drugs

The following were used: adenosine, atropine sulphate, carbachol (carbamylcholine) chloride, 2-chloroadenosine, (±)-isoprenaline hydrochloride

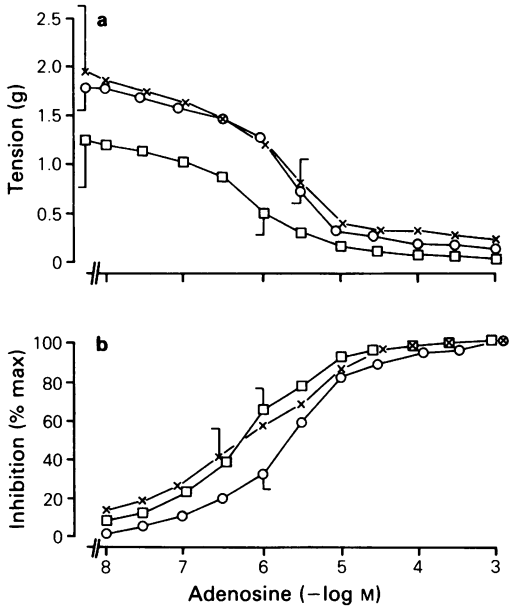

Figure 1 Mean cumulative concentration-response curves ($n > 5$) for the inhibition by adenosine of contractions of the guinea-pig ileum induced by field stimulation (0.2 Hz, 1 ms pulse width, supramaximal voltage). The tension developed was measured at rest and at each concentration of adenosine (a) and the changes from resting level were plotted as a percentage of the maximum effect (b). Concentration-response curves were constructed at 37 (○), 30 (X) or 27°C (□). Tissues were incubated throughout with phentolamine (10^{-6}M) and propranolol (10^{-6}M).

(Sigma), phentolamine mesylate (Ciba) and (±)-propranolol hydrochloride (Sigma). All solutions were freshly prepared in distilled water, a small quantity of ascorbic acid being added to the isoprenaline.

Results

Guinea-pig ileum

Adenosine and 2-chloroadenosine produced concentration-dependent inhibition of the stimulation-induced contractions of the ileum. The twitches were virtually abolished by adenosine (Figure 1a) and 2-chloroadenosine (Figure 2a) at the maximum effect when examined at 37, 30 and 27°C. The resting contractions were significantly reduced ($P < 0.005$) from 2.23 ± 0.40 g ($n = 5$) at 37°C to 0.94 ± 0.19 ($n = 7$) at 27°C (data from experiments with 2-chloroadenosine).

Table 1 Sensitivity of isolated tissues to adenosine and 2-chloroadenosine at different temperatures measured as the pD_2

	Temperature	*Ileum*		*Vas deferens*		*Atria*		*Trachea contracted with carbachol*			
								10^{-5}M		10^{-4}M[1]	
		pD_2	n	pD_2	n	pD_2	n	pD_2	n	pD_2	n
Adenosine	37	5.68 ± 0.14	5	4.17 ± 0.10	8	ND		ND		3.98 ± 0.22	4
	30	5.87 ± 0.33^{NS}	5	4.74 ± 0.16†	8	ND		ND		3.39 ± 0.10*	5
	27	6.32 ± 0.27*	6	4.64 ± 0.12**	4	ND		ND		—	
2-Chloroadenosine	37	7.19 ± 0.10	5	6.18 ± 0.13	8	6.41 ± 0.04	7	4.88 ± 0.10	6	4.48 ± 0.06	4
	30	7.31 ± 0.08^{NS}	5	6.84 ± 0.13†	8	*6.67 ± 0.09*	7	5.10 ± 0.18^{NS}	4	4.38 ± 0.14^{NS}	4
	27	7.70 ± 0.17*	7	6.73 ± 0.14*	4	6.87 ± 0.07††	4	—		4.24 ± 0.06*	4

Mean pD_2 values (± s.e.mean) were determined as the − log molar concentration for a 50% of maximum response. Differences between the values at 30 or 27°C and that at 37°C, determined by an unpaired Student's t test, are depicted as: *$P < 0.05$; **$P < 0.02$; †$P < 0.005$; ††$P < 0.001$ and NS not significant. [1]The values for adenosine in the trachea contracted with carbachol (10^{-4}M) were taken at the EC_{25} level. ND indicates experiment was not performed.

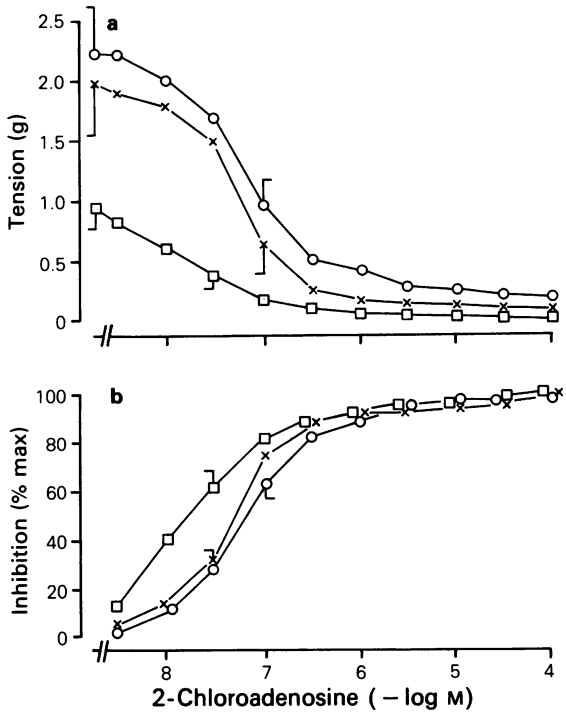

Figure 2 Mean cumulative concentration-response curves ($n > 5$) for the inhibition by 2-chloroadenosine of contractions of the guinea-pig ileum induced by field stimulation (0.2 Hz, 1 ms pulse width, supramaximal voltage). The tension developed was measured at rest and at each concentration of 2-chloroadenosine (a) and the changes from resting level were plotted as a percentage of the maximal effect (b). Concentration-response curves were obtained at 37 (○), 30 (X) or 27°C (□). Tissues were incubated throughout with phentolamine (10^{-6}M) and propranolol (10^{-6}M).

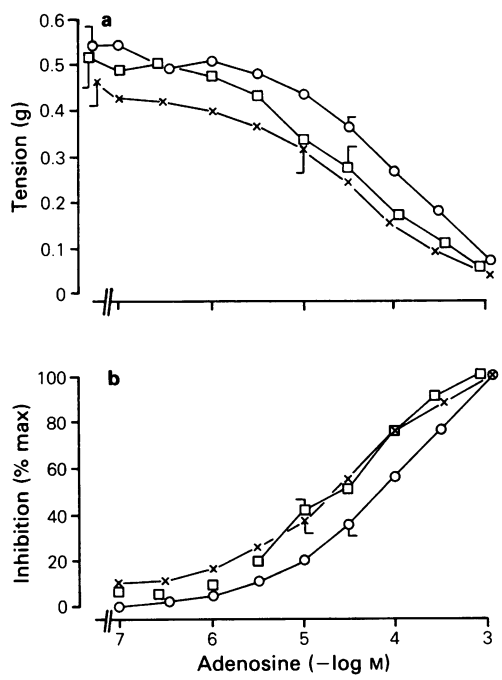

Figure 3 Mean cumulative concentration-response curves ($n > 4$) for the inhibition by adenosine of contractions of the rat vas deferens induced by field stimulation (0.2 Hz, 1 ms pulse width, supramaximal voltage). The tension developed was measured at rest and at each concentration of adenosine (a) and the changes from the resting level were plotted as a percentage of the maximum effect (b). Concentration-response curves were constructed at 37 (○), 30 (X) or 27°C (□). Tissues were incubated throughout with phentolamine (10^{-6}M), propranolol (10^{-6}M) and atropine (10^{-6}M).

When the inhibitory responses were plotted as a percentage of the maximum response, the adenosine curve at 30°C was to the left of that at 37°C with a further shift to the left at 27°C (Figure 1b). The pD_2 value at 30°C was not significantly ($P > 0.05$) different from that at 37°C, but at 27°C the difference was significant ($P < 0.05$) (Table 1). Similarly, with 2-chloroadenosine there was a shift to the left of the concentration-response curves at the lower temperatures (Figure 2b) with a significant ($P < 0.05$) elevation of the pD_2 value at 27°C (Table 1).

Rat vas deferens

The twitch contractions of the vas deferens were inhibited by adenosine (Figure 3a) and 2-chloroadenosine (Figure 4a) in a concentration-dependent manner, with almost complete abolition at the maximum effect. The resting contraction height was progressively reduced by lowering the temperature from 37°C (0.59 ± 0.01 g, $n = 8$) to 30°C (0.53 ± 0.10 g, $n = 8$) and 27°C (0.46 ± 0.08 g, $n = 4$). The concentration-response curves were plotted as a percentage of the maximum response to reveal a shift of the curves to both adenosine (Figure 3b) and 2-chloroadenosine (Figure 4b) to the left on cooling to 30°C. The pD_2 values were significantly ($P < 0.005$) increased with both agonists (Table 1). However, on further cooling to 27°C there was no further shift of the curves or change in pD_2 values in either case.

Rat left atria

The atrial tension was reduced by 2-chloroadenosine in a concentration-dependent fashion achieving at the maximum effect a $62 \pm 3\%$ inhibition of resting

developed tension at 37°C ($n = 7$), 51 ± 5% inhibition at 30°C ($n = 7$) and 38 ± 1% inhibition at 27°C ($n = 4$) (Figure 5a). The resting developed tension was significantly ($P < 0.05$) increased by cooling from 37°C (0.33 ± 0.04 g) to 30°C (0.38 ± 0.04 g) and 27°C (0.69 ± 0.14 g). A progressive shift of the concentration-response curves to the left by cooling was observed when they were plotted as a percentage of the maximum response in each tissue (Figure 5b). The pD_2 values were significantly ($P < 0.05$) greater at 30°C and 27°C than at 37°C (Table 1).

Guinea-pig trachea

Tracheal spirals contracted by carbachol (10^{-5}M) were relaxed by 2-chloroadenosine in a concentration-related manner at 37 and 30°C (Figure 6a). The relaxations to 2-chloroadenosine at the highest concentration used (10^{-4}M) were not complete relaxations, so isoprenaline was added and the responses expressed as a percentage of the maximum response to this agonist (Figure 6b). Isoprenaline was added cumulatively until the maximum effect was obtained, thus overcoming β-adrenoceptor blockade due to the presence of propranolol. The concentration-response curves plotted in this way were virtually superimposed at 37 and 30°C (Figure 6b) and there was no significant ($P > 0.05$) difference between the pD_2 values (Table 1).

Because of the possibility that the responses may be influenced by the magnitude of carbachol-induced contractions, adenosine and 2-chloroadenosine were also examined in tracheal spirals contracted by carbachol at a higher concentration (10^{-4}M) than used previously. The relaxation responses were plotted as a percentage of the maximum responses to isoprenaline

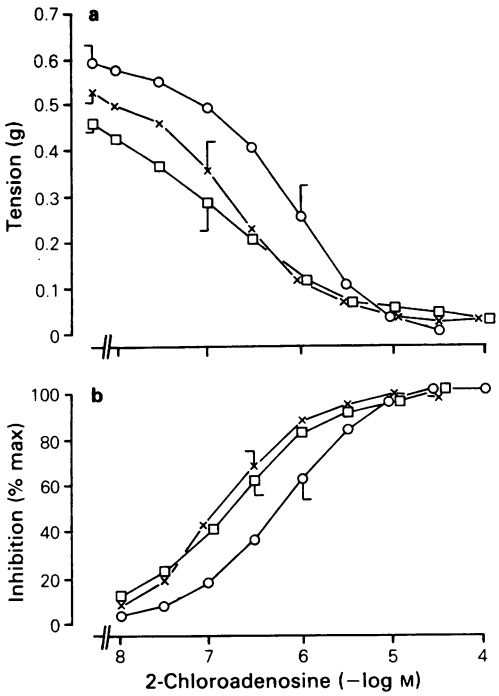

Figure 4 Mean cumulative concentration-response curves ($n > 4$) for the inhibition by 2-chloroadenosine of contractions of the rat vas deferens induced by field stimulation (0.2 Hz, 1 ms pulse width, supramaximal voltage). The tension developed was measured at rest and at each concentration of 2-chloroadenosine (a) and the changes from the resting level were plotted as a percentage of the maximum effect (b). Concentration-response curves were constructed at 37 (○), 30 (X) or 27°C (□). Tissues were incubated throughout with phentolamine (10^{-6}M), propranolol (10^{-6}M) and atropine (10^{-6}M).

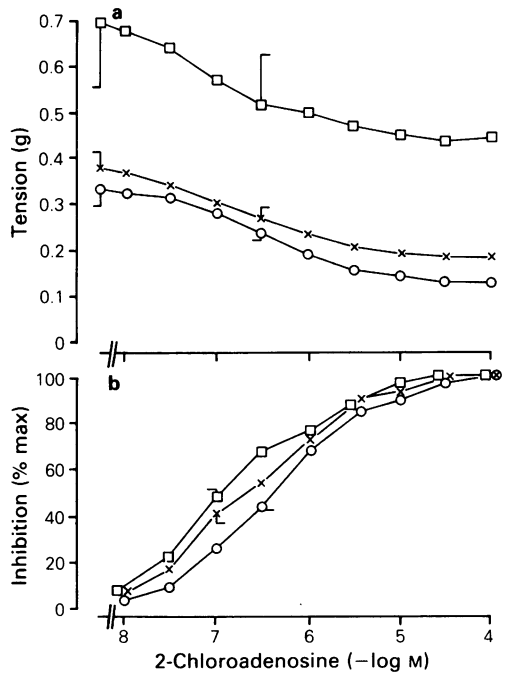

Figure 5 Mean cumulative concentration-response curves ($n > 4$) for the inhibition by 2-chloroadenosine of the contractions of rat left atria paced at 2 Hz (5 ms pulse width, threshold voltage + 30%). The tension developed was measured at rest and at each concentration of 2-chloroadenosine (a) and the changes in tension from the resting level were plotted as a percentage of the maximum effect (b). Concentration-response curves were constructed at 37 (○), 30 (X) or 27°C (□). Tissues were incubated throughout with phentolamine (10^{-6}M), propranolol (10^{-6}M) and atropine (10^{-6}M).

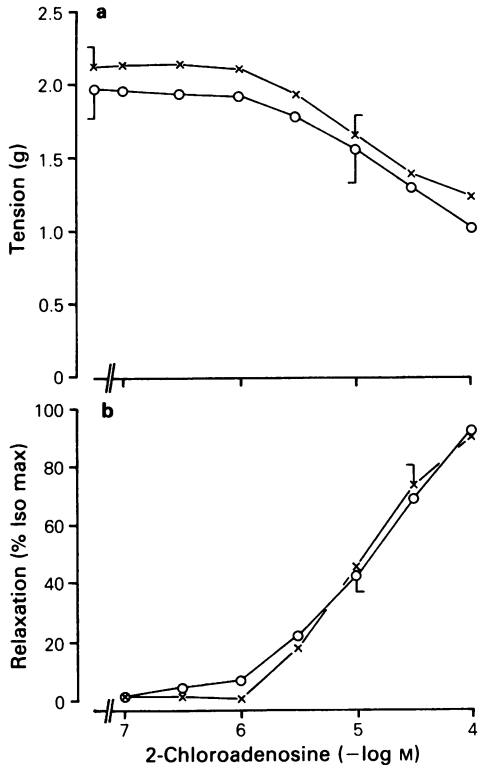

Figure 6 Mean cumulative concentration-response curves ($n > 4$) for the relaxation by 2-chloroadenosine of the guinea-pig trachea contracted by carbachol (10^{-5}M). The tension was measured before and after each concentration of 2-chlorodenosine (a). At the maximum concentration, a supramaximal concentration of isoprenaline (2×10^{-4}M) was added to the bath and the changes in tension were plotted as a percentage of this maximum relaxation (b). Concentration-response curves were constructed at 37 (O) or 30°C (X). Tissues were incubated throughout with phentolamine (10^{-6}M) and propranolol (10^{-6}M).

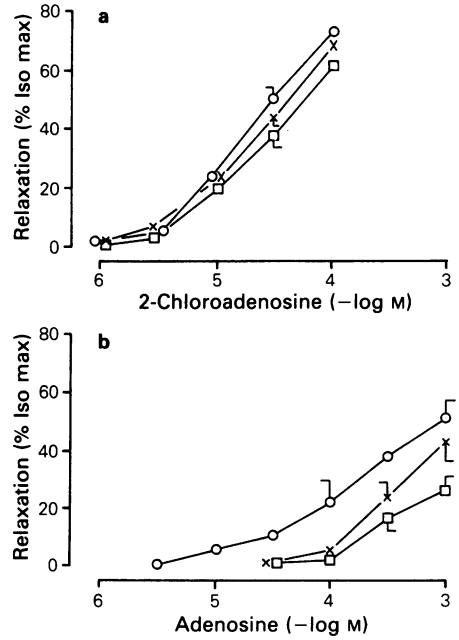

Figure 7 Mean cumulative concentration-response curves ($n > 4$) for the relaxation by 2-chloroadenosine (a) and adenosine (b) of the guinea-pig trachea contracted by carbachol (10^{-4}M). At the maximum concentration of each agonist, a high concentration of isoprenaline (2×10^{-4}M) was added to the bath and the changes in tension were plotted as a percentage of this maximum relaxation. Concentration-response curves were constructed at 37 (O), 30 (X) or 27°C (□). Tissues were incubated throughout with phentolamine (10^{-6}M) and propranolol (10^{-6}M).

(Figure 7). There was a small but progressive displacement to the right of the concentration-response curves for 2-chloroadenosine with cooling (Figure 7a). The pD_2 value at 30°C was not significantly less ($P > 0.05$), but the value at 27°C was significantly less than at 37°C ($P < 0.05$) (Table 1).

The maximum relaxation produced by adenosine at the highest concentration used (10^{-3}M), was less than 50% of the isoprenaline-induced relaxation at all temperatures (Figure 7b). The response to this concentration of adenosine at 27°C (25 ± 5%, $n = 4$) was significantly ($P < 0.05$) less than at 37°C (48 ± 8%, $n = 4$). Although the curves were clearly displaced to the right by cooling, comparisons between them at different temperatures could not be made at the EC_{50} values; instead the EC_{25} values were used for this comparison. The EC_{25} values showed a significant reduction at 30 compared with 37°C ($P < 0.05$) (Table 1).

Discussion

Adenosine and 2-chloroadenosine exerted inhibitory or relaxant responses in the four tissues examined. The reduction of twitch height of field-stimulated guinea-pig ileum is attributed to inhibition by adenosine of acetylcholine release (Sawynok & Jhamandas 1976; Hayashi *et al.*, 1983). Adenosine stimulates presynaptic inhibitory receptors that have been classified as the A_1-subtype (Paton, 1981; 1983). Similarly, the reduction of twitch height of field-stimulated rat vas deferens by adenosine is due to inhibition of noradrenergic transmission via presynaptic adenosine receptors

of the A_1-subtype (Paton, 1981; 1983). The inhibition of the paced left atrium of the rat by adenosine involves a direct negative inotropic effect upon the cardiac muscle (Burnstock & Meghji, 1983) which is mediated via receptors of the A_1-subtype (Paton, 1983), as in the guinea-pig atria (Evans *et al.*, 1982; Collis, 1983). In contrast to the above three tissues, the relaxation of the carbachol-contracted guinea-pig trachea is a direct effect upon the tracheal smooth muscle due to stimulation of A_2-receptors (Brown & Collis, 1982). The responses examined in this study were assumed to be mediated via P-purinoceptors since they have been shown to be antagonized by theophylline either in this laboratory (Paton, 1983) or by others (Brown & Collis, 1982).

The concentration-response curves to adenosine or 2-chloroadenosine were measured as absolute changes in tension and expressed as a percentage of the maximum response to obtain the pD_2 as an index of the tissue sensitivity. The sensitivity to adenosine or 2-chloroadenosine was determined at bath temperatures of 37, 30 or 27°C. The resting states of contraction of each tissue were affected by the bath temperature and this influenced the nature of the concentration-response curves when plotted as the change in tension. For example, the resting developed tension of the paced left atria was greater at 27°C than at 30 or 37°C and confirms the well established effect of temperature upon the cardiac muscle (Blinks & Koch-Weser, 1963). The contractions of the guinea-pig ileum and rat vas deferens were reduced by cooling, so that the extent of the inhibition by adenosine was reduced at the lower temperatures. For comparisons of sensitivity between temperatures it was therefore necessary to convert these to percentage of maximum plots. These revealed shifts of the concentration-response curves to the left by cooling the atria, ileum and vas deferens indicating a hypothermia-induced supersensitivity.

It is unlikely that this supersensitivity is merely due to the temperature-induced changes in resting levels and subsequent conversion to a percentage of maximum plot. This conclusion is supported by examples of similar resting levels such as the atrial contractions at 37 and 30°C, but a significant increase in sensitivity at the lower temperature when measured from the pD_2 value. The resting contractions of the vas deferens were in fact not significantly different at the three temperatures, yet the sensitivity increased on cooling to 30 and 27°C. The supersensitivity occurred in a progressive fashion between 37, 30 and 27°C only in the atria. In the ileum there was a significant increase only between 37 and 27°C, whereas in the vas deferens there was supersensitivity between 37 and 30°C but with no further increase on cooling to 27°C. This was a consistent pattern whether adenosine or 2-chloroadenosine was the agonist.

Therefore hypothermia-induced supersensitivity to both adenosine and 2-chloroadenosine occurred in the atria, ileum and vas deferens. It was possible that lowering the temperature may reduce the breakdown of adenosine by deaminase or its uptake (Plagemann, 1970) and thus explain the increase in sensitivity. However, 2-chloroadenosine is not taken up by the tissues (Muller & Paton, 1979; Collis & Pettinger, 1982) or deaminated (Clarke *et al.*, 1952; Rockwell & Maguire, 1966; Muller & Paton, 1979). The supersensitivity could not therefore be attributed to any change in disposition of either agonist.

The fourth tissue examined was the carbachol-contracted trachea of the guinea-pig. The concentration-response curves for 2-chloroadenosine were virtually superimposed at 37 and 30°C whether the tissue was contracted with 10^{-5} or 10^{-4}M carbachol. On further cooling to 27°C there was in fact a rightwards shift of the curve in tissues contracted with 10^{-4}M carbachol, indicating a reduced sensitivity. This failure to observe the hypothermia-induced supersensitivity seen with the previous tissues was therefore independent of the concentration of carbachol used to contract the tissue. The higher concentration of carbachol was used to examine adenosine, which, as in all tissues used, was less potent than the 2-chloroanalogue. At the maximum concentration of adenosine used (10^{-3}M), the relaxation was only 50% of the maximum relaxation produced by isoprenaline. Since the maximum responses to adenosine or 2-chloroadenosine were not attained, it was not possible to express these curves as a percentage of their own maxima. However, there was a clear reduction in sensitivity as the trachea was cooled, with the concentration-response curves being depressed and displaced to the right. Furthermore, the threshold concentration for relaxation was also increased.

Thus in contrast to the atria, ileum and vas deferens, the trachea did not exhibit hypothermia-induced supersensitivity but displayed a subsensitivity. Relaxation of the guinea-pig trachea by adenosine (Coleman, 1976; Farmer & Farrar, 1976; Jones *et al.*, 1980) is mediated via adenosine receptors which have been classified as the A_2-subtype (Brown & Collis, 1982), which distinguishes it from the atria, ileum and vas deferens. The relaxation of the trachea by 2-chloroadenosine also differed from the other tissues in that 100 fold higher concentrations had to be used. This difference is also apparent from the reports by Collis and colleagues on guinea-pig trachea (Brown & Collis, 1982) and atria (Collis, 1983). These results therefore suggest that hypothermia-induced supersensitivity occurs for responses mediated via A_1-receptors but not A_2-receptor-mediated responses.

The A_1- and A_2-receptor subtypes have been classified in biochemical and pharmacological systems by the rank orders of potency of agonists and by agonist stereospecificity (Paton, 1981; 1983; Daly, 1982).

From biochemical characterization, the A_1-receptor is associated with inhibition of adenylate cyclase whereas the A_2-receptor is associated with stimulation of this enzyme (van Calker *et al.*, 1979; Daly, 1982). The opposing effects of temperature upon the responses mediated by these receptor subtypes suggests that the supersensitivity is a relatively specific phenomenon and probably associated with the receptor or cyclase system that it inhibits. Radioligand binding studies indicate that binding to adenosine receptors is optimal at 37°C, reducing below this temperature (Dutta & Mustafa, 1979; 1980; Schwabe & Trost, 1980). The supersensitivity at A_1-receptors does not therefore appear to involve an increase in binding of the agonist to the receptor.

In conclusion, the present study has demonstrated a hypothermia-induced supersensitivity to adenosine and 2-chloroadenosine in tissues where the responses are mediated via adenosine receptors of the A_1-subtype, but no change or a subsensitivity for responses mediated via A_2-receptors. The responses mediated via adenosine receptors therefore share with those mediated via β-adrenoceptors a common differential effect of temperature. Responses mediated via β_1-adrenoceptors exhibit hypothermia-induced supersensitivity whereas responses mediated via β_2-adrenoceptors do not (Williams & Broadley, 1982). The present observation further substantiates the dual receptor classification for adenosine and suggests a fundamental temperature-dependent difference between the two adenosine receptor subtypes.

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