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Effects of Flumazenil on Function and Metabolism in the Ischemic Working Rat Heart

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Abstract

We examined the effects of benzodiazepine antagonist, flumazenil, on myocardial function and metabolism in the ischemic working rat heart preparation. Animals were divided into three groups. The one was control group and the others were flumazenil, $10^{-5}$and 10^{-6} $M \cdot L^{-1}$ groups. The hearts were perfused with Krebs-Henseleit bicarbonate (KHB) buffer. After the control perfusion, whole heart ischemia was induced by clamping a one-way aortic valve bypass for 15 min followed by reperfusion for 20 min. In the flumazenil groups, flumazenil was administered to KHB buffer during the reperfusion period. The mechanical performance in all groups was impaired after ischemia, but there were no significant defferences in the recovery of cardiac output, peak systolic pressure, coronary flow and LV dP/dT maximum among the groups. Ventricular fibrillation occurred in all hearts except for one heart in the flumazenil 10^{-6}M group. There were also no significant differences in high energy phosphates, glycogen and lactate concentrations among the groups. These results suggest that flumazenil has no adverse effects during post-ischemic reperfusion.

Key words: Flumazenil, Myocardial ischemia, Myocardial metabolism, Working rat heart perparaton

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Introduction

Flumazenil is a new benzodiazepine antagonist. It has been used in reversing benzodiazepine-induced sedation for invasive procedure such as cardiac catheterization, intracardiac catheter ablation, intracoronary thrombolytic therapy and cardioversion[1~3]. It is also known that flumazenil has no adverse effects on the heart[4]. However, there are few reports regarding the effects of flumazenil alone on ischemic hearts. Thus it is important to delineate the cardiac effects of flumazenil in patients with ischemic heart disease.

In the present study, we used the ischemic working rat heart preparation to determine the direct effects of flumazenil on cardiac performance and metabolism, when it was administered during reperfusion.

Materials and methods

Approval from the Animal Ethical Committee of Yamanashi Medical University was obtained prior to initiating this study. Twenty-four 3-month-old male Wistar rats weighing 280–320 g were used. The animals were anesthetized with isoflurane. The hearts were then rapidly excised and perfused as a Langendorff preparation. Non-recirculating modified Krebs-Henseleit bicarbonate buffer solution was used as perfusate. The perfusate was maintained at 37.0± 0.3 ℃ and contained (mM) : NaCl 118, KCl 4.7, $CaCl_2$ 3.0, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, di-NaEDTA 0.5, and glucose 11. During the retrograde perfusion, the left atrium was connected via a pul-

monary vein to an angled steel cannula. After this preliminary perfusion, the heart was converted to working preparation by perfusing the left atrium and releasing the aortic out flow for a stabilization period of 10 min. A non-recirculation Krebs-Henseleit bicarbonate buffer was used also during the working heart perparation.

Left ventricular pressure (LVP) was measured with a transducer (P10EZ, Gould, Oxnard, CA) connected to a thin catheter (18G, Argyle Intramedicut Catheter, Sherwood, Tokyo) inserted into the left ventricle through the mitral valve from the angled steel cannula in the left atrium. Rates of pressure development (LV dP/dT) were measured from the derivatives of LVP obtained electronically. Aortic pressure was recorded with a Gould P10EZ transducer on a polygraph (Nihonkohden, Tokyo). Aortic outflow was recorded with an electromagnetic blood flow meter (MFV-3200, Nihonkohden, Tokyo). Coronary flow was measured by timed collection of the pulmonary artery outflow and surface run-off of the heart. Cardiac output was considered as the sum of the aortic and coronary outflows. At no time was the coronary effluent recirculated.

The solution was equilibrated with a gas mixture of 95% O_2 and 5% CO_2. Aortic oxygen tension was estimated by sampling perfusate from the atrial bubble trap on the left atrial line with a gas-tight syringe. For measurement of oxygen tension of coronary effluent, a catheter was placed in the pulmonary artery, from which samples were obtained with a gas-tight syringe. The oxygen tension was measured in an intermittently self-calibrating blood gas analyzer system (Instrumentation Laboratory Model 1306, Lexington, MA). Myocardial oxygen consumption ($M\dot{V}O_2$), was calculated as O_2 solubility multiplied by coronary flow per gram heart tissue multiplied by the difference between inflow O_2 and outflow O_2 tensions.

After additional 10 min of perfusion, whole heart ischemia was induced by clamping a one-way aortic valve bypass for 15 min. The aortic cannula was provided with sidearms both above the one-way valve. These sidearms were connected by a short length of Tygon tubing, which provided a bypass around the one-way valve for control perfusions. Ischemia was induced in this preparation by simply clamping the bypass tube. Since the largest fraction of coronary flow occurs during diastole, this one way valve severely restricted coronary perfusion, but did not influence aortic output or ventricular afterload[5]. Only during the ischemic period, the hearts were paced at 350 beats·min^{-1}. Reperfusion of the hearts after this ischemic period of 15 min was performed by declamping the one-way aortic valve bypass tube and lasted for 20 min. In the flumazenil groups, the perfusate contained flumazenil, 10^{-5} or 10^{-6} M, was used only during the reperfusion period. The recovery time of cardiac function was measured at the time required for the aortic flow to return to positive from the start of reperfusion.

At the end of perfusion, hearts were quickly frozen by clamping with a Wollenberger clamp cooled in liquid nitrogen and freeze-dried for 6 days. An aliquot was extracted with perchloric acid and centrifuged at 3000 r.p.m. Concentrations of high energy phosphates (ATP, ADP and AMP) were measured by the high liquid performance chromatography [6]. Lactate level was measured spectrophotometrically by standard techniques[7]. Another piece of freeze-dried sample was placed in 30% potassium hydroxide and digested at 100℃. Tissue glycogen was extracted, hydrolyzed and assayed as glucose equivalents [8]. The values were expressed as μmol per gram dry weight.

The data are expressed as means ± S.D. Testing for significant differences among the groups was accomplished by a one-way analysis of variance, followed by Duncan's multiple range test. The incidence of ventricular fibrillation was analyzed by a chi-square test. A probability of $P < 0.05$ was regarded as statistically significant.

Results

Although the mechanical performance of all hearts was impaired after ischemia, there were no significant differences in coronary flow, cardiac output, LV dP/dT max, heart rate and $M\dot{V}O_2$ among the three groups (Fig. 1, Table 1). The ventricular fibrillation occurred during reperfusion in all hearts except for one heart in

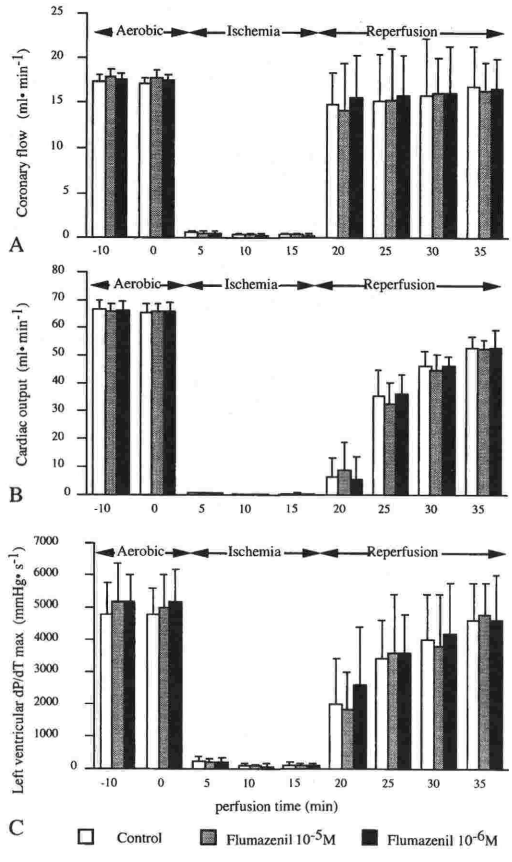

Fig. 1 Changes in coronary flow (A), cardiac output (B) and left ventricular dP/dT max. (C) of the control and flumazenil groups as a function of time. At zero time, whole heart ischemia was induced for 15 min followed by reperfusion for 20 min. Each bar represents mean ± S.D. for 8 hearts.

the flumazenil 10^{-6} M group. The duration of ventricular fibrillation and the recovery time of cardiac function are shown in Table 2. No significant differences were detected among the groups. There were also no significant differences in myocardial ATP, ADP, AMP, lactate and glycogen levels among the groups (Fig. 2).

Discussion

We demonstrated that ten to 100 times higher than usual dosage of flumazenil did not make any hemodynamic and metabolic differences during postischemic reperfusion period. These results are consistent with those of the studies indicating that flumazenil up to 10 mg·kg^{-1} of flumazenil administered intravenously did not change blood pressure, heart rate and left ventricular end-diastolic pressure in anesthetized dog and that flumazenil up to 10^{-4} M had no effects for heart rate in the isolated right atrium and the contractility in the isolated guinea pig papillary muscle[4]. The lack of hemodynamic response to administration of flumazenil may be related to the fact that no significant increases in levels of plasma catecholamine, cortisol, vasopressin and beta-endorphins have been observed[9~11]. But, we cannot conclude because our preparation is denervated, free from the neurotransmitter and intrinsic hormones originated from other organs and at a constant pre-load and after-load.

Flumazenil, the only benzodiazepine antagonist in clinical use, has an imidabenzodiazepine structure

Table1. Heart rate (HR), Myocardial oxygen consumption ($M\dot{V}O_2$) in the working rat heart

		ischemia			
Time (min)	-10	0	20	25	35
HR (beats·min^{-1})					
Control	318±40	320±36	123±89	220±62	306±46
Flumazenil 10^{-5} M	306±33	310±32	142±78	236±83	299±52
Flumazenil 10^{-6} M	312±46	310±38	146±90	242±69	310±58
$M\dot{V}O_2$ (μmoles·min^{-1}·gram^{-1})					
Control	41± 6	40± 5	22± 7	27± 8	33± 6
Flumazenil 10^{-5} M	39± 8	40± 6	23± 5	29± 5	34± 6
Flumazenil 10^{-6} M	39± 5	39± 6	23± 5	28± 5	34± 7

Each value is the mean ± S.D.

Table2. Duration of ventricular fibrillation (Vf) and recovery time of cardiac function in the working rat heart

	Control	Flumazenil10^{-5} M	Flumazenil10^{-6} M
Duration fo Vf (second)	185±55	197±43	201±66
Recovery time (second)	465±98	471±87	450±59

Each value is the mean ± S.D.
Recovery time; the time requred for the aortic flow to return to positive from the strat of reperfusion

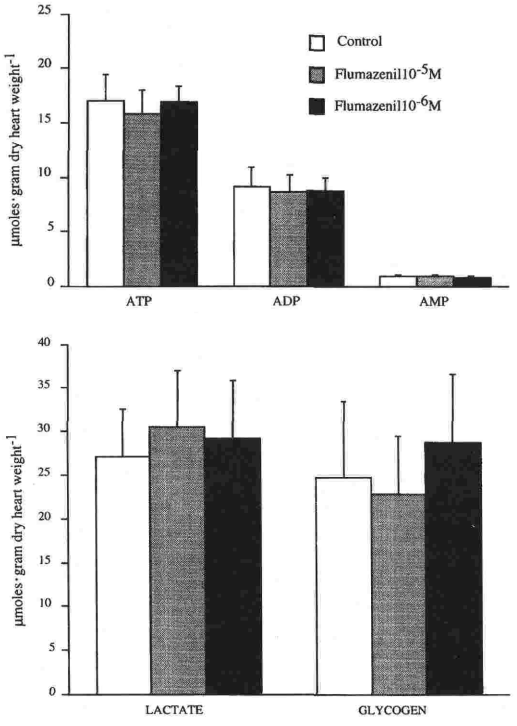

Fig. 2 Myocardial concentrations of ATP, ADP, AMP (upper panel), lactate and glycogen (lower panel). Each bar represents mean±S.D. for 8 hearts.

similar to midazolam. Flumazenil has a high affinity for the benzodiazepine receptor [12~14]. There are two types of benzodiazepine binding sites. One is the brain type binding site that is gamma aminobutyric acid (GABA)-dependent and the other is peripheral type that is unaffected by GABA. The brain type receptor is part of a supramolecular complex containing the $GABA_A$ receptor and possesses anticonvulsive, anoxiolytic, muscle relaxant and anterograde amnestic effects [12, 14]. The peripheral type receptor exists in kidney, heart, mast cell, platelet, adrenals and even in the brain. The peripheral type benzodiazepine agonist decreases the duration of intracellular action potential and the contractility in guinea pig papillary muscle [15]. Flumazenil inhibits the central effects of benzodiazepine by competitive interaction at brain type receptors and does not have affinity for peripheral type receptors [13~15]. Thus, it is likely that flumazenil had no effects mediated by peripheral receptor on myocardial function and metabolism.

In the human experiments, the reversal of benzodiazepine-induced sedation or anesthesia did not influence hemodynamics [16, 17]. The intravenous bolus doses of flumazenil up to 100 mg were well tolerated in healthy volunteers [18]. In patients with cardiac disease, Geller et al [19]. reported that reversal of diazepam sedation did not modify cardiac performance. Moreover, Croughwell et al [20, 21]. did not observe significant hemodynamic changes by flumazenil receiving or not receiving benzodiazepine. On the other hand, Marty et al [22]. observed that flumazenil led to a moderate increase in blood pressure and a more marked elevation of left ventricular end-diastolic pressure compared with the effect of placebo. However these changes represented mainly a return towards presedation values. In that study, the conceivable mechanism, by which flumazenil may induce hemodynamic alterations, would be related to direct antagonism of the pharmacologic effects of benzodiazepines in central nervous system.

In the present study, flumazenil had neither arrhythminogenic nor antiarrhythmic effects. In general, benzodiazepines have potentially antiarrhythmic effects. For example, diazepam has been used in the management of arrhythmias associated with chloroquine poisoning [23]. These effects are mediated by peripheral benzodiazepine receptor, thus it is unlikely that flumazenil has antiarrhythmic effects. On the contrary, several side effects such as ventricular arrhythmias and complete heart block have been reported [24~26]. However, these reports have indicated that flumazenil induced arrhythmias under benzodiazepine overdose when combined with cardiotoxic

drugs such as tricyclic antidepressant, beta-receptor antagonist and chloral hydrate. Therefore, flumazenil seems to have no effects on precipitation and prevention of arrhythmias.

In summary, we examined the effects of flumazenil on myocardial function and metabolism in the ischemic working rat heart. Although this model is polymorphonuclear cell-free preparation, flumazenil has no adverse effects on recovery of cardiac performance, arrhythmia during the reperfusion period and myocardial metabolism in the ischemic heart.

Acknowledgments

We wish to thank Mr. Koshimizu and Miss. Amemiya for their valuable technical assistance.

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