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Peripheral benzodiazepines potentiate the effect of adenosine in rat vas deferens

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Abstract—Ro 5-4864, diazepam, clonazepam and PK 11195 inhibit the responses of rat vas deferens following electrical stimulation in the rank order Ro 5-4864 > PK 11195 = diazepam > clonazepam. The adenosine-inhibited electrically-induced response was potentiated by Ro 5-4864 or diazepam, but not by clonazepam or PK 11195. This potentiation is due to an inhibition of the adenosine uptake system.

In the mammalian central nervous system, benzodiazepines produce their effects by the facilitation of γ-aminobutyric acid (GABA)-mediated transmission, opening the chloride ion channels in the cell membrane (Olsen 1982).

Another class of binding sites for benzodiazepines has been characterized in several peripheral tissues (Das et al 1987; Olson et al 1988). These are known as peripheral-type benzodiazepine receptors for which some specific ligands with nanomolar affinity have been described, e.g. Ro 5-4864 with agonist properties and PK 11195 (a non-benzodiazepine) with antagonist properties. At the present time the behavioural and clinical relevance of those recognition sites is uncertain.

Previous studies from our laboratory have evaluated the activity of three benzodiazepines, Ro 5-4864 (specific peripheral agonist), diazepam (with affinity for central and peripheral binding sites) and clonazepam (specific central agonist), and PK 11195 on noradrenaline and KCl-induced contractions in rat vas deferens (Camarasa et al 1988, 1989).

Those drugs inhibited the responses to agonists in a non-competitive manner. The three benzodiazepines had Hill coefficients significantly different from unity. PK 11195 did not reverse the effects of benzodiazepines; rather it showed the same antagonistic effect on noradrenaline- and KCl-induced contractions (Hill coefficient not significantly different from unity).

We also observed that when the calcium concentration in the Krebs-Henseleit solution was raised to 3·8–5·7 mM, the antagonistic effect of the three benzodiazepines, but not PK 11195, was reduced significantly, suggesting that the benzodiazepine effect is partially coupled to calcium channels. This could explain the difference of the values of the Hill coefficients between benzodiazepines and PK 11195, but the common mechanism of action of the four compounds needs to be explained further.

Clanachan & Marshall (1980) found that diazepam potentiated the effects of adenosine on isolated cardiac and smooth muscle, suggesting that the diazepam effects were related to adenosine uptake inhibition and not to a receptor mechanism. In guinea-pig left atria, some central benzodiazepines potentiate the cardiac responses to the purine, the rank order of potency being diazepam ≫ oxazepam > clonazepam (Kenakin 1982).

Our aim has been to determine if the potentiation of the adenosine effect also occurs with the peripheral-type benzodiazepines and to investigate the interaction between adenosine and peripheral benzodiazepines to explain the common mechanism of action shown by PK 11195, Ro 5-4864 and diazepam in rat vas deferens.

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Materials and methods

Drugs. Adenosine, 2-chloroadenosine and 2-hydroxy-5-nitrobenzyl-6-thioguanosine (HNBTG) were obtained from Sigma Chem Co.; Ro 5-4864 (7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one) from Fluka AG; diazepam and clonazepam were gifts from Hoffman LaRoche and PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methyl propyl)-3-isoquinoline carboxamide) a gift from Pharmuka-Rhône Poulenc. Adenosine and HNBTG solutions were prepared in Krebs-Henseleit. Benzodiazepine and PK 11195 solutions were prepared in ethanol and diluted with Krebs-Henseleit. The final concentration of ethanol was maintained below 1% and did not interfere with the responses.

Organ preparations, recording and statistics. Male Sprague Dawley rats, 300 to 350 g, were killed by a blow to the head followed by exsanguination. Vasa deferentia were quickly removed and placed in Krebs-Henseleit solution of the composition (mM): NaCl 119; KCl 4·60; $NaHCO_3$ 25; $CaCl_2$ 2·50; KH_2PO_4 1·20; $MgSO_4$ 1·20 and glucose 11·10, bubbled with 95% O_2: 5% CO_2. All vasa were bisected and 2 cm of the epididymal portion was mounted in transmural electrodes and placed in a 20 mL tissue chamber, suspended under a resting tension of 400 mg at 37°C and allowed to stabilize for 30 min.

Contractions were elicited by trains of pulses (2 Hz) for 5 s every 100 s (Letica stimulator LI12100) at supramaximal voltage (30 V) and pulse widths of 0·1 ms. These contractions were recorded isometrically with force-displacement transducers (Letica TRI 010) and displayed on a polygraph recorder (Letica 4000).

The purine remained in contact with the tissues for 1 min before electrical stimulation. The tissues were exposed to different concentrations of drugs for 10 min before dose-response curves were redetermined.

A method described by Kenakin (1982) was used to quantitate sensitization of rat vas deferens to adenosine. Sensitivity of vas deferens to adenosine (quantitated as the IC50) was then measured in the presence of various concentrations of benzodiazepines or HNBTG. The estimates of sensitization were then applied to Kenakin's equation yielding the pK_i values (−log equilibrium dissociation constant of the inhibitor for the site of uptake).

Results are expressed as mean ± s.e.m. IC50 values (concentration producing 50% inhibition) were calculated by a non-linear regression analysis program NKMODEL (Holford 1982). Significance levels for the difference between groups were estimated using ANOVA followed by Scheffé's test. The difference was judged to be significant for $P < 0.05$.

Results and discussion

Ro 5-4864 (6.27×10^{-6}–4.69×10^{-5} M), diazepam (2.92×10^{-6}–1.75×10^{-4} M), clonazepam (4.46×10^{-5}–1.74×10^{-4} M) and PK 11195 (3.40×10^{-5}–7.37×10^{-5} M) inhibited the responses elicited by electrical stimulation. Adenosine (10^{-5}–10^{-4} M) also inhibited the responses of rat vas deferens following electrical stimulation (Table 1).

Table 1. IC50 of tested drugs and adenosine on electrically induced contractions in rat vas deferens (mean ± s.e.m., n = number of averaged dose-response curves).

Drug	n	IC50 (μM)
Ro 5-4864	5	26·58 ± 3·90
Diazepam	5	46·87 ± 12·62
Clonazepam	6	92·21 ± 20·26
PK 11195	6	44·10 ± 14·62
Adenosine	5	53·57 ± 5·56

In concentrations which had no direct effect on rat vas deferens electrically-induced contractions, Ro 5-4864 (10^{-7}–5×10^{-6} M) and diazepam (10^{-7}–2×10^{-6} M) significantly potentiated, in a concentration-related manner, the inhibitory action of adenosine. Fig. 1 shows an example in which the concentration-response curve for adenosine was shifted to the left by Ro 5-4864 and diazepam. Clonazepam and PK 11195 did not potentiate the adenosine responses. The estimates of pK_i were 5·69 ± 0·37 for Ro 5-4864 and 6·01 ± 0·41 for diazepam.

HNBTG, as an irreversible inhibitor of adenosine uptake (Clanachan & Marshall 1980), potentiated significantly the effects of adenosine (Fig. 1). Following inhibition of adenosine uptake by HNBTG (10^{-5} M), Ro 5-4864 and diazepam, at different concentrations, both failed to produce potentiation of the inhibitory effects of adenosine.

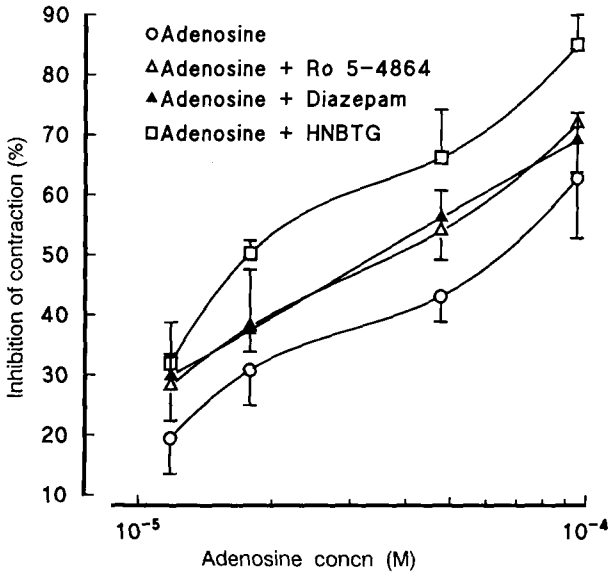

FIG. 1. Potentiation of adenosine inhibitory effect by Ro 5-4864 (3×10^{-6} M), diazepam (10^{-6} M) and HNBTG (10^{-5} M) on electrically induced contractions in rat vas deferens. Vertical bars denote s.e.m. (n = 5–6 experiments).

The fact that in the presence of HNBTG, Ro 5-4864 and diazepam did not potentiate the effects of adenosine, lends support to a mechanism of action based on an inhibition of adenosine uptake. This suggestion is supported by the observation that the inhibitory action of 2-chloroadenosine ($3{\cdot}5 \times 10^{-7}$–10^{-5} M) (a purine agonist which is not removed from the receptor compartment by adenosine uptake and/or degradative processes), on electrically-induced contractions of rat vas deferens, was not potentiated by Ro 5-4864 or diazepam.

PK 11195 and clonazepam did not show any potentiation of adenosine concentration-response curves either in the presence or in the absence of HNBTG.

Additionally, the inhibitory effect of PK 11195 on stimulated rat vas deferens was not observed if adenosine remained in previous contact with the tissue (data not shown), indicating that adenosine could prevent the interaction of PK 11195 with its binding sites. These binding sites are not thought to be identical to those labelled by Ro 5-4864 or diazepam, as the analogous contact of the purine with the tissue did not affect the Ro 5-4864 and diazepam responses. This is in agreement with the results of Eshleman & Murray (1989) in trout and mouse brain membranes.

Finally, the inhibition of the adenosine uptake system cannot explain the common inhibitory effect of the three benzodiazepines studied and PK 11195 on noradrenaline, KCl and electrically-induced contractions in rat vas deferens. However, the interaction between the adenosine uptake system and centrally acting benzodiazepines can be extended to peripheral-type benzodiazepines such as Ro 5-4864.

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