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COMMUNICATIONS

Effects of putative endogenous benzodiazepine receptor ligands on the potentiation of adenosine by benzodiazepines in isolated smooth muscle

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Benzodiazepines potentiate GABA-mediated inhibitory neurotransmission in the c.n.s. (Costa et al 1975; Keller et al 1976) by a mechanism that probably begins at the specific benzodiazepine receptor (Möhler & Okada 1977; Squires & Braestrup 1977). Benzodiazepines may either mimic, or displace from the receptor, endogenous ligands (Marangos et al 1979). The putative endogenous ligands identified so far in brain are nicotinamide and the purines inosine and hypoxanthine (Marangos et al 1978; Skolnick et al 1978; Möhler et al 1979). It has since been proposed that the benzodiazepine receptor is purinergic (Damm et al 1979). Inosine, hypoxanthine and nicotinamide are reported to possess benzodiazepine-like actions in the c.n.s. (Marangos et al 1979; Möhler et al 1979).

Benzodiazepines may also act by potentiating adenosine, a proposed intercellular mediator in the c.n.s. (Phillis 1979). It has not been established whether this action is mediated via benzodiazepine receptors. There are no behavioural models of c.n.s. purinergic function but purinergic receptors exist in smooth muscle and adenosine is a ligand for these receptors (Burnstock 1979). We have used the rat vas deferens preparation, which has presynaptic purinergic receptors (Clanachan et al 1977) to investigate whether the putative benzodiazepine ligands either have benzodiazepine-like effects on purinergic systems, or interfere with the potentiation of adenosine by benzodiazepines.

Materials and methods

Vasa deferentia of Sprague Dawley rats (100–120 g) were dissected and mounted through ring electrodes in a pair of 25 ml double jacketed baths, maintained at 32 °C. The baths contained Krebs solution of the following composition (mM): NaCl 118, KCl 4·75, $MgCl_2$ 1·17, $CaCl_2$ 2·54, KH_2PO_4 0·93, $NaHCO_3$ 25 and glucose 11, gassed with 95% O_2 plus 5% CO_2. The tissues were connected to isotonic transducers and equilibrated for 30 min with 250 mg tension before use. Contractions were elicited by trains of pulses (train frequency 0·1 Hz, duration 100 ms) delivered at supramaximal voltage (50–60 v), 50 Hz and 1 ms duration from a Grass 588 stimulator and isolation units. Non-cumulative concentration-effect curves were prepared for adenosine which was in contact with the vas for 1 min before stimulation. The contraction recorded after each addition of adenosine was expressed as a percentage of the initial reponse and the percentage inhibition calculated. Concentration-effect curves were repeated (usually 3 times) until entirely consistent responses were obtained. Benzodiazepines and putative benzodiazepine receptor ligands were added to the tissue 2 min before each addition of adenosine and the concentration-inhibition curves were redetermined.

Adenosine, inosine, hypoxanthine and nicotinamide were freshly dissolved in Krebs solution. Stock solutions of diazepam (Roche) and clobazam (Hoechst) were made in an aqueous solution containing 40% propylene glycol and 10% ethanol; solutions for use were made by diluting stock solutions with Krebs.

IC50 values (concentration producing 50% inhibition) were calculated by linear regression analysis. Significance levels for the difference between groups were determined using Student's *t*-test.

Results

Adenosine (10^{-7}–10^{-4} M) inhibited the electrically stimulated vas deferens; the log concentration-inhibition curve is shown in Fig. 1. Theophylline partly antagonized adenosine. Adenosine (IC50 = 66 ± 7 μM, n = 15) was significantly ($P < 0.01$) less potent in the presence of 10^{-5}M theophylline (IC50 = 48 ± 5 μM n = 10) and 3×10^{-5} M theophylline (IC50 = 18 ± 4 μM, n = 10) (Fig. 1). Diazepam (10^{-6}–3×10^{-5} M) alone, weakly inhibited the smooth muscle contractions; the inhibition was not affected by theophylline (10^{-5} M) The inhibitory effect of adenosine (IC50 = 85 ± 6 μM, n = 8) on the tissue contractions was potentiated by diazepam. The effect of 3×10^{-6} M diazepam on the adenosine dose-inhibition curve (IC50 = 50 ± 4 μM, n = 8, $P < 0.01$) is shown in Fig. 2. The diluted benzodiazepine solvent had no effect on the adrenosine-induced inhibition of the tissue contraction.

The effect of inosine on the inhibitory action of adenosine and its potentiation by diazepam was investigated. Inosine (3×10^{-5} M) had no effect on either the

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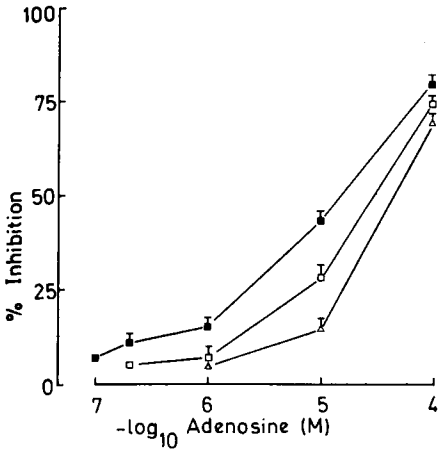

FIG. 1. Effect of theophylline on the inhibition by adenosine of the contractions of the rat vas deferens. Log dose-inhibition curves were obtained with adenosine alone (10^{-7}–10^{-4} M, ■——■), adenosine plus 10^{-5} M theophylline (□——□), adenosine plus 3×10^{-5} M theophylline (△——△). Vertical bars indicate the s.e.m.

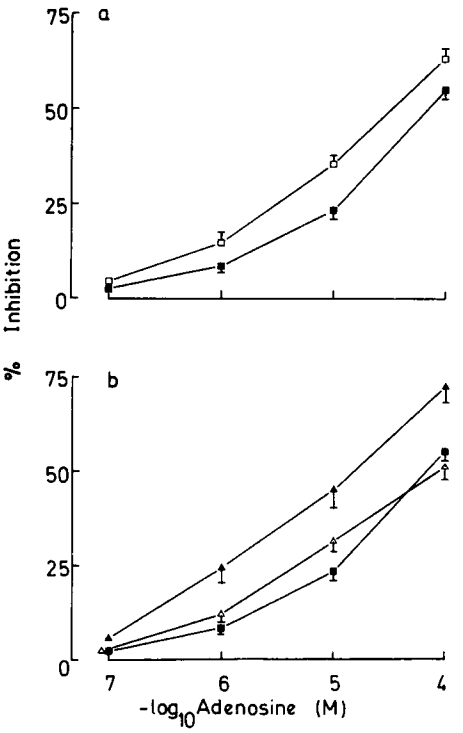

FIG. 2. Effects of inosine and diazepam on the inhibition by adenosine of the contractions of the rat vas deferens. Log dose-inhibition curves were obtained (a) with adenosine alone (■——■), adenosine plus 3×10^{-6} M diazepam (□——□) and (b) with adenosine alone (■——■), adenosine with 3×10^{-5} M inosine (△——△) and adenosine plus inosine and diazepam (▲——▲). Vertical bars indicate the s.e.m.

adenosine-induced inhibition of the vas deferens (IC50 = 93 ± 7 μM, n = 14) or the diazepam-induced potentiation of adenosine (IC50 = 16 ± 9 μM, n = 14, $P < 0.01$) (Fig. 2). Inosine alone had no effect on the contractions of the smooth muscle.

The effect of nicotinamide on the inhibitory action of adenosine (IC50 = 43 ± 7 μM, n = 10) and its potentiation by diazepam was investigated. Nicotinamide (3×10^{-5} M) had no effect on the adenosine-induced inhibition of the vas deferens (IC50 = 58 ± 9 μM, n = 10) and the diazepam-induced potentiation of adenosine (IC50 = 30 ± 3 μM, n = 10, $P < 0.01$) (Fig. 3). Nicotinamide alone had no effect on the contractions of the vas.

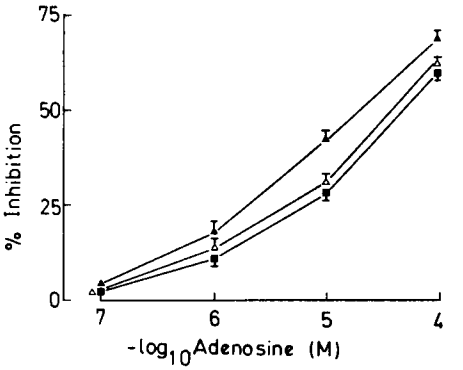

FIG. 3. Effects of nicotinamide and diazepam on the inhibition by adenosine of the contractions of the rat vas deferens. Log dose-inhibition curves were obtained with adenosine alone (■——■), adenosine plus 3×10^{-5} M nicotinamide (△——△) and adenosine plus nicotinamide and 3×10^{-6} M diazepam (▲——▲). Vertical bars indicate the s.e.m.

The interaction between adenosine (IC50 = 43 ± 9 μM) and hypoxanthine was investigated. Hypoxanthine (3×10^{-5} M) had no effect on the adenosine-induced inhibition of the vas deferens (IC50 = 30 ± 7 μM). The effect of hypoxanthine on the benzodiazepine-adenosine interaction was studied using clobazam, a 1′,-5′-benzodiazepine. Clobazam (10^{-5} M) potentiated adenosine on the vas deferens (IC50 values 25 ± 4 μM and 8 ± 2 μM n = 10, $P < 0.01$) (Fig. 4). Hypoxanthine (3×10^{-5} M) had no effect on the clobazam-adenosine interaction (IC50 = 8 ± 4 μM). Hypoxanthine alone had no effect on the contractions of the vas deferens.

Discussion

The present study was undertaken to determine firstly whether three compounds which may be endogenous benzodiazepine-like agents, inosine hypoxanthine and nicotinamide (Asano & Spector 1979; Marangos et al 1979; Möhler et al 1979) can mimic the action of benzodiazepines on adenosine uptake. The findings have confirmed that benzodiazepines potentiate adenosine in the vas deferens, probably by preventing its uptake (Clanachan et al 1977), at concentrations similar to those

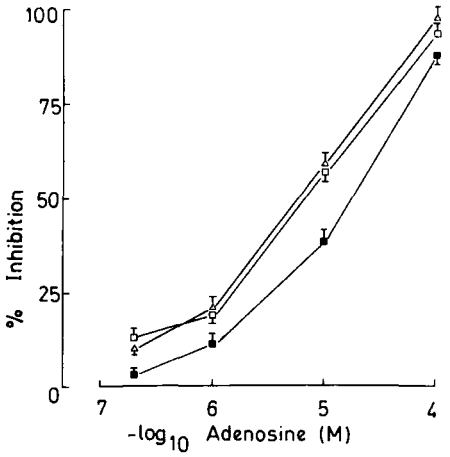

FIG. 4. Effects of hypoxanthine and clobazam on the inhibition by adenosine of the contractions of the rat vas deferens. Log dose-inhibition curves were obtained with adenosine alone (■——■), adenosine plus 10^{-6} M clobazam (□——□) and adenosine plus hypoxanthine and clobazam (△——△). Vertical bars indicate the s.e.m.

needed to prevent seizures in vivo (Geller et al 1978). However, none of the 3 compounds tested were able to potentiate adenosine in the peripheral model. The findings may be relevant to the c.n.s. because a brain adenosine-benzodiazepine interaction was proposed (Phillis 1979). Diazepam inhibits adenosine uptake by brain slices and glial cells (Mah & Daly 1976; Hertz et al 1979) and potentiates the depressant action of adenosine on cortical neurones (Phillis 1979). A second aim of the present study was to determine whether the putative endogenous benzodiazepine ligands could modify in any way the benzodiazepine-adenosine interaction in vitro. This possibility arose because although it has been assumed that benzodiazepines mimic endogenous c.n.s. ligands, it is equally possible that they may act by displacing such ligand(s). Neither inosine nor nicotinamide affected the diazepam-adenosine interaction and hypoxanthine had no effect on the potentiation of adenosine produced by the 1′, 5′-benzodiazepine, clobazam. Thus none of the putative ligands had any effect on the interaction in the periphery between benzodiazepines and adenosine.

Considerable caution is required in extrapolating these findings to the c.n.s. In particular, benzodiazepine receptors have not been found outside the c.n.s. and it is believed that at least one important action of this group of drugs in the c.n.s., namely potentiation of GABA (Costa et al 1975; Geller et al 1978) depends on the specific receptors.

Thus, the potentiation of adenosine in the c.n.s. by benzodiazepines may involve the specific receptors, although this has not been proved. The effects of the putative ligands on central actions of adenosine such as increased cyclic AMP production (Sattin & Rall 1970) and depression of neuronal firing (Phillis et al 1974) have not been investigated.

Unless it can be shown that c.n.s. adenosine-benzodiazepine interactions rely on benzodiazepine receptors, it is unlikely that the 'benzodiazepine-like' central actions which the 3 putative ligands have been attributed with (Marangos et al 1979; Möhler et al 1979) are mediated by c.n.s. purinergic mechanisms.

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