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The negative inotropic effect of diazepam in rat right ventricular strips

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Abstract—The effect of diazepam on cardiac contractility was investigated in electrically-driven right ventricular strips of the rat. Diazepam produced a concentration-dependent negative inotropic effect which was antagonized by either flumazenil, a benzodiazepine central-type receptor antagonist, or PK 11195, a benzodiazepine peripheral type receptor antagonist. The results suggest that the inhibitory effect of diazepam on cardiac contractility in the rat is mediated by both central and peripheral benzodiazepine receptors.

There are diverse reports on the effect of diazepam on cardiac contractility. Negative (Daniell 1975), positive (Castillo-Ferrando et al 1985) and biphasic (Gonzalez et al 1990) inotropic effects have been described. The mechanism involved in the inotropic actions of diazepam is also unclear. There is general agreement that diazepam acts by binding to specific receptors, which are mainly localized in the central nervous system. These central benzodiazepine receptors are coupled to the γ-aminobutyric acid (GABA) receptor and are linked to chloride channels. They are inhibited by the specific antagonist flumazenil or the convulsant agent picrotoxin (for review see Haefely et al 1985). Recently, a second class of diazepam binding site has been identified which is not coupled to the GABA receptor-chloride channel complex (Marangos et al 1982). These so-called peripheral-type receptors have been identified in kidney, heart and adrenals (Anholt et al 1985). It also appears that there are benzodiazepine receptors of the central-type in peripheral tissues (Luzzi et al 1986) as well as peripheral-type receptors in the brain (Benavides et al 1983). The non-benzodiazepine PK 11195 specifically binds to the peripheral sites and seems to antagonize the action of diazepam on these receptors (Mestre et al 1984).

The aim of the present study was to analyse the inotropic effect of diazepam and test for the involvement of central- and peripheral-type benzodiazepine receptors.

Materials and methods

Drugs. The drugs used were diazepam and flumazenil (a gift from Roche, Spain) and PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide, generously supplied by Pharmuka Lab., Gennevilliers Cedex, France). These drugs were freshly dissolved in dimethylsulphoxide (DMSO obtained from Probus, Barcelona, Spain) and saline (4 DMSO:6 saline); this stock was diluted into prewarmed and preaerated bathing solution to achieve the desired final concentration.

Sprague-Dawley rats of either sex, 250–400 g, were stunned and exsanguinated. The chest was opened and the heart was rapidly removed and placed in Tyrode solution saturated with 95% O_2–5% CO_2 and the free wall of the right ventricle was excised. All procedures were performed in the presence of Tyrode solution of the following composition (mM): NaCl 136·9, KCl 5·0, $CaCl_2$ 1·8, $MgCl_2$ 1·5, NaH_2PO_4 0·4, $NaHCO_3$ 11·9, dextrose 5·0. Right ventricular strips were mounted longitudinally between two platinum electrodes under 1 g tension in Tyrode solution maintained at 34°C and gassed with 95% O_2–5% CO_2. The preparations were electrically stimulated (Grass

SD-9 stimulator), at a frequency of 1·5 Hz; duration of 4 ms and supramaximal voltage (30 V) were given for 30 min before the start of the experiments. Contractions were measured using a force-displacement transducer (Grass FT 03) and recorded on a Dynograph Beckman Polygraph.

Cumulative concentration-response curves to diazepam were performed by increasing the concentration stepwise as soon as the response to the previous dose had levelled off. To study the interaction between this drug and either flumazenil or PK 11195, concentration-response curves to diazepam were made twice for each preparation, first alone and then, after a wash-out period, in the presence of the antagonist. Only one antagonist was tested in each muscle strip.

The effect of diazepam alone is expressed as percent change. To ascertain whether the effect of diazepam is modified by the presence of flumazenil or PK 11195, we measured the ID50 for diazepam in the absence and presence of each compound; this value was defined as the mean drug concentration that produces a 50% inhibition of the amplitude of the contraction.

Data were analysed with Student's *t*-test and an analysis of variance. Linear regression analysis of the concentration response curves was used to determine the ID50. $P < 0.05$ was considered to indicate statistical significance.

Results

A typical effect of diazepam on cardiac contractility in rat isolated right ventricular strips is shown in Fig. 1A. Diazepam reduced the amplitude of contraction in a concentration-dependent manner producing a maximal decrease of 54·1 ± 6·5% at 60 μM. The negative inotropic effects of diazepam were rapidly reversed after the wash-out. DMSO applied in the same concentrations as those present in the diazepam solution did not significantly affect ventricular contractility (Fig. 1B).

Concentration-response curves for diazepam were constructed in the absence and in the presence of either flumazenil or PK 11195. Flumazenil at a concentration of 1 μM (virtually devoid of any effect on ventricular contractility) was applied to the preparation 20 min before addition of diazepam. This drug produced a rightward shift in the diazepam concentration-response curve (Fig. 2). The peripheral benzodiazepine receptor antagonist, PK 11195 (1 μM) also caused a rightward shift in the

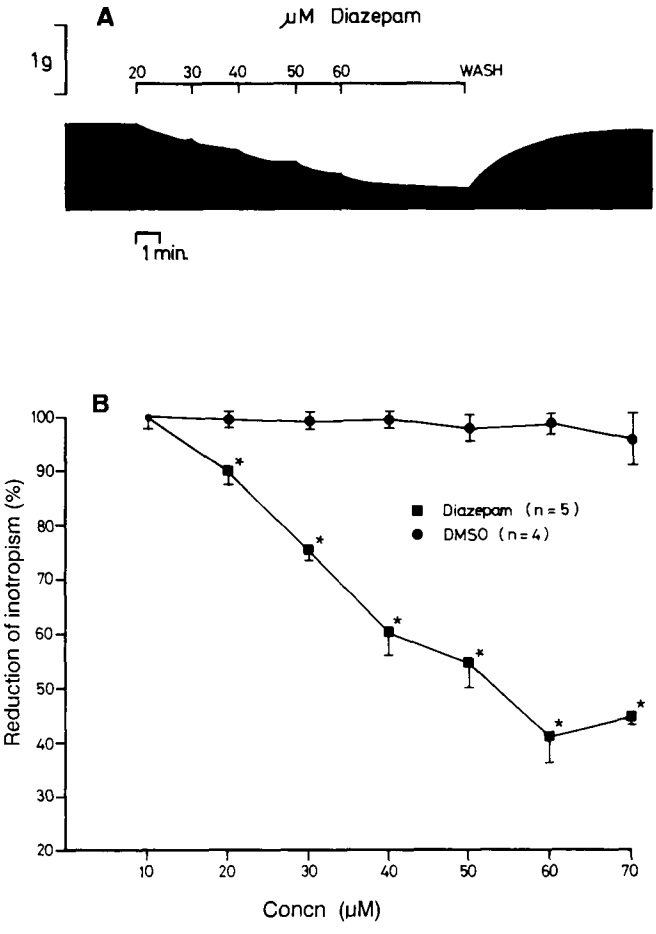

FIG. 1. A. Effects of diazepam on isometric contractions produced by electrical stimulation (1·5 Hz) in rat isolated right ventricular strips. B. Cumulative concentration-response curve for diazepam and dimethylsulphoxide (DMSO) on electrically driven right ventricular strips of the rat. Concentrations of DMSO were the same as those present in the diazepam solution. The results are expressed as percentage changes of basal contractions. Each point represents the mean ± s.e.m. (vertical bars). * $P < 0.05$.

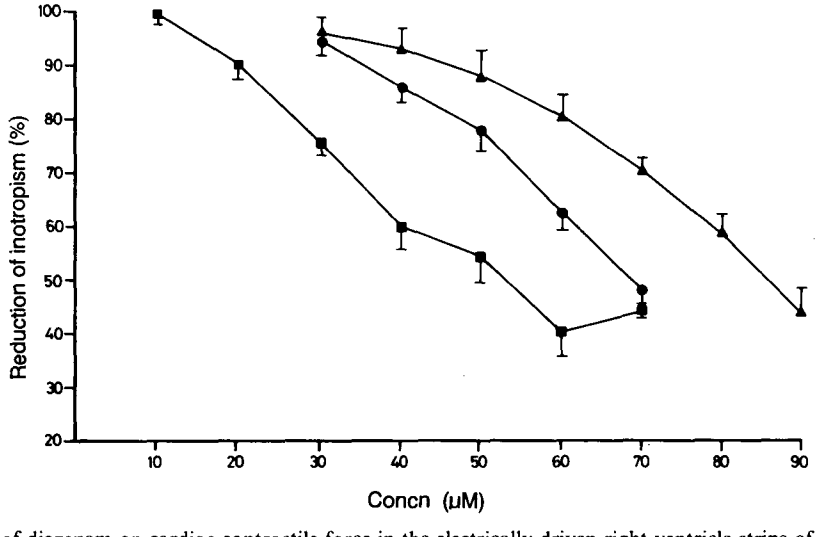

FIG. 2. Effect of diazepam on cardiac contractile force in the electrically-driven right ventricle strips of the rat in the absence (■; n = 5) and in the presence of PK 11195 (1 μM ●; n = 5) of flumazenil (1 μM ▲; n = 4). Further details as in legend to Fig. 1.

concentration-response curve to diazepam. The ID50 of diazepam ($51{\cdot}8 \pm 5{\cdot}3$ μM) also was significantly increased in the presence of either flumazenil ($86{\cdot}0 \pm 2{\cdot}2$ μM) or PK 11195 ($67{\cdot}8 \pm 2{\cdot}2$ μM). These changes in the ID50 values were statistically significant ($P < 0{\cdot}05$).

Discussion

The present results show that diazepam (10–60 μM) reduces cardiac contractility in the isolated right ventricle of the rat. This effect was antagonized by both flumazenil and PK 11195 which are respectively central- and peripheral-type benzodiazepine antagonists.

The negative inotropic effects observed are in contrast with the positive inotropic effect reported by others (Castillo-Ferrando et al 1985; Gonzalez et al 1990). It is difficult to explain this discrepancy but it may be related to the different cardiac preparations used in these studies. Castillo-Ferrando et al (1985) used isolated left atria whilst a Langendorff preparation was used by Gonzalez et al (1990).

The effect of diazepam was antagonized by PK 11195. This suggests that activation of peripheral-type receptors are involved in this effect of diazepam. The existence of peripheral receptors for diazepam in the rat ventricular myocardium is well established (Anholt et al 1985) and they seem to be involved in the pharmacological actions of diazepam on the heart (Mestre et al 1984). Interestingly, the central benzodiazepine receptor antagonist flumazenil also antagonized the negative inotropic action of diazepam. This is at variance with other reports indicating that the central-type benzodiazepine receptors are not involved in the cardiac actions of diazepam. For instance, it has been shown that diazepam decreases in a dose-dependent manner the duration of the cardiac action potential of the guinea-pig heart preparation (Mestre et al 1984) as well as ventricular automaticity in the rat (Ruiz et al 1989). These effects were antagonized by PK 11195, but not by flumazenil. Furthermore, both diazepam and ROS-4864, which is a selective agonist of the peripheral-type receptors, reduced the force of contraction in guinea-pig papillary muscles. Flumazenil failed to block these effects which are effectively antagonized by PK 11195 (Mestre et al 1984). However, it has been recently shown that receptors like those found in the central nervous system could also mediate the inotropic effect of diazepam. Gonzalez et al (1990) showed that flumazenil (10 μM) antagonizes the inotropic effects of diazepam in the Langendorff rat heart. Our data thus agree with these results and also support an involvement of the central benzodiazepine receptors in the effects of diazepam on cardiac contractility. The possibility that some endogenous negative inotropic agents could be involved in the cardiac effects of diazepam should not be discarded. In fact, it has been shown that benzodiazepines produce sensitization of rat heart to the negative inotropic effect of adenosine (Kenakin 1982). However, acetylcholine is probably not involved in this effect, since benzodiazepines seem to produce a vagolytic action in the rat heart (DiMicco 1987). An interference with calcium transport could also mediate this effect since benzodiazepine seems to have some calcium antagonist action in the heart (Mestre et al 1984; Ruiz et al 1989).

In conclusion, our results indicate that diazepam reduces cardiac contractility in the rat and this effect seems to be mediated by both peripheral and central type benzodiazepine receptors. This could mediate the diazepam induced reduction in cardiac output (Rao et al 1973) and may have some clinical relevance because of the wide therapeutic use of benzodiazepines.

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