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Short communication

Reversal of benzodiazepine-induced renal vasculature relaxation with flumazenil

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Abstract

The effects of the central-type benzodiazepine receptor antagonist flumazenil on renal vascular tone and its ability to reverse the benzodiazepine-induced vasodilation were investigated. The isolated and perfused rat kidney model was used. Flumazenil was unable to modify renal vascular resistance under basal conditions and in noradrenaline-pretreated kidneys. Relaxation induced by diazepam or clonazepam of noradrenaline-preconstricted renal vasculature was blunted by 10 μM flumazenil. These results suggest that central-type benzodiazepine receptors could be involved in benzodiazepine-induced renal vasodilation.

Keywords: Benzodiazepine; Kidney; Vasculature; (Rat)

1. Introduction

Benzodiazepines are widely used anxiolytic, sedative, hypnotic, anticonvulsant and muscle relaxant drugs. In addition to their therapeutic actions, they have been shown to exert peripheral pharmacological effects, including reduction of noradrenaline-induced chronotropic responses in rat atria (Elgoyhen and Adler-Graschinsky, 1989) and relaxation of the smooth muscles of different animal tissues (French et al., 1989; Escubedo et al., 1992; Yamakage et al., 1999). In previous works, we reported the ability of benzodiazepines to promote direct effects on renal function, such as diuresis and natriuresis (Monasterolo and Elías, 1993; Monasterolo et al., 1995). Moreover, clonazepam, which has selective affinity for central-type benzodiazepine receptors, and the mixed agonist diazepam, which has affinity for both peripheral- and central-type receptors, induced relaxation of the preconstricted renal vasculature (Monasterolo et al., 1995).

Flumazenil is a specific antagonist at the central-type benzodiazepine receptor with minimal positive intrinsic efficacy (Haefely, 1988). It is therapeutically used to reverse the effects of benzodiazepine overdose (Weinbroum et al., 1997).

This study investigated the effects of flumazenil on the renal vasculature and its ability to reverse the diazepam- and clonazepam-induced vasodilation. Since the isolated perfused kidney model offers the advantage of circumventing non-renal factors that influence the "in vivo" model (Bekersky, 1983), it is an appropriate model to attain our aim. The results obtained show that flumazenil does not exert direct effects on renal vascular resistance and blunts the diazepam- or clonazepam-induced relaxation of the noradrenaline-preconstricted renal vasculature.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 300 to 350 g, were housed at 21–24 °C under a 12-h dark–light cycle and maintained on a standard diet and water ad libitum. Experiments were performed in accordance with the European Community guidelines for the use of experimental animals and were approved by our institutional ethics committee.

2.2. Experimental procedures

Animals were anesthetized with sodium thiopental (70 mg/kg body wt., i.p.). The right kidney was prepared and

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perfused as previously described (Elías et al., 1985, 1987). The perfusion medium (pH=7.40) consisted of Ringer–Krebs solution supplemented with dextran 2% (Sigma, St. Louis, MO; average MW 82,200) as a colloid-osmotic agent, 10 mM glucose, 5 mM sodium pyruvate, 5 mM sodium lactate, 0.5 mM cysteine, 0.5 mM glutamic acid and 2.3 mM glycine. The entire system operated under thermostatic control at 37 °C. Perfusion through the isolated kidney "in situ" was performed with the use of a peristaltic pump (Masterflex, USA). Flow rate was measured with a flowmeter (Gilmont, USA) inserted in the arterial line. Perfusion pressure was continuously measured at the tip of the arterial cannula by means of a pressure transducer (Gould P231D, USA) and recorded on a multipen recorder (Rikadenki, Japan).

Basal flow rates and perfusion pressures of the isolated rat kidneys reached a stable plateau 15–20 min after the onset of artificial perfusion. After a 30-min equilibration period, noradrenaline was added to the perfusion medium to induce vasoconstriction. A perfusate noradrenaline concentration of 1 μM, which induced ~75% of the maximal contractile response to noradrenaline, was provided throughout the experiments. A stabilization period of 15 min with 1 μM noradrenaline was allowed. The subsequent experimental time was 25–35 min, during which perfusion pressure at constant flow was measured in the presence of increasing concentrations of diazepam (0.5–580 μM), clonazepam (2–500 μM) or flumazenil (0.2–500 μM). The final concentrations of benzodiazepines were attained by adding cumulative amounts to the perfusion medium at the beginning of each perfusion pressure measurement period. Perfusion pressure measurements were performed when the recording graph reached a steady state (up to 5 min) after the addition of each dose. Other series of experiments were carried out to evaluate the renal vascular response to diazepam or clonazepam in the presence of 10 μM flumazenil. The latter was added to the perfusion medium 10 min prior to the addition of noradrenaline (20 min after the onset of perfusion, when flow rate and perfusion pressure had reached stable plateaus).

Complete reversal of the increase in perfusion pressure produced by 1 μM noradrenaline was considered 100% relaxation. Concentration–response curves were fitted by using a computer program (Pharmacologic Calculation System 4.0). For each curve, the concentration required for 35% relaxation (EC_{35}) was obtained. All data are expressed as means±S.E.M. Differences between means were analyzed by Student's *t*-test. *P* values less than 0.05 were considered significant.

2.3. Chemicals

Chemicals were of the highest grade available commercially. Diazepam, clonazepam and flumazenil were generous gifts from Productos Roche (Buenos Aires, Argentina). All benzodiazepines were freshly prepared in ethanol. A group of preparations was treated with the corresponding volumes of vehicle.

3. Results

3.1. Renal perfusion parameters

After the equilibration period, basal perfusion conditions were characterized by a flow of 22±1 ml/min and a pressure of 125±4 mm Hg. Constant flow was maintained throughout the experiments. Addition of noradrenaline to the perfusion medium caused a rise in perfusion pressure, which reached a value of 246±6 mm Hg after the stabilization period. No differences in basal or noradrenaline-induced perfusion pressure between experimental groups were found.

3.2. Effects of diazepam, clonazepam and flumazenil on noradrenaline-preconstricted renal vasculature

In the control group, the vasoconstriction achieved with 1 μM noradrenaline remained unchanged throughout the experiments and was not modified by the addition of the corresponding volume of the benzodiazepine vehicle. Diazepam and clonazepam induced a concentration-dependent relaxation of the noradrenaline-contracted renal vasculature (Fig. 1). The responses to flumazenil did not differ from those obtained with the vehicle.

3.3. Effects of diazepam and clonazepam in the presence of flumazenil

The basal perfusion pressure (123±1 mm Hg) of these preparations (*n*=8) was not modified by the addition of

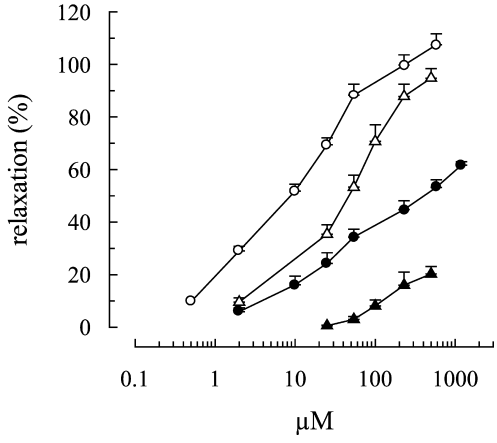

Fig. 1. Effects of benzodiazepines on the noradrenaline-preconstricted renal vasculature. Values are expressed as means±S.E.M. (*n*=4–7). One hundred percent relaxation represents the complete reversal of the increase in perfusion pressure produced by 1 μM noradrenaline. ○, diazepam; △, clonazepam; ●, diazepam+flumazenil; ▲, clonazepam+flumazenil.

Table 1
Relative potency of diazepam and clonazepam to relax noradrenaline-precontracted renal vasculature, in the absence and in the presence of 10 μM flumazenil

	EC_{35} (log μM)[a]
Diazepam	0.49±0.04[b]
Diazepam+flumazenil	1.85±0.18[c]
Clonazepam	1.39±0.05
Clonazepam+flumazenil	>3

Flumazenil was added 10 min before the addition of 1 μM noradrenaline and was present up to the end of the experiment.
[a] Concentration of benzodiazepine required to reach 35% relaxation.
[b] Values are expressed as the means±S.E.M. (n=4–7).
[c] P<0.01 vs. diazepam.

flumazenil (10 μM) to the perfusate. Concentration–response curves for diazepam and clonazepam were recorded in the presence of flumazenil. This agent caused a rightward shift in both curves, as compared with those obtained in the absence of flumazenil (Fig. 1). EC_{35} values for diazepam and clonazepam were significantly increased in the presence of flumazenil. These data are collected in Table 1.

4. Discussion

The ability of micromolar concentrations of benzodiazepines to induce relaxation of vascular smooth muscle was previously described by our group (Monasterolo et al., 1995) and other investigators (French et al., 1989; Elgoyhen et al., 1993; Gimeno et al., 1994).

The present experiments show that, in addition to the reported vasodilator effects of benzodiazepines, the central-type benzodiazepine receptor antagonist, flumazenil, antagonizes the renal vascular relaxation induced by diazepam or clonazepam. To our knowledge, this is the first report that describes the reversal by flumazenil of benzodiazepine-induced vasodilation in a peripheral isolated preparation. It is noteworthy that flumazenil did not modify renal vascular resistance under basal conditions or in noradrenaline-pretreated kidneys. In view of these findings, central-type benzodiazepine receptors appear to be involved in the benzodiazepine-induced renal vasodilation. In accordance with these results, it has been reported that the lorazepam-induced changes in regional cerebral blood flow are reversed by flumazenil, indicating that this effect is mediated through GABA-benzodiazepine receptors (Matthew et al., 1995). Further support for our assumption comes from observations that the $GABA_A$ antagonist bicuculline increases renal perfusion pressure (Monasterolo et al., 1996). Despite these results, other matters should be considered. The rank order of potency of several benzodiazepines to compete with the mixed benzodiazepine [^{3}H]flunitrazepam for its renal binding site indicates that [^{3}H]flunitrazepam binds to peripheral-type benzodiazepine receptors in kidney membranes (Beaumont et al., 1984). These authors described that unlabeled clonazepam was not effective in displacing [^{3}H]flunitrazepam specific binding except in micromolar concentrations. The low density or absence of central-type benzodiazepine receptors in peripheral tissues is a property that distinguishes these receptors from peripheral-type binding sites (Krueger, 1991). However, Hernández (1991) found that the negative inotropic effect of diazepam in rat ventricular strips was antagonized by flumazenil, suggesting the involvement of central-type benzodiazepine receptors in this diazepam-induced effect. Other studies showed that flumazenil antagonized the diazepam-induced potentiation of GABA contractions in guinea-pig ileum (Luzzi et al., 1986). The discrepancy between the results of pharmacological studies and receptor characterization studies may be because certain experimental situations in saturation and displacement experiments may not allow the detection of receptor heterogeneity or the real proportion of receptors (Swillens et al., 1995). The existence of a low-affinity (micromolar) benzodiazepine binding site in peripheral tissues, like that identified in the central nervous system (Taft and DeLorenzo, 1984), cannot be precluded.

In summary, the fact that flumazenil "per se" does not affect renal vascular resistance but can antagonize diazepam- or clonazepam-induced effects encourages us to propose that benzodiazepine-evoked renal vasodilation could be mediated by an interaction with a specific binding site. Further investigations to properly characterize this binding site are required. Elucidation of the mechanism of action involved in benzodiazepine-induced vascular relaxation would be helpful in designing more effective and selective compounds.

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