Protective Effects of *N*-n-butyl Haloperidol Iodide on Myocardial Ischemia-Reperfusion Injury in Rabbits

Fen-Fei Gao[1], Gang-Gang Shi[1], Jin-Hong Zheng[1], and Bing Liu[2]

[1]*Department of Pharmacology*
Shantou University Medical College
Shantou 515041, P.R.C.
[2]*Institute of Physiology*
Ruhr-University Bochum
D-44780 Bochum, Germany

Abstract

***N*-n-butyl haloperidol iodide (F_2), a novel compound derived from haloperidol, was synthesized by our drugs research lab. The present study aims to evaluate the protective effects of F_2 on myocardial ischemia-reperfusion injury *in vivo*, and to try to find the protective mechanism of F_2. The animal model of myocardial ischemia-reperfusion injury was established by ligaturing rabbit's left ventricular branch of coronary artery for 40 min and removing the ligation later to reperfuse for 40 min. Different doses of F_2 were intravenously injected before the onset of ischemia. The changes of hemodynamics were recorded during the experiment, and the activities of superoxide dismutase (SOD), creatine kinase (CK), Ca^{2+}-ATPase, Na^+,K^+-ATPase and the level of malondialdehyde (MDA) of myocardial tissue were detected after reperfusion. Administration of F_2 could dose-dependently ameliorate the hemodynamics of ischemia-reperfusion injured myocardium. During the course of reperfusion, MAP, LVSP, $\pm dP/dt_{max}$ in all F_2 groups were obviously higher than those in the ischemia-reperfusion control group, and LVEDP were lower. F_2 could also reduce the production of MDA, and maintain the activities of SOD, Ca^{2+}-ATPase, Na^+,K^+-ATPase, and minimize the leakage of CK out of myocardial cells in a dose-dependent manner. These results suggested that F_2 had apparent protective effects against myocardial ischemia-reperfusion injury.**

Key Words: ***N*-n-butyl haloperidol iodide, myocardial ischemia-reperfusion injury, hemodynamics, enzymes**

Introduction

Haloperidol is a classical antipsychotic drug of butyrophenones. Shi *et al.* have reported that haloperidol antagonizes the contraction of coronary artery induced by phencyclidine and agonist of opioid receptor (7, 8, 9, 11). Our advanced studies have also shown that haloperidol can antagonize noncompetitively the contraction of coronary artery strips induced by potassium chloride (KCl), histamine, noradrenaline in concentration-dependent manner (10). Haloperidol of clinical dose relieved unstable angina pectoris and improved the ischemic change of ECG in patients, but its extrapyramidal adverse reactions inhibited large sample observation and further study (12). So we thought to alter the chemical structure of haloperidol and synthesized a series of quaternary ammonium salt derivative of haloperidol, with polarity increased so that they do not pass the blood-brain barrier. One of these compounds of serial number F_2 (*N*-n-butyl haloperidol iodide, see Fig. 1) was screened, and was found to maintain the vasodilating effects but have no extrapyramidal side reactions (13). Our previous studies have shown that F_2 can not only antagonize the pig coronary arterial strips contraction induced by KCl (16) and decrease

Corresponding author: Dr. Gang-Gang Shi, Department of Pharmacology, Shantou University Medical College, Shantou 515041, P.R.C. Phone: +86-754-8900301, Fax: +86-754-8557562, E-mail: ggshi@stu.edu.cn
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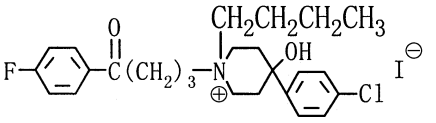

Fig.1 *N*-n-butyl haloperidol iodide (F_2)

the intracellular calcium fluorescence intensity of aortic vascular smooth muscle cells under the laser scanning confocal microscopy (17), but also open potassium-channel of aortic vascular smooth muscle cells (15) and block calcium-channel of ventricular myocytes (4) in whole-cell patch clamp experiment. Since F_2, a novel compound, is different from any current calcium-channel blockers and potassium-channel openers in structure, we have acquired the Chinese national inventive patent (No. ZL 96 1 19098. 1). Further research and development of F_2 is needed because it can develop a new way to study calcium-channel blockers and potassium-channel openers, and may become a new potential drug for treating myocardial ischemia. This experiment was designed planned to study the effects of F_2 on hemodynamics *in vivo* and enzymes of myocardial tissue in ischemia-reperfusion injury of rabbit heart.

Materials and Methods

Surgical Preparation

Zelanian rabbits of either sex weighing between 2.0-2.6 kg were anesthetized with intravenous pentobarbital sodium (30 mg/kg). A cannula with 40 IU/ml of sodium heparin linked to pressure transducer (PT14M2, Fudan university, China) was inserted into the femoral artery to monitor mean arterial pressure. A heparinized catheter was inserted into left ventricle through right common carotid artery to measure the left ventricular pressure. Thoracotomy was performed through the fourth left intercostal space adjacent to sternum, and the pericardium was widely opened and sewn to thoracic wall. With caution not to break pleura, this could be done without using an animal artificial respirator (2, 20). A silk ligature was placed under the left ventricular branch of the coronary artery (LVBCA) approximately 5 mm below left auricle of heart. Ischemia was induced by ligated the LVBCA. 40 minutes later, the ligature was cut and reperfusion was obtained for another 40 minutes. The ECG of limb lead II was used to document the process of myocardial ischemia and reperfusion. The notable raising of ST segment of ECG is an indication of myocardial ischemia, and the raised ST segment lowered a half and the appearance of deep and large Q wave is an indication of myocardial reperfusion injury.

Drugs Preparation

F_2 (synthesized by chemical group of Drugs Research Lab of Shantou University Medical College, and identified by Shanghai Organic Chemistry Institute of Chinese Academy of Sciences) was dissolved to the concentration required with 35% (v/v) polyethylene glycol 400 (PEG 400, Shanghai Chemical Corporation, China) before experiment. The concentrations of verapamil (Knoll AG, Germany) and each group F_2 were dispensed to ensure the same volume of drug liquid administered as 1 ml/kg.

General Experimental Protocol

Ninety Zelanian rabbits were randomly assigned to one of the nine groups. ① Sham-operated group (Sham, n=10): The LVBCA was surrounded by a silk ligature but not ligated. ② Control group (n=10): LVBCA occlusion for 40 min followed by reperfusion for 40 min. Saline (1ml/kg) was injected through the limbic auricular veins 5 min before ischemia. ③ PEG group (n=10): PEG 400 was substituted for saline. The others are treated the same as those of the control group. ④ Verapamil group (Ver, n=10): Verapamil (0.25mg/kg) was substituted for saline. ⑤~⑨ F_2 of five different dosage group (n=10, respectively): F_2 (0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, and 4 mg/kg, respectively) was substituted for saline. The changes of hemodynamic, including heart rate (HR), mean aortic pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and $\pm dP/dt_{max}$, were recorded with MS302 recording-and-analyzing system software (Guangdong Medicine College, China) at baseline, 3 min after adminstration of drugs and before ischemia, 40 min of ischemia and 40 min of reperfusion. At the end of the experiment, ischemic myocardial tissue samples were used to determine the activities of superoxide dismutase (SOD), creatine kinase (CK), ATPase and the level of malondialdehyde (MDA).

Tissue Preparation and Measurement

Tissue samples from ischemic zones were rinsed off blood with cold saline, then absorbed the water with filter paper, and weighed. Adding 9 times of saline, they were homogenized to 10% tissue homogenate with a glass-grinder in ice water. After centrifugation at 2500 xg for 10 min, the supernatants were decanted for measurement. According to the commercial test kits (Jiancheng Bioengineering Institute, Nanjing, China), the tissue protein is measured spectrophotometrically (spectrophotometer, UV-120-02, SHIMADZU, Japan) by using Coomassie bright-blue (CBBG250) stain method, the activity of

Table 1. Hemodynamic data measured in control and treatment groups.

Time	group	MAP	LVSP	LVEDP	$+dP/dt_{max}$	$-dP/dt_{max}$	HR
Baseline	Sham	11.19±1.34	14.06±1.67	0.63±0.29	547.0±105.4	401.6±84.3	271.4±23.6
	Control	11.08±1.88	14.38±2.27	0.61±0.41	550.1±95.3	361.4±123.3	257.7±21.7
	PEG	11.53±1.53	14.47±1.72	0.52±0.29	523.8±132.7	410.0±114.5	266.7±29.5
	Ver	10.99±1.98	13.86±2.47	0.82±0.41	498.6±145.7	380.3±108.4	262.2±39.1
	F2(0.25)	11.56±1.46	14.01±2.19	0.52±0.26	503.8±110.9	396.8±109.2	265.0±28.9
	F2(0.5)	11.11±1.01	14.25±1.84	0.80±0.46	564.7±161.8	397.5±147.9	268.3±26.0
	F2(1.0)	11.35±1.36	14.09±1.90	0.66±0.42	541.0±120.0	383.0±82.2	256.2±33.9
	F2(2.0)	10.93±1.71	14.04±1.58	0.64±0.32	554.0±146.5	427.4±84.3	268.7±32.7
	F2(4.0)	11.12±1.77	13.91±2.56	0.62±0.37	519.2±121.6	378.8±119.0	262.8±36.0
Adminstration of drugs and before Ischemia	Sham	11.16±1.12	13.95±1.97	0.71±0.45	539.9±117.4	405.3±99.4	267.8±22.4
	Control	11.09±1.90	14.42±2.43	0.61±0.38	541.1±103.3	358.4±116.1	252.2±22.6
	PEG	12.03±1.73	14.93±2.02	0.63±0.32	536.1±122.9	408.0±116.5	268.6±31.3
	Ver	10.16±1.65	12.63±2.37	0.98±0.46	415.8±134.7#*	351.3±113.4	231.2±34.3#
	F2(0.25)	11.42±1.53	13.87±2.25	0.54±0.19	498.5±106.9	396.7±113.6	266.2±31.4
	F2(0.5)	11.06±1.42	14.03±1.73	0.85±0.41☆	565.8±169.7	382.5±136.6	266.3±27.3
	F2(1.0)	10.61±1.20	13.78±1.56	0.68±0.46	506.3±131.6	385.2±95.6	249.2±30.6
	F2(2.0)	10.05±1.83	12.31±1.32#*	0.69±0.41	480.0±113.6	365.4±65.6	221.2±28.7#*☆
	F2(4.0)	9.98±1.65	11.62±1.86#*☆	0.79±0.37	441.3±142.3	311.1±109.3#	215.8±36.4#☆
I 40min	Sham	10.96±.01	13.74±1.11	0.76±0.32	517.2±142.1	373.9±88.2	252.8±20.2
	Control	9.23±2.09#	11.55±2.34#	1.19±0.50#	339.7±146.7#	251.6±87.5#	245.0±26.2
	PEG	10.05±1.79	12.03±1.80#	0.99±0.33	352.4±120.8#	273.4±66.5#	224.2±30.2#
	Ver	8.53±1.85#	11.51±1.98#	1.19±0.51#	351.8±134.2#	292.8±67.9#	254.7±31.8
	F2(0.25)	9.67±1.96	11.48±1.97#	0.90±0.35	354.1±139.3#	289.1±109.5	255.4±35.7
	F2(0.5)	9.80±1.33#	11.78±1.93#	1.14±0.38#	374.9±139.4#	287.0±137.5	262.4±32.9
	F2(1.0)	9.40±1.82#	11.45±2.30#	1.10±0.40#	368.5±125.5#	279.4±117.9	239.3±26.3
	F2(2.0)	8.72±1.61#	10.79±1.83#	0.84±0.34	368.6±164.9#	283.7±109.6	262.7±39.7
	F2(4.0)	9.09±1.80#	10.68±1.84#	0.98±0.25	339.7±125.8#	241.2±104.2#	240.7±39.2
R 40min	Sham	10.39±1.19	13.13±1.36	0.70±0.38	474.6±138.3	341.5±91.7	231.1±27.7
	Control	8.55±2.03#	9.65±2.09#	1.52±0.48#	184.9±96.7#	183.1±85.5#	214.8±27.4
	PEG	8.64±1.28#	9.91±1.70#	1.33±0.32#	209.8±115.9#	201.4±99.6#	234.8±32.5
	Ver	9.36±1.99	11.71±2.19*	0.89±0.30*	360.1±140.5*	281.3±91.5*	250.8±23.6*
	F2(0.25)	9.05±1.26#	10.81±1.40#	1.19±0.32#	304.0±94.6#*	248.2±79.1#	238.7±24.8*
	F2(0.5)	9.47±1.01	11.73±1.25#*	1.04±0.41*	359.8±125.8*	282.4±102.1*	252.0±36.2*
	F2(1.0)	9.55±1.93	12.19±1.24*☆	0.77±0.38*☆	388.3±104.6*	283.7±92.5*	236.6±26.8
	F2(2.0)	9.38±1.45	11.62±1.29#*	0.76±0.31*☆	397.5±131.6*	293.1±104.5*	247.6±24.1*
	F2(4.0)	9.41±1.69	11.58±1.97*	0.81±0.34*☆	403.2±112.3*☆	288.3±105.3*	229.5±26.0

MAP, mean aortic pressure (kPa); LVSP, left ventricular systolic pressure (kPa); LVEDP, left ventricular end-diastolic pressure (kPa), $\pm dP/dt_{max}$,(kPa/s), HR, heart rate (beats/min). Values are expressed as mean±S.D. (n=10). #P<0.05 vs Sham; *P<0.05 vs Control; ☆P<0.05 vs F_2 (0.25)

SOD using xanthine oxidase method, the level of MDA using thiobarbituric acid (TBA) stain method, CK activity using molybdenum acid ammonium method, and the activities of Ca^{2+}-ATPase and Na^+, K^+-ATPase through detecting the contents of phosphorus (14).

Statistical Analysis

All values are expressed as mean±standard deviation (S.D.). Comparison among groups was assessed by one-way analysis of variance (ANOVA). If significant difference existed among groups, the Student-Newman-Keul's test was used to detect differences between any two of them. A P value< 0.05 was accepted as statistically significant.

Results

Changes in the Time Course of Hemodynamics

Hemodynamic data for MAP, LVSP, LVEDP, $\pm dP/dt_{max}$ and HR in the nine groups are shown in Table 1. After administration of verapamil and F_2,

Table 2. MDA and enzymes in ischemia-reperfusion myocardial tissue measured in all groups.

	MDA (nmol/mgprot)	SOD (NU/mgprot)	CK (U/mgprot)	Na^+, K^+-ATPase (μmolPi/mgprot/hr)	Ca^{2+}-ATPase (μmolPi/mgprot/hr)
Sham	0.41±0.10	11.27±2.88	239.8±101.8	4.06±1.54	3.10±1.23
Control	1.48±0.67#	7.56±1.94#	79.1±31.2#	1.74±0.48#	0.84±0.29#
PEG	1.29±0.39#	7.46±2.34#	84.7±35.0#	1.49±0.91#	0.82±0.32#
Ver	0.75±0.19 #*	10.18±2.72*	135.1±42.4#*	3.12±1.07**	1.95±0.64#*
F_2 (0.25)	1.08±0.30#	8.26±1.31#	100.8±38.2#	2.19±0.60#*	1.16±0.90#
F_2 (0. 5)	0.90±0.34 #*	9.32±2.06	110.0±42.3#	2.32±0.73#	1.89±0.51#*☆
F_2 1.0)	0.77±0.22 #*☆	10.24±2.25 *☆	145.6±47.2 #*☆	3.09±0.96 *☆	2.14±0.75 #*☆
F_2 (2.0)	0.79±0.19 #*☆	10.19±2.30 *☆	157.8±54.6 #*☆★	3.21±1.39 *☆	2.45±0.72 *☆
F_2 (4.0)	0.70±0.22 #*☆	10.26±2.70 *☆	164.5±51.1 *☆★	3.66±1.26 *☆★	2.60±0.96 *☆

Values are expressed as mean±S.D. (n=10). #P<0.05 vs Sham; *P<0.05 vs Control; ☆P<0.05 vs F_2 (0.25); ★P<0.05 vs F_2 (0.5)

MAP, LVSP, ±dP/dt_{max} and HR tended to be less and LVEDP tended to be greater. Coronary occlusion significantly caused an decrease in MAP, LVSP and ±dP/dt_{max} and an increase in LVEDP compared with Sham group (P<0.05). However, there was no significant difference among the Control, PEG and all treatment groups (P>0.05). After reperfusion, MAP, LVSP and ±dP/dt_{max} in Control and PEG groups decrease progressively and LVEDP increase progressively, but there was no group difference (P> 0.05). However, compared with Control group, these hemodynamic indices in verapamil and each F_2 groups have obvious improvement, but they were still worse than those of the Sham group. In addition, HR tended to be less during ischemia and reperfusion course, but this phenomenon wasn't found among all groups.

Changes of Enzymes in Myocardial Tissue

The protective effect of F_2 on SOD, CK, Na^+, K^+-ATPase and Ca^{2+}-ATPase is shown in Table 2. After reperfusion, the level of MDA in myocardial tissue in Control and PEG groups increased significantly and the activities of SOD, CK, Na^+, K^+-ATPase and Ca^{2+}-ATPase decreased significantly, as compared with Sham group (P<0.05), but there was no group difference between Control and PEG groups (P>0.05). However, verapamil could reduce the product of MDA significantly, and maintain the activities of SOD, Ca^{2+}-ATPase, Na^+,K^+-ATPase, and minimize the leakage of CK out of myocardial cells, as compared with Control group. F_2 also had a similar effect as verapamil in a dose-dependent manner, but the parameters of verapamil and each F_2 groups were worse than those of the Sham group.

Discussion

The present study demonstrated that F_2 could significantly ameliorate the hemodynamics of ischemia-reperfusion injured myocardium and maintain the activities of enzymes in myocardial cells.

Verapamil was chosen as the positive control drug because our previous study has shown that F_2 can antagonise extracellular Ca^{2+} flowing into cell induced by KCl in the bioassay of coronary artery strips (16) and was observed under the laser scanning confocal microscopy (17). Moreover, in another experiment of whole-cell patch clamp of rat ventricular myocytes, the results demonstrated that F_2 could block L-type calcium-channel. So the calcium-channel blocker has been selected as the positive control drug. The present experimental results showed that verapamil could ameliorate the cardiac function and reduce the yield of MDA, protect the activities of SOD, Ca^{2+}-ATPase, Na^+, K^+-ATPase, and minimize the leakage of CK out of myocardial cells. Obviously, the myocardial ischemia-reperfusion injury animal model was set up successfully, too.

The results of solvent control group excluded the effect of the solvent on experiment. The results of every F_2 groups have suggested that F_2 could exert a protective effect on hemodynamics and the enzyme of ischemia-reperfusion heart, moreover in a dose-dependent manner.

The experimental results have shown that the protective effect of verapamil and F_2 mostly displayed in reperfusion period, and that verapamil and F_2 did not have obvious ameliorative effect on cardiac function in ischemic period, and hence having no

significant difference, as compared with Control group. At 40 min of reperfusion, LVSP tended to be greater in dose-dependent manner in F_2 0.25 mg/kg group, 0.5 mg/kg group and 1mg/kg group. However, LVSP in F_2 2 mg/kg group and 4 mg/kg group are less, as compared with that in F_2 1 mg/kg group. The reason may be that F_2 has strong effect on decrease cardiac function in 2 mg/kg and 4 mg/kg, as can be seen in Table 1. After administration of F_2 2 mg/kg or 4 mg/kg and before ischemia, LVSP and HR decrease significantly. But the recovery of LVSP after reperfusion from ischemia (LVSP at 40 min of reperfusion minus that at 40 min of ischemia) tended to increase as the dose of F_2 increased. The experimental results have also suggested that the indices of HR and MAP are both insensitive in reflection of left ventricular function.

ATPase is a protease in the biomembrane that is important in material transporting, energy exchanging and information convecting. The level of ATPase is an important marker for damage to energy metabolism and function of cells. The experimental results indicated that F_2 could preserve the activity of ATPase of myocardial cells. Lipid peroxidation metabolite MDA and superoxide dismutase (SOD) could also reflect the level of oxygen-derived free radicals, the activity of SOD reflecting the power of cells cleaning oxygen-derived free radicals and the quantity of MDA indirectly reflecting the degree of free radicals attacking cells. Ischemia or hypoxia causes ATP content of cells less, Ca^{2+} enters into mitochondrion, mitochondrial cytochrome oxidase system arises functional disorder, and oxygen entering into cell changes into oxygen-derived free radical by way of mono-electron deoxidization. Calcium antagonist F_2 can decrease the production of MDA and preserve the activity of SOD *via* saving ATP consumption, which relieves damage to myocardium by oxygen-derived free radicals. The change of CK content in myocardial cell can estimate the extent myocardial cell damage and is evaluated as a quantitative marker of myocardial infarction dimension (19), because CK in myocardial cell exudes and decreases owing to myocardial plasmalemma is damaged to result in permeability increase. The present study indicated that F_2 could definitely attenuate myocardial cell necrosis.

The mechanisms of calcium antagonists attenuate ischemia-reperfusion injury have been thought to be resulted from calcium antagonists, antagonizing calcium overload, but the present study has demonstrated that the main reason for calcium overload is Na^+/Ca^{2+} exchanging abnormally (1, 3, 6). So another opinion is that calcium antagonists inhibit rapidly the electrical and contractile action of myocardium in ischemic period, decrease the consumption of ATP energy during ischemia by way of negative myodynamia and heart rate and decrease after loading, preserve energy for cellular plerosis after ischemia so as to protect myocardium. Nayler (5) reported that calcium antagonists can reduce the calcium content of ischemic myocardium and ameliorate cardiac function, and thought that its protective mechanism is preservation of Ca^{2+}-ATPase activity *via* preservation of myocardial energy. Yanagida *et al*. (18) suggested that verapamil could preserve high-energy phosphate compound in ischemic and reperfused myocardium and attenuate myocardial ischemia-reperfusion injury. The present study supported this argument. After administration of verapamil and F_2 and before ischemia, MAP, LVSP, $\pm dP/dt_{max}$ and HR tended to decrease and LVEDP tened to increase in our experiment, which is an indication of depressor and bradycardiac effect of verapamil and F_2.

Furthermore, F_2, as a potassium-channel opener, could expedite K^+ outflow to inhibit cell membrane depolarization and to decrease amplitude and lasting time of action potential. So it could shorten the opening time of voltage-dependent calcium-channels, decrease the level of free Ca^{2+} in cells, inhibit electrical and contractile action of myocardial cell and show down the consumption of ATP. Thus F_2 could attenuate the damage of ischemia to cells and enhance the power of cells anti-damage. This may be a factor of F_2 to resist ischemia-reperfusion injury.

In summary, the present data of experiments demonstrated that F_2 could significantly ameliorate the hemodynamics of ischemia-reperfusion injured myocardium and maintain the activities of enzymes in myocardial cells. These results suggested that F_2 is a potent compound in attenuation of reperfusion injury.

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