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Flunarizine but not theophylline modulates inotropic responses of the isolated rat heart to diazepam

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Abstract

Diazepam ($2 \cdot 10^{-5}$–$6 \cdot 10^{-4}$ M) induced a concentration-dependent positive inotropic effect on the perfused rat heart which was preceded by a transient concentration-dependent negative inotropic response. The influence of the Ca^{2+}-entry blocking drug, flunarizine, and the adenosine receptor blocking drug, theophylline on these inotropic responses was studied. Flunarizine in concentrations of 10^{-9}–10^{-6} M antagonized the positive inotropic response to diazepam significantly; the negative inotropic response was reduced as well. At the lower concentrations of diazepam the negative inotropic response was completely abolished in the presence of flunarizine. The actions of the Ca^{2+}-entry blocker were related to the concentrations used. Theophylline in concentrations up to $5 \cdot 10^{-5}$ M did not interfere with either inotropic response to diazepam. The results suggest that Ca^{2+} currents in the myocardium are involved with the response of the isolated heart to diazepam. It is concluded that the finding that the negative inotropic effect of diazepam was almost abolished by flunarizine suggests that the site of this response must be associated with Ca^{2+}-current mechanisms.

Keywords: Benzodiazepine; Diazepam; Langendorff heart; Inotropy; Ca^{2+}-entry blocking drug; Adenosine receptor blocking drug

1. Introduction

Benzodiazepines are frequently prescribed for the treatment of anxiety in patients in which the primary pathological process involves cardiovascular function, especially after a recently experienced myocardial crisis (Greenblatt et al., 1983). Moreover, during the past decade, these drugs have become indispensable for anaesthesia (Rall, 1990), which may also affect heart conditions.

After ingestion of therapeutic dosages of diazepam, virtually no cardiac effects are observed in otherwise healthy humans. Intravenous injection of 5–10 mg diazepam in humans, however, may cause a decrease of ventricular force (Rall, 1990).

Animal studies have revealed that benzodiazepines may induce cardiovascular effects, but the data and their interpretation are rather contradictory. In animal models, both positive and negative inotropic actions or no effects of the drugs have been observed (Daniell, 1975; Castillo-Ferrando et al., 1985; Haskins et al., 1986; Akahane et al., 1987).

More recently, Leeuwin et al. (1993) have shown that benzodiazepines elicit both positive and negative inotropic responses in isolated rat heart preparations and that the nature of the response depends on the type of derivative employed and concentrations that a preparation is exposed to. Diazepam [7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one] induces a biphasic response, i.e., it has a transient concentration-related negative inotropic action preceding a positive inotropic response which proceeds in two steps and is also related to the concentration.

Leeuwin et al. (1996) have subsequently shown that, after simultaneous exposure of the isolated rat heart to diazepam and PK 11195 [1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide], the positive inotropic response to diazepam is suppressed concentration-dependently. In the presence of $5 \cdot 10^{-5}$ M PK 11195, this response is abolished completely. The negative inotropic response to diazepam, however, does not change at all. Those authors proposed the concept that the location of the positive inotropic response to diazepam must be at the site of peripheral-type benzodiazepine receptors, and that, in the case of the negative inotropic response, intervention of some other mechanism related to these receptors must be considered.

The physiological significance of the peripheral type of

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benzodiazepine receptors is still unknown, but they were at least shown to be associated with Ca^{2+}-dependent events. Large densities of Ca^{2+}-channels were demonstrated in cardiac, vascular and skeletal muscles (Triggle and Janis, 1984a,b). There are also indications for a functional interaction between peripheral-type benzodiazepine receptors and voltage-operated Ca^{2+} channels in the spontaneously beating guinea-pig atrium (Bolger et al., 1989).

Adenosine is a potent coronary vasodilator. It is well known that adenosine and adenosine receptor agonists may evoke negative inotropic responses in the isolated heart, which are reversed by adenosine antagonists (Belardinelli et al., 1989; Shryock et al., 1992; Mudumbi et al., 1993). Moreover, it has been shown that benzodiazepines potentiate pharmacological responses to adenosine in cardiac muscles (Clanachan and Marshall, 1980). Thus, the possibility was considered of involvement of Ca^{2+}- and/or adenosine-dependent events with inotropic actions of benzodiazepines. Therefore, the present study was initiated to investigate the effects of a Ca^{2+}-entry blocking drug, i.e., flunarizine, which is selective, but has no perceived actions on the slow inward Ca^{2+} current in myocardium (Godfraind et al., 1986), on the inotropic response of the isolated rat heart in the presence of diazepam. Theophylline, an adenosine receptor antagonist (Mudumbi et al., 1993), was also included in the investigation. The results presented here may contribute to a better understanding of actions of benzodiazepines at peripheral sites.

2. Materials and methods

Cpb: WU(WI) female Wistar rats weighing 160–180 g, anaesthetized with pentobarbitone, and heparinized with 500 IU/kg heparin i.v. were used throughout. The hearts were perfused in the Langendorff setup. After thoracotomy, the aorta was cannulated rapidly in order to allow perfusion (retrograde). The heart was excised, placed in the experimental setup, and instantly perfused with Tyrode's solution (NaCl 118.4 mM, KCl 4.7 mM, $CaCl_2$ 1.3 mM, $MgSO_4$ 0.65 mM, $NaHCO_3$ 25.0 mM, KH_2PO_4 0.6 mM, glucose 11.1 mM) at pH 7.0 and gassed with 95% O_2/5% CO_2 at 37°C, under a constant hydrostatic pressure of 60 cm. The perfusion rate was 8 ml/min. Heart contractile force was measured as left ventricular pressure. A latex balloon filled with water was inserted in the left ventricle to measure oscillations in force of heartbeat, and was connected to a Gould Statham pressure transducer (P231D). Ventricular pressure was recorded continuously on a Gould (2-channel) recorder, model 8188.2202.06.

After equilibration and recording for 20 min (= control level in advance of the first administration), the experiment started with the exposure of the preparation to a drug. This was followed by a subsequent recovery period and control recording, lasting 10 min, after the previous administration. Diazepam ($2 \cdot 10^{-5}$–$6 \cdot 10^{-4}$ M) was administered (in 10 s) in increasing concentrations after each 10 min of control recording. Identical experiments were performed in the continuous presence of flunarizine (10^{-9}–10^{-6} M) or theophylline (10^{-6}–$5 \cdot 10^{-5}$ M) in the perfusate. The inotropic response – after each exposure of the preparation to a benzodiazepine – was expressed as percentage change of contractile force measured when either maximum increase or depression was manifest, as compared to the force measured immediately before administration of a drug.

Statistics. Data collected before and after exposure to a benzodiazepine were analyzed statistically using Student's *t*-test. Each drug or combination of drugs was tested in 7 experiments. In order to quantify the effect of the Ca^{2+}-en-

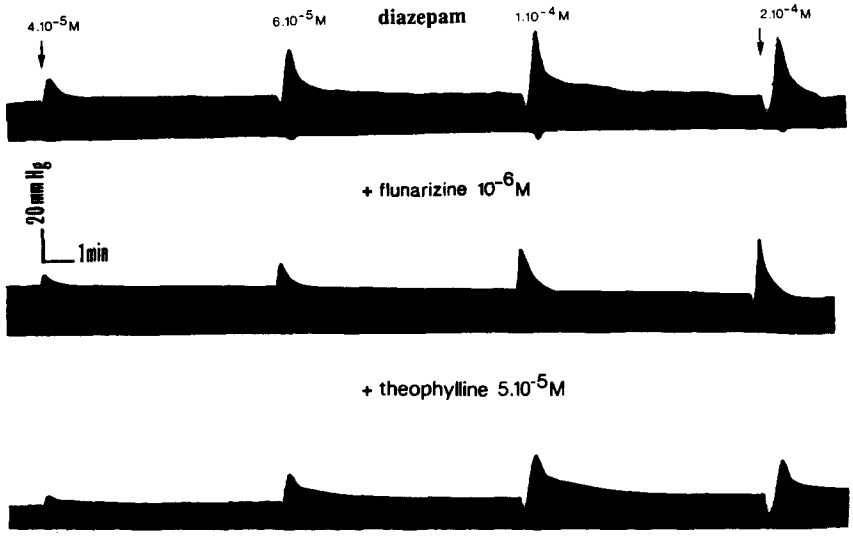

Fig. 1. Representative recordings of inotropic responses of the isolated rat heart to diazepam administered in stepwise increasing concentrations – alone and in the presence of 10^{-6} M flunarizine or $5 \cdot 10^{-5}$ M theophylline.

try blockers on the diazepam-induced negative inotropic response, values of EC_{50} and maximal effect were compared in the absence and presence of the blockers. Cumulative concentration-inhibition relationships were analyzed with least-squares curve-fitting, and IC_{50} was calculated as the drug concentration causing a half-maximal effect.

The drugs tested were: diazepam (Valium, Roche Nederland) dissolved in propylene glycol; flunarizine (Janssen) dissolved in distilled water and subsequently diluted with Tyrode's solution; and theophylline (Fluka Chemie) dissolved in distilled water.

3. Results

Propylene glycol alone, when added to the perfusion fluid, did not alter inotropy of the isolated heart. Flunarizine or theophylline in concentrations used in this study also did not interfere with the spontaneous contractile force. Characteristic recordings of the inotropic response of the heart to diazepam before and after exposure to flunarizine or theophylline are shown in Fig. 1. In the presence of flunarizine (10^{-6} M), strong suppression, and even elimination, of the negative inotropic response to diazepam was observed and in addition the positive inotropic action was reduced. Theophylline ($5 \cdot 10^{-5}$ M) did not affect either inotropic response to diazepam. The quantitative results are summarized in Figs. 2 and 3.

Fig. 2 shows that the positive inotropic response to diazepam in two steps – i.e., a concentration-related increase of contractile force, lessened at still higher concentrations – was also manifest in the presence of 10^{-9}–10^{-6} M flunarizine. This response to diazepam however, was significantly depressed ($P < 0.05$), the degree of depres-

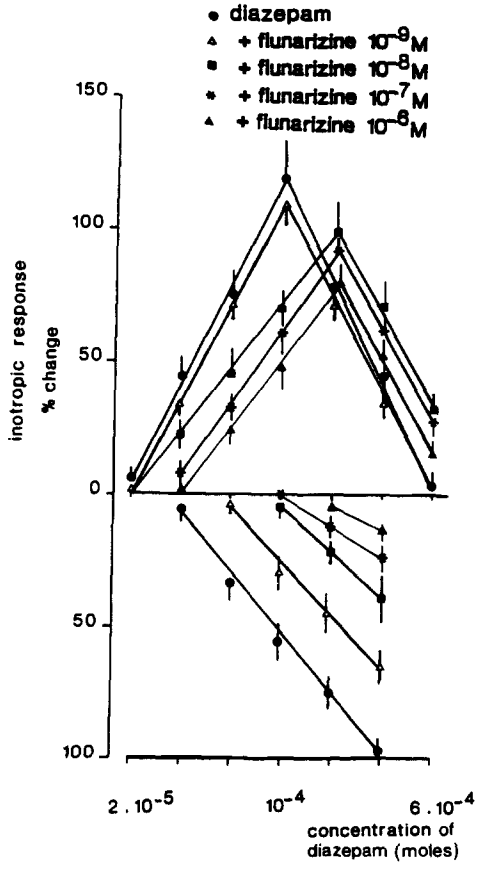

Fig. 2. Concentration-response curves for the inotropic responses of the isolated rat heart to diazepam alone and in the presence of flunarizine shown as percentage changes in force of contraction. Percentage effect was calculated after *each* exposure of the preparation to diazepam, measured when either maximum increase or depression was manifest, and compared to the contractile force measured in the absence of diazepam immediately before it was administered. Baseline contraction force (mmHg): 23.5 ± 1.2; on exposure to diazepam ($1 \cdot 10^{-4}$ M) $10.8 + 0.8$ (negative inotropy) and 61.5 ± 4.7 (positive inotropy), respectively. Upper part of the abcissa: the effect of diazepam (means $\pm$ S.E.M., $n = 7$) as positive inotropic response. Lower part of the abcissa: the effect of diazepam (means $\pm$ S.E.M., $n = 7$) as a negative inotropic response.

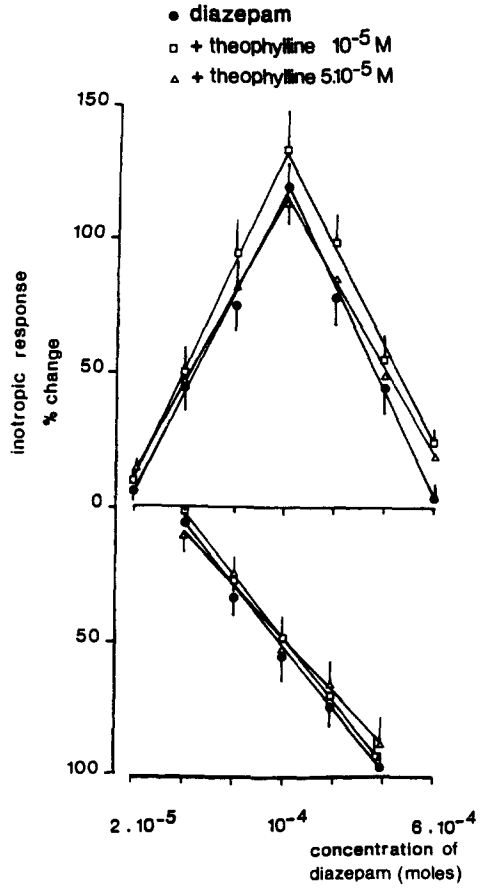

Fig. 3. Concentration-response curves for the inotropic responses of the isolated rat heart to diazepam alone and in the presence of theophylline shown as percentage changes in force of contraction. Percentage effect was calculated after *each* exposure of the preparation to diazepam, measured when either maximum increase or depression was manifest, and compared to the contractile force measured in the absence of diazepam immediately before it was administered. Baseline contraction force (mmHg): 23.5 ± 1.2; on exposure to diazepam ($1 \cdot 10^{-4}$ M): 10.8 ± 0.8 (negative inotropy) and 61.5 ± 4.7 (positive inotropy), respectively. Upper part of the abcissa: the effect of diazepam (means $\pm$ S.E.M., $n = 7$) as positive inotropic response. Lower part of the abcissa: the effect of diazepam (means $\pm$ S.E.M., $n = 7$) as a negative inotropic response.

sion depending on the concentration of the Ca^{2+}-entry blocker employed. The negative inotropic response was markedly reversed ($P < 0.05$) as well and shifted to the right in the presence of 10^{-9}–10^{-6} M flunarizine in the perfusate. At the lowest concentrations of diazepam the negative inotropic response was completely abolished by flunarizine. The IC_{50} value for flunarizine was $2.4 \cdot 10^{-9}$ M.

As can be seen in Fig. 3, neither the positive nor the negative inotropic responses to diazepam were affected in the presence of 10^{-6}–$5 \cdot 10^{-5}$ M theophylline ($P > 0.05$).

4. Discussion

Over the concentration range of 10^{-9}–10^{-6} M, flunarizine significantly depressed the positive inotropic response of the isolated Langendorff rat heart. The positive inotropic response to diazepam was not affected in the presence of concentrations of theophylline up to $5 \cdot 10^{-5}$ M. Flunarizine markedly reduced the negative inotropic response to diazepam, even abolishing it at the lower concentrations of diazepam. Theophylline did not interfere with the negative inotropic response.

In a recent study, Leeuwin et al. (1996) have shown that the positive inotropic response induced by diazepam is suppressed completely by adequate concentrations of PK 11195, an antagonist of the peripheral type of benzodiazepine receptors (Le Fur et al., 1983; Triggle and Janis, 1984a,b; Doble et al., 1985; Awad and Gavish, 1987; Bolger et al., 1989; Verma and Snyder, 1989; Farges et al., 1993; Rao et al., 1994). Over the same concentration range (10^{-7}–$5 \cdot 10^{-5}$ M), however, PK 11195 did not change the negative inotropic response. It is, thus, tempting to argue that the positive inotropic response of the heart to diazepam results from binding of PK 11195 at the site of the peripheral type of benzodiazepine receptor. The fact that the negative inotropic response was not affected by PK 11195 would mean that some other mechanism must be involved.

Results of electrophysiological and pharmacological studies suggest an association between voltage-dependent Ca^{2+} channels – which occur richly in cardiovascular tissue – and the peripheral type of benzodiazepine receptor in the cardiovascular system (Mestre et al., 1985, Mestre et al., 1986; Abraham et al., 1987; Bolger et al., 1989). Moreover, Le Fur et al. (1985) demonstrated that high Ca^{2+} concentrations inhibit the coronary dilatation induced by Ro 05-4864 [7-chloro-5-(-4-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one; 4′-chlorodiazepam].

The present results at least indicated the involvement of Ca^{2+}-current mechanisms with diazepam-induced inotropic responses of the isolated heart. The Ca^{2+}-entry blocking drug, flunarizine, modulated these responses. The positive inotropic response was antagonized by flunarizine. The Ca^{2+}-entry blocker also strongly reduced the negative inotropic response to diazepam, even abolishing it at the lowest concentrations of the benzodiazepine. Thus, the negative inotropic response to diazepam may be associated with a site possessing affinity for Ca^{2+}-entry blocking drugs of the type of flunarizine.

It was argued earlier (Leeuwin et al., 1996) that adenosine receptor mechanisms may be involved as well in the cardiac actions of diazepam. Adenosine has important actions in the heart, among them a negative inotropic action (Belardinelli et al., 1989). Cardiac effects of adenosine are reversed by adenosine antagonists (Belardinelli et al., 1982; Shryock et al., 1992; Mudumbi et al., 1993). The actions of adenosine on cardiac muscle are mediated by externally located adenosine receptors, which are xanthine-sensitive (Belardinelli et al., 1982; Collis, 1983; Hughes and Stone, 1983). Benzodiazepines potentiate pharmacological responses to adenosine in cardiac muscles (Clanachan and Marshall, 1980). In the present study, the effects of diazepam on inotropy were studied in the presence of theophylline, an adenosine receptor antagonist, at different concentrations. Neither the positive nor the negative inotropic response to diazepam was modulated. It seems, thus, that the possibility of intervention of adenosine receptor mechanisms with these actions must be excluded.

In conclusion, the finding that the negative inotropic response to diazepam was reduced and almost completely suppressed, at least at relatively low concentrations of diazepam, by the Ca^{2+}-entry blocking drug, flunarizine, could suggest that Ca^{2+}-current mechanisms, which are functionally related to peripheral type of benzodiazepine receptors, may be involved in the response.

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