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The Effect of Diazepam on Ventricular Automaticity Induced by a Local Injury. Evidence of Involvement of "Peripheral Type" Benzodiazepine Receptors

F. RUIZ,* J. HERNÁNDEZ AND D. PÉREZ*

*Department of Pharmacology, University of Murcia, Medical School, Murcia, *Department of Biostatistics, University of Murcia, Medical School, Murcia, Spain*

Abstract—The effect of diazepam on ectopic cardiac automaticity has been examined in rats. We also investigated whether "central" or "peripheral type" benzodiazepine receptors are involved, as well as the role of calcium, on the possible effect of diazepam, by studying the interaction of this drug with either GABA, picrotoxin, RO 15-1788, PK 11195, diltiazem or Bay K 8644. A local injury of the rat isolated right ventricle produced a sustained abnormal rhythm which was completely abolished by diazepam (30–50 μM). This effect was not modified by the presence of either GABA (100 μM) picrotoxin (2 μM) or RO 15-1788 (5 μM) but it was reduced by the antagonist of "peripheral type" benzodiazepine receptors PK 11195 (0·1 μM). On the other hand the calcium channel blocker diltiazem (5 μM) and the calcium channel activator Bay K 8644 (3 nM), respectively, potentiated and reduced the effect of diazepam. It is concluded that diazepam effectively reduces ectopic cardiac automaticity in the rat. The "central type" benzodiazepine receptors are not involved in this effect, but it seems to be, at least, partially mediated by "peripheral type" receptors and is a calcium-dependent phenomenon.

There is experimental (Ando et al 1979) and clinical (Van Loon 1968) evidence that diazepam possesses antiarrhythmic actions. Different mechanisms such as inhibition of sympathetic influences (Gillis et al 1974) or local anaesthetic action (Ando et al 1979), have been proposed to explain this antiarrhymic effect. However, the mechanism responsible is still 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 with chloride channels. They display high affinity binding for diazepam and are inhibited by the specific antagonist RO 15-1788 or the convulsant agent picrotoxin (for review see Haefely et al 1985). Recently, a second class of diazepan binding site has been identified which is not coupled to the GABA receptor chloride channel complex (Marangos et al 1982) but seems to be associated with calcium channel function (Cantor et al 1984; Bender & Hertz 1985), the affinity of diazepam for these receptors is lower (Rampe & Triggle 1986). These so-called "peripheral type" receptors have been identified in kidney, heart and adrenals (Taniguchi et al 1982; Benavides et al 1983a). 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 1983b). The non-benzodiazepine PK 11195 (an isoquinoline carboxamide derivative), specifically binds to the peripheral sites and seems to antagonize the action of diazepam on these receptors (Mestre et al 1985).

We attempted to obtain further data supporting the antiarrhythmic activity of diazepam by studying its effect of an experimental model of ectopic automaticity in-vitro. A sustained abnormal rhythm was induced by a local injury of the isolated right ventricle of the rat (Hernández et al 1987). We also studied whether "central" or "peripheral" type receptors are involved in the effect of diazepam by investigating the interaction between this drug and either GABA, picrotoxin, RO 15-1788 or PK 11195 in this preparation. The possible role of calcium was also studied by investigating the interaction of diazepam with either an agonist or antagonist of calcium channels. We chose for this purpose the typical calcium antagonist, diltiazem, and the calcium channel facilitator, Bay K 8644. This agent is a novel dihydropiridine analogue which increases the transmembrane current through the slow channels by binding to the dihydropyridine receptor in or near voltage operated slow calcium channels (Schramm & Towart 1985).

A brief account of some of the results has already been reported (Hernández et al 1987).

Materials and Methods

The experiments were carried out on isolated preparations taken from Sprague-Dawley rats of either sex and set up as previously described (Hernández & Serrano 1982). We used a 30 mL organ bath with a porous plate at the bottom to ensure an effective aeration. The bathing solution (Tyrode) contained (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. The bathing solution was maintained at 37°C, pH 7·4 and bubbled with a mixture of 95% O_2 and 5% CO_2. The animals were killed by a blow on the head, the heart was removed and placed in warm Tyrode solution. The right ventricle was cut out and the atrial end was damaged by crushing it with a pair of Starling forceps and fixed to a metallic support. The apical end was attached to a Grass FT-03 force-displacement transducer by a nylon thread. The force of contractions was recorded on a

Correspondence to: F. Ruiz, Martínez-Corbalan, Departamento de Fisiología y Farmacología, Unidad Docente Farmacología, Facultad de Medicina, Espinardo (Murcia), Spain.

* F. Ruiz holds a studentship of Consejeria de Cultura y Educación de la Comunidad Autónoma de Murcia.

Dynograph Beckman poligraph. The resting tension was set at 1 g. For each experiment the preparation was allowed to stabilize for at least 10 min. Only ventricles which had a stable basal rate, with a variation of less than 10 beats min^{-1}, and contractile activity at the end of the stabilization period were accepted for study.

Drugs

The following drugs and compounds were used in this study: diazepam and RO 15-1788 (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate, generously supplied by Roche, Spain), GABA (Sigma UK), picrotoxin (Aldrich Milwaukee, USA), Diltiazem (gift from Lab. Esteve. Barcelona, Spain), PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(l-methylpropyl)-3-isoquinolinecarboxamide, gift from Pharmuka Lab. Gennevilliers Cedex, France), Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate, kindly supplied by Bayer AG, Wuppertal, FRG). Diazepam, RO 15-1788, Bay K 8644 and PK 11195 were freshly dissolved in dimethylsulphoxide (DMSO obtained from Probus. Barcelona, Spain) and saline (4 DMSO:6 saline); GABA and picrotoxin were dissolved in saline. This stock was diluted into prewarmed and preaerated bathing solution to achieve the desired final concentration. As Bay K 8644 was sensitive to light, all experiments with this agent were carried out in a dark room using red light.

Drugs were added to the bath in 0·1 mL aqueous solution. The range of concentrations tested was from 10^{-9} M to 5×10^{-5} M. A drug effect was defined as the maximal change observed in the contractile frequency of the preparation recorder in the first minute that followed drug addition to the bathing solution.

Statistics

To ascertain whether the inhibitory effect of diazepam is modified by the presence of the different agents, we measured the corresponding ID50 for diazepam in the absence and presence of each compound. ID50 is defined as the mean drug concentration that reduced ectopic ventricular automaticity by 50% and was calculated by analysis of the linear regression of the logit of response versus log dose. In the analysis of differences between doses of drugs and between different drugs, we used a two-way analysis of variance and then Turkey's test for individual comparisons. P values of 0·05 or less were considered to represent significant differences.

Results

We performed 60 experiments in which a persistent spontaneous automaticity appeared using the technique described above. The mean ventricular rate was 81 ± 3 beats min^{-1}.

The effect of diazepam

Figure 1, shows a typical response to addition of diazepam (30 μM) to a bathing solution containing a right ventricle with ectopic automaticity. As can be seen, diazepam abolished the automatic activity of the preparation during the time this drug was present in the medium, the suppressive effect appearing within the first minute after drug administration.

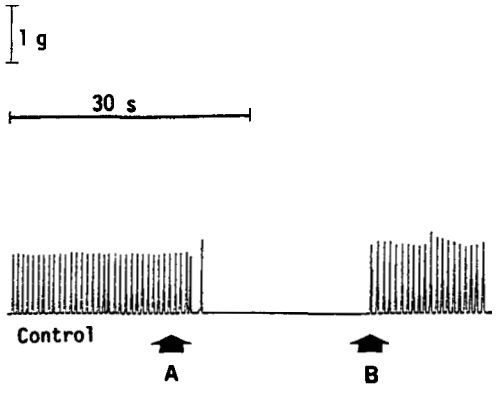

FIG. 1. (A) Effect of diazepam (30 μM) on the induced automaticity in isolated right ventricle of the rat. (B) after washing, recovery of spontaneous rate of discharge.

After washing out diazepam, the ventricular rate returned to control values.

The effect of diazepam was concentration-dependent as can be concluded from results summarized in Fig. 2; the reduction caused by diazepam (10 μM) on ventricular automaticity was about 10-15% of the control rate, 30 μM almost completely abolished ventricular automaticity and 50 μM of diazepam consistently abolished ventricular automaticity. Concentrations of diazepam lower than 10 μM were virtually devoid of effect on ventricular automaticity.

The effect of diazepam cannot be attributed to its solvent

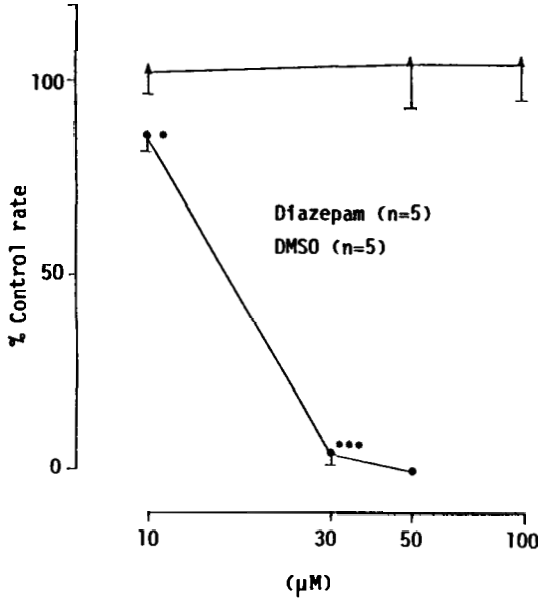

FIG. 2. Dose-response curve for the effect of diazepam (▲) and DMSO (●) on ventricular automaticity. Both agents were applied in a cumulative manner. The concentrations of DMSO were the same as those present in the diazepam solutions. A drug's effect was defined as the maximal change observed in the automatic frequency of the preparation recorded in the first min which followed the addition of each drug into the bathing solution. The results are expressed as percentages of the control frequency recorded in the min that preceded the addition of the drug (pre-drug period) into the bath. Each point represents the mean ± s.e.m. (vertical bars). The average ventricular rate ± s.e.m. during the pre-drug period for diazepam was $93{\cdot}4 \pm 3{\cdot}3$ beats min^{-1} and for DMSO was $98{\cdot}4 \pm 3{\cdot}3$ beats min^{-1}. * Indicates $P < 0{\cdot}05$ *** Indicates $P < 0{\cdot}001$.

DMSO since, as shown in Fig. 2, this solvent did not cause significant changes in the ectopic ventricular rate.

Diazepam in the presence of either GABA, picrotoxin or RO 15-1788

To know whether the effect of diazepam could be attributed to activation of a receptor like that found in the central nervous system, it was decided to study the action of diazepam in the presence of either GABA (100 μM) or the antagonists of the benzodiazepine/GABA/channel chloride receptor complex, picrotoxin (2 μM) or RO 15-1788 (5 μM). Each of these agents was devoid of effect on ventricular automaticity at those concentrations and was added to the organ bath 3–5 min before the concentration-response curve for diazepam.

The inhibitory effect of diazepam on ectopic automaticity was not modified by the presence of either GABA or picrotoxin (Fig. 3). In the presence of 5 μM RO 15-1788, diazepam (10 μM) causes a small, but not statistically significant, increase in ventricular automaticity. Higher concentrations of diazepam in the presence of RO 15-1788 inhibited ventricular automaticity similarly to their effects in the absence of RO 15-1788 (Fig. 4). On the other hand, higher concentrations (50–100 μM) of RO 15-1788 itself reduced ventricular automaticity by about 28% at each concentration ($P < 0.05$).

Diazepam in the presence of PK 11195

Fig. 5 illustrates a concentration-response curve for diazepam obtained in the absence and in the presence of 0·1 μM PK 11195. This concentration of PK 11195 was virtually devoid of effect on ventricular automaticity, but shifted to the right the concentration-response curve obtained for diazepam. For example, diazepam 30 μM which decreased ventricular rate by 95% when compared with the control rate, only reduced it by 34% in the presence of 0·1 μM of PK 11195-($P < 0.001$). The ID50 for diazepam was significantly changed in the presence of PK 11195 (Table 1).

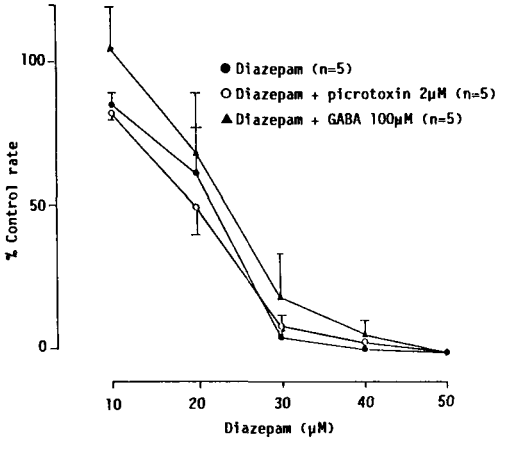

FIG. 3. Effect of diazepam on ventricular automaticity in the absence (●) and in the presence of GABA (▲) or picrotoxin (○). Average ventricular rate ± s.e.m. during the pre-drug period for diazepam-+GABA was 73·8 ± 7·8 beats min^{-1}, and for diazepam + picrotoxin 74·2 ± 8·3 beats min^{-1}. Further details as in legend to Fig. 2.

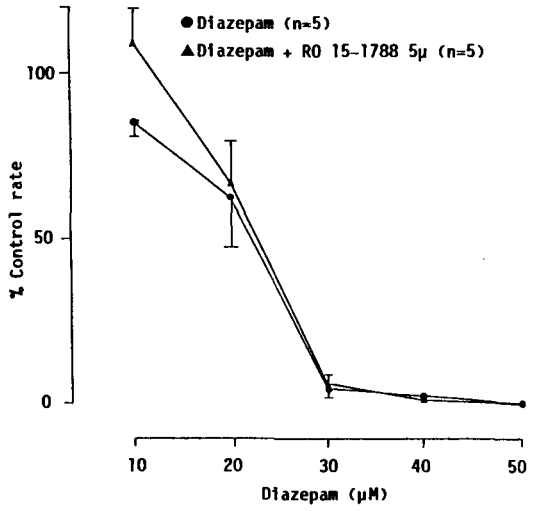

FIG. 4. Effect of diazepam on ventricular automaticity in the absence (●) and in the presence of RO 15-1788 (▲). Average ventricular rate ± s.e.m. during the pre-drug period for diazepam + RO 15-1788 was 64·4 ± 7·6 beats min^{-1}. Further details as in legend to Fig. 2.

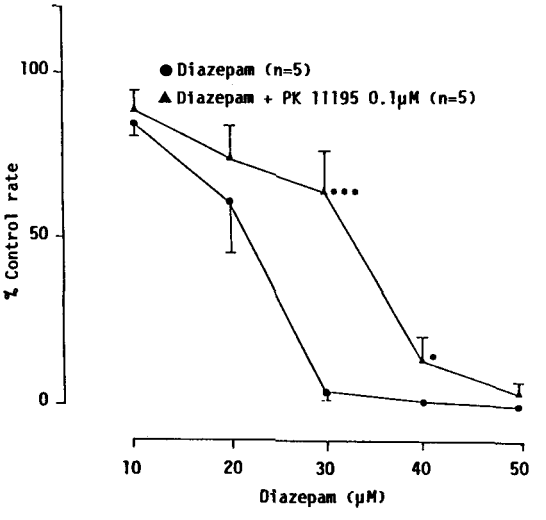

FIG. 5. Effect of diazepam on ventricular automaticity in the absence (●) and in the presence of PK 11195 (▲). Average ventricular rate ± s.e.m. during the pre-drug period for diazepam + PK 11195 was 74·2 ± 11·5 beats min^{-1}. * Indicates $P < 0.05$, *** Indicates $P < 0.001$. Further details as in legend to Fig. 2.

Table 1. ID50 of diazepam in absence and in the presence of different drugs.

Drug	n	ID50 × 10^{-5}M (mean ± s.e.)	P
Diazepam	5	1·77 ± 0·17	
+			
GABA (100 μM)	5	2·15 ± 0·27	N.S.
Picrotoxin (2 μM)	5	1·72 ± 0·09	N.S.
RO 15-1788 (5 μM)	5	1·99 ± 0·10	N.S.
PK 11195 (0·1 μM)	5	2·52 ± 0·22	< 0·05
Diltiazem (5 μM)	5	1·11 ± 0·17	< 0·05
Bay K 8644 (3 nM)	5	4·871 ± 1·07	< 0·02

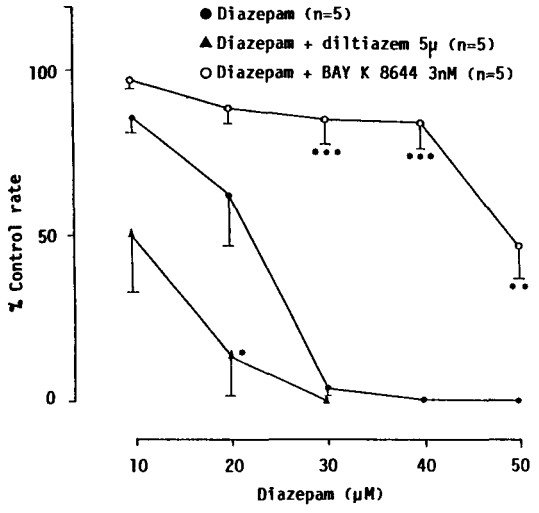

FIG. 6. Effect of diazepam on ventricular automaticity in the absence (●) and in the presence of diltiazem (▲) or Bay K 8644 (○). Average ventricular rate ± s.e.m. during the pre-drug period for diazepam + diltiazem was 76·9 ± 14·6 beats min^{-1} and for diazepam + Bay K 8644 94·6 ± 13·3 beats min^{-1} * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Further details as in legend to Fig. 2.

Diazepam in the presence of diltiazem or Bay K 8644

Dose-response curves for diazepam were constructed in the absence and in the presence of either diltiazem or Bay K 8644 in concentrations devoid of effect on ventricular automaticity.

Diltiazem (5 μM) as shown in Fig. 6, caused a consistent potentiation of the inhibitory action of diazepam on ventricular automaticity. The ID50 of diazepam was also significantly reduced in the presence of diltiazem (Table 1). On the contrary, the calcium channel facilitator Bay K 8644 (3 nM) reduced the effect of diazepam and significantly increased its ID50 (Fig. 6, Table 1).

Discussion

The present results show that diazepam in concentrations higher than 1 μM abolishes ectopic ventricular automaticity induced by a local injury. The effect was not modified by addition of either GABA, picrotoxin or RO 15-1788. However, PK 11195 reduced the effect of diazepam on ventricular automaticity.

Two different binding sites for diazepam have been identified. The "central" receptor has nanomolar affinity for diazepam and the effect of diazepam on this receptor is potentiated by GABA and antagonized by RO 15-1788 or picrotoxin (Haefely et al 1985). The "peripheral" receptor is activated by micromolar concentrations of diazepam (Driessen et al 1984). In the present work the effect of diazepam was unaffected by GABA but was antagonized by PK 11195. This suggests that activation of "peripheral" receptors are involved in these effects of diazepam. The existence of "peripheral" receptors for diazepam in the rat heart is well established (Taniguchi et al 1982) where they are distributed through the ventricular tissue (Anholt et al 1985). These receptors seem to be involved in the pharmacological actions of diazepam on the heart. For instance, it has been shown that diazepam decreases in a dose-dependent manner the duration of the cardiac action potential and the contractility of the guinea-pig heart preparation. These effects were not modified by the presence of GABA, and were antagonized by PK 11195 but not by RO 15-1788 (Mestre et al 1984).

The central benzodiazepine antagonist RO 15-1788, which did not antagonize the effect of diazepam in the present work, when applied on its own also reduced the spontaneous frequency. This effect could probably be related to the capacity of RO 15-1788 to depress cellular electrophysiological activity (Polc et al 1981).

There is evidence pointing to an association between calcium channels and the peripheral diazepam binding site (Holck & Osterrieder 1985). In the present study, the effect of diazepam on cardiac automaticity was potentiated by the calcium channel antagonist diltiazem and inhibited by the calcium channel activator Bay K 8644. Thus, diazepam seems to have some calcium antagonist action. This agrees with the fact that diazepam depresses the calcium-dependent action potential, an effect which is independent of GABA (Cherubini & North 1985), and is also consistent with the finding that selective stimulation of the "peripheral type" diazepam receptor mimics both the mechanical and electrical properties of the calcium channel blocker nifedipine in the isolated guinea-pig papillary muscle (Mestre et al 1984). Furthermore, it has been shown that diazepam caused a significant inhibition of the endogenous calcium-calmodulin-stimulated phosphorylation of several major membrane proteins. This effect is stereospecific and produced by membrane-bound diazepam (De Lorenzo et al 1981). It has also been demonstrated that nitrendipine, which specifically interacts with the voltage-dependent calcium channel receptor could be displaced by both diazepam (Bender & Hertz 1985) or the selective agonist of the "peripheral type" receptor, RO 5-4864 (Cantor et al 1984; Rampe & Triggle 1987). Thus, our data appear to suggest an involvement of calcium channels in the inhibitory effects of diazepam.

Other effects of diazepam might also contribute to its inhibitory action on ventricular automaticity, since diazepam enhances the inhibitory effect of exogenous adenosine on ventricular automaticity (Ruiz et al 1988) and also has some local anaesthetic activity (Ando et al 1979).

In conclusion, our results indicate that diazepam abolishes ectopic automaticity induced by local injury in the isolated right ventricle of the rat. This effect was unaffected by GABA but seems to be, at least partially mediated by peripheral benzodiazepine sites and is a calcium-dependent phenomenon.

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