Therapeutic concentrations of diazepam potentiate the effects of adenosine on isolated cardiac and smooth muscle

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Although the primary central action of the benzodiazepines is considered to involve a potentiation of the actions of γ-aminobutyric acid, it has also been demonstrated that diazepam is a potent inhibitor of adenosine uptake into brain slices (Mah & Daly, 1976). It was thus of interest to investigate whether diazepam also modifies the peripheral actions of adenosine. We examined the effects of clinical concentrations of diazepam (10^{-6}–10^{-5} M) (Hillestad, Hansen, Melsom & Drivenes, 1974) on (a) adenosine mediated inhibition of neurotransmission in the rat vas deferens (Clanachan, Johns & Paton, 1977) and (b) the negative inotropic action of adenosine in electrically driven (2 Hz) guinea-pig left atria.

Both adenosine (10^{-7}–3×10^{-4} M) and 2-chloroadenosine (10^{-8}–10^{-5} M) produced concentration-dependent inhibition of the isometric contractions of the rat vas deferens (Table 1), effects thought to be mediated via stimulation of presynaptic adenosine receptors (Clanachan, 1979). Diazepam (10^{-6} and 10^{-5} M) significantly potentiated (2.3× and 7.5× respectively) the inhibitory effect of adenosine but failed to modify the action of 2-chloroadenosine. Similarly, in atria, the negative inotropic action of adenosine (10^{-6}–10^{-3} M) was significantly potentiated by diazepam (10^{-6} and 10^{-5} M; 3.4× and 16.5× respectively) whereas the effect of 2-chloroadenosine (10^{-8}–10^{-7} M) was unaffected (Table 1).

Comparable concentrations of diazepam solvent did not significantly modify adenosine concentration-effect curves in either tissue (Table 1). In addition, the concentrations of diazepam used exhibited no direct effects on contractions of either vas or atria.

The mechanism of the potentiation of adenosine seems likely to be due to inhibition of adenosine uptake or degradation since the 2-chloro analogue of adenosine, which has been reported not to be a substrate for the adenosine uptake system (Muller & Paton, 1978), was not affected by diazepam. This inhibition of adenosine uptake may explain the well documented coronary vasodilator action of diazepam in dogs (Abel, Reis & Staroscik, 1970; Daniell, 1975) and in man (Ikram, Rubin & Jewkes, 1973).

Table 1 The effect of diazepam on the responses of adenosine and 2-chloroadenosine on the rat vas deferens and guinea-pig atria

	Rat vas deferens§			*Guinea-pig atria*		
	n	IC_{50} ± s.e. mean	*P*	*n*	IC_{50} ± s.e. mean	*P*
Adenosine (μM)						
Control	20	23 ± 3	—	24	191 ± 37	—
Time Control	10	15 ± 2	—	7	165 ± 70	—
Diazepam 10^{-6} M	6	6 ± 1	<0.01	7	60 ± 8	<0.05
Diazepam 10^{-5} M	6	2 ± 1	<0.001	6	18 ± 7	<0.01
Solvent†	6	10 ± 3	NS	5	73 ± 25	NS
2-Chloroadenosine (*nM*)						
Control	6	180 ± 10	—	7	50 ± 5	—
Diazepam 10^{-5} M	5	180 ± 40	NS	7	69 ± 11	NS

IC_{50} values (concentration producing 50% inhibition) were calculated by linear regression analysis.
P values represent the significance of the difference of the IC_{50} from its corresponding control value as calculated by Student's *t*-test.
† The concentration of solvent used was equivalent to that present in a 10^{-5} M diazepam solution.
§ Only the IC_{50} values for the twitch response of the vas deferens are given.

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Histamine H_2-receptor antagonists in the mouse isolated vas deferens

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Histamine inhibits the electrically stimulated twitch response of the mouse isolated vas deferens (Marshall, 1978). The inhibition was antagonized by cimetidine and not by mepyramine suggesting a histamine H_2-receptor was involved. Quantitative studies of the interaction between histamine H_2-receptor agonists and antagonists have now been completed.

Mice vasa deferentia were suspended in magnesium-free Krebs solution and responses to stimulation (0.2 Hz, 2.0 ms) recorded isometrically. For each preparation cumulative concentration-inhibition curves to histamine or selective histamine H_2-receptor agonists were obtained. After recovery the vas deferens was equilibrated with a single concentration of histamine H_2-receptor antagonist for 40 min before a second application of agonist.

Cimetidine (10, 30, 100, 300 and 1000 μM), burimamide (30, 100, 300, 1000 and 3000 μM) and metiamide (10, 30 and 100 μM) shifted the histamine-inhibition curve (control IC_{50}, concentration to inhibit the twitch by 50%, 4.76 ± 0.22 μM, mean ± s.e. mean) to the right with no change in the maximum inhibitory effect (greater than 95% twitch inhibition). Higher concentrations of metiamide (300 μM and 1 mM), unlike those of cimetidine, did not shift the curve for histamine, dimaprit (control IC_{50} 21.3 ± 2.2 μM) or 4-methyl histamine (control IC_{50} 49.8 ± 4.2 μM) further to the right but the maximum inhibition was unaltered. This 'ceiling effect' of metiamide was unaffected by a longer equilibration period (80 or 120 min). Metiamide and cimetidine appear to compete for a single receptor site because combinations of the two drugs (e.g. metiamide 300 μM with cimetidine 300 μM or 1 mM) produced dose ratio shifts of the histamine inhibition curve consistent with $DR_1 + DR_2 - 1$ (DR_1 and DR_2 are the dose ratio shifts produced separately by the two antagonists) (Paton & Rang, 1965).

From the dose ratios based on the IC_{50} in each experiment, pA_2 values were determined (Black, Duncan, Durant, Ganellin & Parson, 1972). When all five concentrations of either metiamide or cimetidine were used the slope of the plot of log (DR-1) against log molar concentration of antagonist was less than unity. However, when the three lowest concentrations of antagonist were used a slope not differing from unity was obtained with a pA_2 value for metiamide against histamine of 5.05 (3.97–5.72, 95% confidence limits; slope 0.91 ± 0.39, ±95% confidence limits) and a pA_2 value for cimetidine against histamine of 4.97 (3.94 – 5.61; slope 0.84 ± 0.34). These pA_2 values are similar to those reported for the mouse stomach (Angus, Black & Stone, 1978) and are significantly lower than those reported for the guinea-pig atrium and rat uterus (Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975; Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973).

The results with the lower concentrations of antagonist suggest that the histamine receptor in the mouse vas deferens is of the H_2-type. Higher concentrations of antagonist appear to produce additional effects.

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