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Flumazenil does not antagonize the cardiac effects of midazolam in the isolated rat heart-lung preparation

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Abstract: We examined the effects of midazolam and flumazenil on cardiac function and metabolism in the isolated rat heart-lung preparation. Wistar rats were divided into five groups (each group: $n = 8$) as follows: (1) control (saline); (2) flumazenil (1.3×10^{-5}M); (3) flumazenil (10^{-4}M); (4) midazolam ($60\,\mu g \cdot ml^{-1}$); and (5) midazolam ($60\,\mu g \cdot ml^{-1}$) and flumazenil (1.3×10^{-5}M). Systolic blood pressure and calculated left ventricular dP/dt maximum in the midazolam or midazolam conbined with flumazenil groups increased significantly in comparison with those in the control group. Heart rate in the midazolam group was lower than that in the control group. However, in the flumazenil group, there were no effects on the hemodynamics. There were no significant differences in the myocardial tissue concentration of ATP, lactate, and glycogen in all groups. In this study, midazolam decreased heart rate; however, flumazenil had no effect on the heart, nor did it antagonize the cardiac effects of midazolam. These results suggest that flumazenil has no effect on the peripheral-type benzodiazepine receptor of the myocardium.

Key words: Flumazenil, Midazolam, Negative chronotropic effect, Heart-lung preparation

Introduction

Midazolam is a water-soluble intravenous benzodiazepine used for sedation and induction of anesthesia [1–3]. The effect of midazolam on the systemic circulation is minimal in dogs [4,5] and humans [6,7]. However, Reves et al. [8] have reported that as a negative inotropic drug, midazolam is $2\frac{1}{2}$ times as potent as diazepam in the isolated rat heart.

Flumazenil is the first highly specific benzodiazepine antagonist, and it immediately reverses the central nervous effects of benzodiazepine. Intravenous doses ranging from 2.5 mg to 100 mg administered as a bolus injection to healthy subjects did not cause any changes of vital functions [9]. In another study [10], however, a significant decrease of blood pressure and pulse rate values was seen relative to the placebo after oral administration of flumazenil 30 mg–100 mg.

Thus, it is interesting to investigate whether midazolam or flumazenil affects cardiac function, and whether there is an interaction between midazolam and flumazenil. This study was undertaken to examine the direct effects of high-dose midazolam and flumazenil, and to investigate the interaction between high-dose midazolam and flumazenil with respect to function and metabolism in the isolated rat heart-lung preparation which eliminates any confounding neurohumoral effects of in vivo studies.

Materials and methods

The experiment was approved by the Animal Care Committtee of Yamanashi Medical University. The techniques were almost identical to those used in previous studies [11,12]. Briefly, 40 male Wistar-ST rats (300–320 g) were anesthetized with 4% isoflurane during the preparation. Tracheostomy was performed, and intermittent positive pressure ventilation was instituted with air. The chest was opened and the heart was arrested with ice-cold saline during the preparation. Cannulae were inserted into the aorta and the superior and inferior vena cavae. The cannula of the superior vena cava was used to monitor right atrial pressure. The heart-lung preparation was perfused with a solution containing red blood cells collected from another rat and Krebs-Ringer bicarbonate buffer with a hematocrit and pH of 25% and 7.4, respectively. The perfusate blood pumped from the aorta was passed through a pneumatic resistance, collected in a reservoir kept at

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37°C using a water jacket, and then returned to the inferior vena cava. No other organs except the heart and lung were perfused in this model, so cardiac output was determined by the inflow, provided the heart did not fail, and afterload was regulated by the pneumatic resistance.

All hearts were perfused initially at a cardiac output of 30 ml·min^{-1} and a mean arterial pressure of 70 mmHg by regulating the venous return and the pneumatic resistance. The value of pneumatic resistance was unchanged during the experiment. All animals were divided into five groups (each group: $n = 8$) as follows: (1) control (saline); (2) flumazenil (1.3×10^{-5} M, about 4 μg·ml^{-1}); (3) flumazenil (10^{-4} M, about 30 μg·ml^{-1}); (4) midazolam (60 μg·ml^{-1}); and (5) midazolam (60 μg·ml^{-1}) and flumazenil (1.3×10^{-5} M). Each drug was diluted in the saline and the volumes of administered drugs were equal. Midazolam was administered in the reservoir 8 min after the start of perfusion. Saline or flumazenil was administered in the reservoir 9.5 min after the start of the perfusion.

Heart rate (HR) was recorded with a bioelectric amplifier AB-621G, Nihonkohden, Tokyo, Japan) and cardiac output was measured with an electromagnetic blood flowmeter (MFV-1200, Nihonkohden). The aortic pressure and right atrial pressure were measured with carrier amplifiers (AP-601G, Nihonkohden) using transducers (TP-101T and LPU-0.1A, Nihonkohden). The rates of ventricular tension development (LV dP/dt_{max}) were calculated from aortic blood pressure obtained electronically [12].

Thirty minutes after the perfusion, the heart was removed and freeze-dried for 6 days. Adenosine triphosphate (ATP) was measured by reversed-phase high-performance liquid chromatography according to a modified version of the methods described by Wynants and Van Belle [13]. Lactate was measured spectrophotometrically by a standard technique [14]. Another portion of freeze-dried sample was placed in 30% potassium hydroxide and digested at 100°C. Tissue glycogen was extracted, hydrolyzed, and assayed as glucose equivalents [15]. The values were expressed as μmol·g^{-1} dry heart weight^{-1}.

Intergroup comparisons were made using one-way analysis of variance followed by the Dunnett test for comparisons with the control values. Intragroup comparisons were made using two-way analysis of variance with repeated measures followed by paired t-test with Bonfferoni modification. A probability of $P < 0.05$ was regarded as statistically significant. The data are given as mean ± SD.

Results

As cardiac output was kept constant by regulating the inflow, there was no significant difference in cardiac output among the groups during the perfusion. There was also no significant difference in right atrial pressure among the groups because not all hearts failed (Table 1). All hemodynamic parameters in the low- and high-dose flumazenil groups and the control group did not differ significantly during the perfusion. However, in the midazolam and midazolam combined with flumazenil groups, systolic blood pressure (SBP) and calculated LV dP/dt_{max} increased and HR decreased after the administration of drugs (Fig. 1). There were no significant differences myocardial ATP, lactate, and glycogen levels among the groups (Fig. 2).

Discussion

Reves et al. [8] have demonstrated a dose-related decrease in LV dP/dt_{max} after the administration of midazolam in a modified Langendorff rat heart. In addi-

Table 1. Effects of flumazenil and midazolam on cardiac output and right atrial pressure in the isolated heart-lung preparation

Time (min)	5	10	15	20	25	30
Cardiac output (ml·min^{-1})						
Control	30.5 ± 0.7	30.0 ± 0.9	30.3 ± 0.9	30.6 ± 0.9	30.7 ± 0.8	30.7 ± 0.8
Flumazenil (1.3×10^{-5} M)	30.5 ± 0.7	31.3 ± 1.3	31.5 ± 0.9	31.3 ± 0.7	31.5 ± 0.7	31.2 ± 1.0
Flumazenil (10^{-4} M)	29.8 ± 0.8	30.2 ± 0.8	30.5 ± 1.1	30.7 ± 1.1	30.6 ± 1.3	30.8 ± 1.3
Midazolam (60 μg·ml^{-1})	30.0 ± 0.5	30.7 ± 0.7	30.8 ± 0.3	30.6 ± 0.5	30.5 ± 0.5	30.5 ± 0.5
Midazolam + flumazenil (1.3×10^{-5} M)	30.6 ± 0.5	31.6 ± 1.1	31.8 ± 0.9	31.8 ± 0.9	31.7 ± 0.8	31.6 ± 1.0
Right atrial pressure (kPa)						
Control	3.6 ± 0.5	3.5 ± 0.5	3.4 ± 0.4	3.4 ± 0.4	3.4 ± 0.5	3.4 ± 0.5
Flumazenil (1.3×10^{-5} M)	3.8 ± 0.7	3.6 ± 0.5	3.5 ± 0.5	3.6 ± 0.6	3.6 ± 0.6	3.5 ± 0.5
Flumazenil (10^{-4} M)	3.6 ± 0.4	3.5 ± 0.5	3.3 ± 0.4	3.3 ± 0.4	3.3 ± 0.4	3.4 ± 0.4
Midazolam (60 μg·ml^{-1})	3.3 ± 0.5	3.2 ± 0.4	3.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.5
Midazolam + flumazenil (1.3×10^{-5} M)	3.5 ± 0.3	3.5 ± 0.2	3.4 ± 0.3	3.4 ± 0.3	3.4 ± 0.2	3.4 ± 0.3

Each group: $n = 8$.
Values are expressed as mean ± SD.

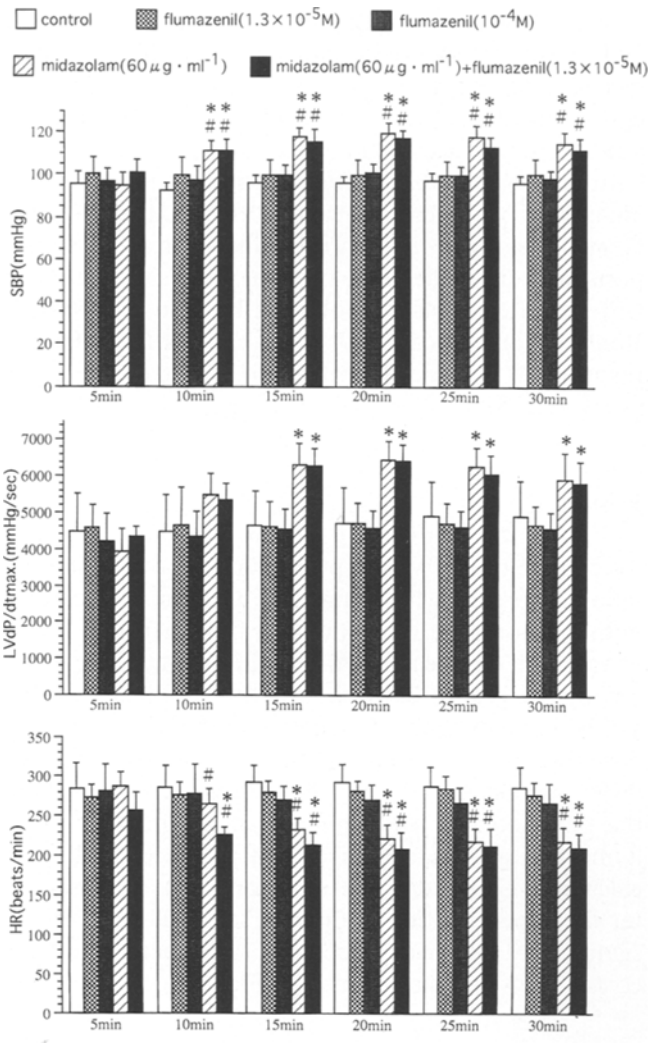

Fig. 1. Changes in systolic blood pressure, calculated ventricular tension development (LV dP/dt_{max}), and heart rate. $*P < 0.05$ as compared with control values. $^{\#}P < 0.05$ as compared with values at 5 min

tion, midazolam depresses cardiac function somewhat more strongly than thiopental and ketamine in isolated guinea pig hearts [16]. However, in concentrations necessary for induction of anesthesia, midazolam has minimal effects on cardiovascular function in dogs [5]. In clinical situations, midazolam, when used alone, did not show severe negative inotropic effects and did not induce regional myocardial dysfunction in patients with chronic coronary artery disease [17].

In contrast to these reports, our results indicate that 100 times the therapeutic doses [18] of midazolam did not show any negative inotropic effects and had negative chronotropic effects, but that flumazenil had no effect on the heart. In this preparation, midazolam decreased HR, and as the cardiac output and afterload (the pneumatic resistance) were constant, the stroke volume might have increased. This might in turn have increased the SBP and the calculated LV dP/dt_{max}. However, this explanation is not definitive. In a previous study [12], ketamine also decreased HR and increased SBP significantly but did not increase the calculated LV dP/dt_{max} in the same preparation. Although the mechanisms responsible for these actions remain unclear, it is likely that the central nervous system (CNS) is the primary target organ for the reported cardiac depressant effect of midazolam, and that the CNS plays a central role in the mediation of cardiovascular systemic effects of midazolam.

The reversal of benzodiazepine-induced sedation or anesthesia did not influence hemodynamics [19,20]. Intravenous bolus doses of flumazenil up to 100 mg were well tolerated in healthy human volunteers [9]. Moreover, in patients undergoing cardiac surgery, flumazenil did not modify cardiovascular parameters [21]. In experimental studies, flumazenil did not change blood pressure and HR at doses of up to 10 mg·kg^{-1} in anesthetized dogs and had no effects on HR and the contractility in guinea pig papillary muscle at up to 10^{-4} M [22]. These results, which are consistent with our study, indicate that flumazenil does not cause any hemodynamic changes at about 100 to 1000 times the usual dosage.

So-called peripheral-type benzodiazepine binding sites have been described in kidney, heart, mast cells, platelets, adrenals, and even in the brain. Flumazenil inhibits the central effects of benzodiazepine by competitive interaction at brain-type receptors and does not have an affinity for peripheral-type receptors [23–25]. Thus, it is likely that there were no effects mediated by peripheral receptors on cardiac function in the isolated rat heart-lung preparation.

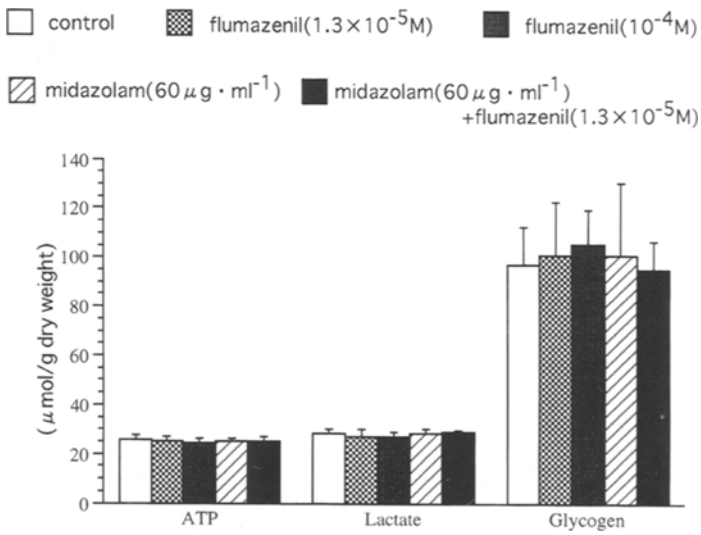

Fig. 2. Myocardial tissue concentrations of adenosine triphosphate (*ATP*), lactate, and glycogen

We have previously reported that 100 times the therapeutic dose of lidocaine induced myocardial depression and decreases of myocardial ATP content [11]. In this study, there were no significant differences in myocardial ATP, lactate, and glycogen content among the groups (Fig. 2). These results indicate that high doses of midazolam and flumazenil did not have any myocardial depressant effects.

A limitation of our use of the isolated heart preparation is that it is not an entirely suitable model for projection of clinical impressions. However, there is evidence that 100 times the therapeutic dose of midazolam did not show any negative inotropic effects. Therefore, the reported cardiodepressant effect of midazolam may be due to a lesser extent to its central nervous effect. Flumazenil, even at high doses, has no direct effect on cardiac function mediated by the peripheral-type benzodiazepine receptor.

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