Effect of Sigma Receptor Ligand Haloperidol on Guinea Pig Isolated Heart Electrogram

M Nováková[1], J Bardoňová[2], I Provazník[2]

[1]Department of Physiology, Faculty of Medicine, Masaryk University Brno, Czech Republic
[2]Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Czech Republic

Abstract

Cardiac sigma receptors are involved in fine modulation of contractility in mammalian myocardium. Their role in the changes of cardiac excitability is studied using various experimental models. In this study, the effect of sigma receptor ligand, psychotropic drug haloperidol, was examined in isolated guinea pig hearts perfused according to Langendorff. Electrogram and coronary flow changes were followed in control and during two administrations of haloperidol. Prolongation of QT interval was found in the first, but not in the second haloperidol application. It is concluded, that sigma ligand haloperidol affects repolarization phase of cardiac electrogram and this process may explain various rhythm disturbances reported in patients treated with haloperidol.

1. Introduction

1.1. Sigma receptors

Sigma receptors are a relatively novel group of receptors originally discovered in the central nervous system of mammals in 1976 [1]. Various pharmacological studies and later also successful identifying and cloning of these binding sites proved that these structures are a unique group of receptors different from any opioid or similar ones. Later, they were found also in many nonneural peripheral tissues of numerous species and also in humans. One of the tissues which contain significant number of sigma receptors is the heart muscle. Two subtypes of sigma receptors were found up to now, sigma-1 and sigma-2.

In our previous studies, we showed that in the isolated rat cardiac cells sigma receptors cause increase in contractility (e.g. the force of contraction increases after binding of sigma ligands to their cardiac receptors). This positive inotropic effect is mediated by increased production of the second messengers - inositol-tris-phosphate (IP3) and di-acyl-glycerol (DAG). The former causes massive release of calcium – acting as activator and modulator of muscle contraction – from its intracellular store – sarcoplasmic reticulum. This considerable increase in cytoplasmic concentration (or better availability) of calcium is probably responsible for some adverse effects of drugs binding to sigma receptors on cardiovascular system [2], [3].

However, the other second messenger – DAG – causes activation and translocation of important enzyme in the heart cell – proteinkinase C. This enzyme triggers the whole set of phyphorylation processes which – in turn – affect various proteins and their function in the cell. One of the phosphorylated proteins is the sigma receptor itself. After phoshorylation it undergoes the process of desensitization – which simply spoken means that adding more ligand to sigma receptor population does not cause expected reaction. On the contrary, the effects mediated by binding of sigma ligands to their receptors are either very small or none [4].

1.2. Sigma ligands

Sigma ligands – drugs binding to sigma receptors – are chemically diverse group of compounds, such as benzomorphans, morphinans, phenothiazines, etc. Many of them can be indicated as so-called psychotropic drugs. Most of them are used in everyday clinical practice in the treatment of various psychoses. Many of them show severe cardiovascular side effects – palpitation, high blood pressure, syncope. Among all, most frequently various arrhythmias occur during the treatment with sigma ligands, some of them being really life-threatening (ectopic ventricular extrasystoles, ventricular fibrillation, sudden cardiac death). QT interval prolongation is very often the underlying mechanism or at least the starting point of these electrical disturbances.

Haloperidol is a psychotropic drug used for several decades in the treatment of schizophrenia and acute

attacks of some other psychoses. Its cardiovascular side effects – mainly arrhythmias are well known and were repeatedly reported. However, its therapeutic potential is high and it is still considered the first choice remedy in certain clinical situations (for instance calm down of agitated patients after cardiac surgery) although its injection can cause even sudden death for lethal arrhythmias [5].

2. Aim of the study

Aim of this study was to examine the effect of haloperidol on electrogram recorded in isolated guinea pig hearts with respect to its repolarization phase since the underlying mechanism of most of haloperidol triggered arrhythmias is QT-interval prolongation.

3. Material and methods

3.1. Isolated heart technique

The technique of perfusion of isolated mammalian heart was introduced by Oscar Langendorff in 1895. Since then, it has been widely used and modified mainly in physiology and pharmacology. The technique is appropriate for hearts of homoiothermic animals, e.g. with a coronary vascular system. The principle is to perfuse the heart with oxygenated solution (perfusate) containing all necessary substrates and ions through a cannula inserted into the ascending aorta. The hydrostatic pressure in the reservoir closes the aortic valves, the perfusate is driven into the coronary system, coronary sinus and widely opened atrium. Two modes of perfusion are used, either at constant pressure or at constant coronary flow.

An improved perfusion set for small animal hearts based on Langendorff technique was reported by Curtis et al. in 1986. Later, it has been modified in our laboratory for employing several drugs or several concentrations of one drug. At any rate, more than one reservoir is needed. The common bath is used for keeping appropriate temperature in four reservoirs, volume of 100 ml, each oxygenated separately. A four-way stop-cock allows rapid switching of solutions. Small diameter of the connecting tubes accounts for a small dead space. A special system keeps the perfusion pressure constant and equal in all four reservoirs in spite of different amount of solution in each (Fig.1).

Left-ventricular (LV) pressure is measured by a latex balloon inflated to approximately 10 mmHg. The special software has been developed for monitoring and recording of LV pressure and touch-free recording of electrogram from the thermostatically controlled bath in which the heart is immersed. The programme allows 45 minute record of systolic pressure, diastolic pressure, and interbeat interval, and 5-second snap-shots with electrogram and corresponding LV pressure curve recordings (Fig.2) [6].

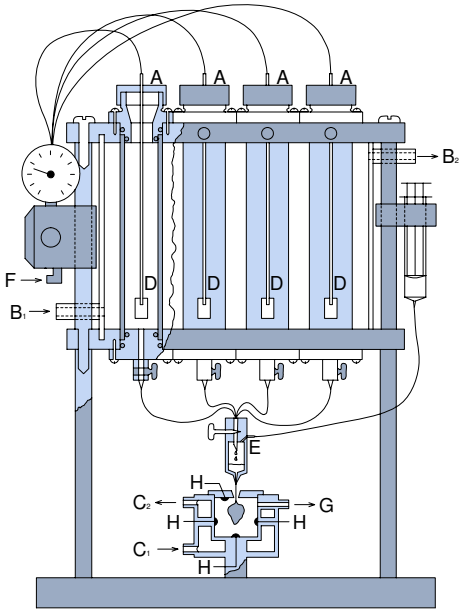

Figure 1: Langendorff perfusion set for small animal heart adapted for pharmacological studies. A – reservoirs, B – connection to thermostat, C – connection to thermostat, D – bubbling stones, E – bubble trapper, F – pressure-keeping system, G – overflow, H – electrodes for touchless electrogram recording.

3.2. Experimental setup

Six adult male guinea pigs were sacrificed under deep anesthesia by xylazine and ketamine. Only male animals were used because the endogenous ligand of sigma receptors has not been found yet and since one of the hot candidates for it is progesterone the males are more reliable model. The chests were then quickly opened, the hearts cut out with sufficiently long piece of aorta, placed into cold (5°C) Krebs-Henseleit solution (K-H, composition see below) and the aorta was cannulated.

The hearts were then perfused at Langendorff set-up with K-H solution of following composition (in mM): NaCl 118, $NaHCO_3$ 24, KCl 4.2, KH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 5.5, Taurine 10 and $CaCl_2$ 1.2. The solution was equilibrated with 95% O_2 and 5% CO_2. The perfusion was

done at constant perfusion pressure (80mmHg) and 37°C. First, period of stabilization was run for 30 minutes (control period). All hearts exhibiting any arrhythmias during this period were excluded from next experiment. Then the first haloperidol administration followed. It was applied in K-H solution for 30min at a concentration of 10nM. This concentration was based on dose-response curve and also on the results from binding studies and previous experiments of our group. This concentration is close to binding constant of sigma receptors. Then, period of wash-out with K-H followed for 30 minutes. In order to examine possible down-regulation (desensitization) of sigma receptors, second administration of 10nM haloperidol followed for another 30 minutes.

3.3. Electrogram and coronary flow recording

During the whole experiment electrogram was recorded and mean coronary flow monitored. The recording of electrogram was carried out by the touch-less method. Six silver-silver chloride disc electrodes (4 mm in diameter) are placed on the inner surface of the bath in which the heart is placed during the whole course of experiment. ECG signals are recorded from three orthogonal bipolar leads (X, Y, and Z). The signals are amplified and digitized at a sampling rate of 500 Hz by a three-channel, 16-bit AD converter. The maximum amplitude of recorded signals varies between 100 μV and 500 μV, depending on the subject. From all experiments, fifteen successive QT intervals were measured and averaged in the 25th min of each period (control, haloperidol I., washout and haloperidol II.) and incidence of arrhythmias was monitored.

The mean coronary flow was measured every fifth minute during the whole experiment by collecting of perfusate leaving the bath and measuring its volume. It was later normalized to control (the value at the end of control was taken as 100% and all values during the rest of experiment were expressed with respect to this value).

4. Results

No significant occurrence of arrhythmias was observed, except of sporadic ventricular extrasystole during the first haloperidol period. During the second haloperidol administration there were not rhythm disturbances at all.

The QT-intervals were prolonged in the first haloperidol period (168.3 vs. 184.7ms; NS). This effect was irreversible (average QT-interval in wash-out was 185ms). Moreover, during the second haloperidol administration additional QT-interval prolongation was not found.

Figure 2 shows original recording in control and haloperidol I. period. Figure 3 gives a schematic overview of the changes in QT interval during our experiments. In figure 4, the coronary flow changes are summarized. It is obvious that slight tendency to decrease coronary flow is insignificant.

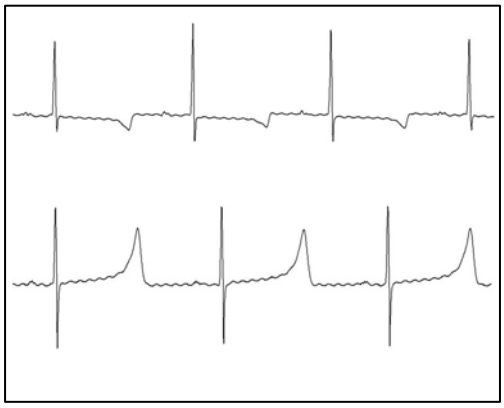

Figure 2: Original recording of electrogram of isolated guinea pig heart under control conditions (top) and under the effect of 10nM haloperidol during its first administration (bottom).

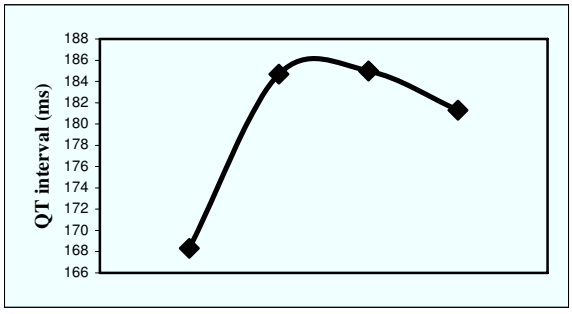

Figure 3: QT interval changes during the experiments.

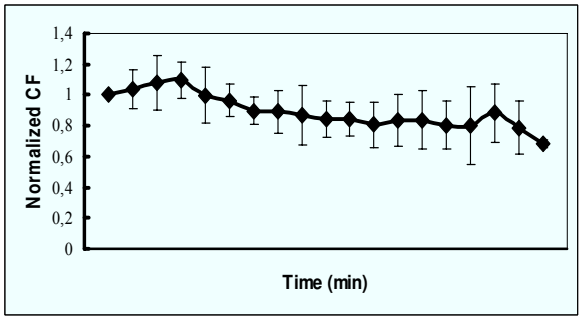

Figure 4: Averaged coronary flow (ml/min) normalized to the end of control period (100%).

5. Discussion

There is a lot of evidence from literature that haloperidol causes severe cardiovascular problems. In our experiments, rhythm disturbances appeared only scarcely. This minimal arrhythmia score in our records is in agreement with the fact that concentration in our study is lower than usual therapeutic plasma level of haloperidol, which is within micromolar range. However, nanomolar concentration is close to binding constant of sigma receptors and we consider it clinically more relevant. Plasmatic levels of drugs are not always reached in the tissue in which the effect is expected. The concentrations of sigma ligands can be (and probably are) lower than the reported plasmatic ones.

The situation with QT interval prolongation is clearer and fits better to our hypothesis how most arrhythmias caused by sigma ligands originate. They are related to repolarization phase of cardiac cells. Recently, it has been reported that sigma receptors are so-called metabotropic receptors, bound to certain (in this case potassium) ionic channel, thus changing excitability of the heart. Some of our recent experiments are focused on direct effects of haloperidol on transient outward currents, one of potassium currents in the heart cell. Its affecting causes electrical instability of the heart.

Also, the fact that QT interval stays prolonged during the wash-out period and that it does not change during the second haloperidol administration, is in agreement with our idea of how binding of sigma ligands to the sigma receptors affects the heart cells. Obviously, after the signaling pathway is triggered, the sigma receptors are desensitized and 30 minutes of washout is time not long enough to see receptors again "working". The effect of haloperidol is thus only minimally (insignificantly) reversible and during the second haloperidol administration, there is even no effect at all.

Following the coronary flow changes is a certain safety-catch in our experiments – if the heart muscle becomes deteriorating during experiments or some experimental set-up is wrong, the coronary flow significantly decreases or changes rapidly.

6. Conclusion

Thus, we can conclude that the effects of haloperidol in this study are mediated by binding of the drug to cardiac sigma receptor and not by direct effect on ionic channels on the membrane. However, more studies are needed to elucidate how these receptors affect cardiac cell membrane.

Acknowledgements

This project was supported by the Grant Agency of the Czech Republic, grant no. 305/04/1385, partially by the grant no. 102/04/0472 and by MŠMT ČR, grant no. MSM0021622402.

References

[1] Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE. The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. Journal of Pharmacology and Experimental Therapeutics 1976; 197(3):517-532.

[2] Nováková M, Ela C, Barg J et al. Inotropic action of sigma receptor ligands in isolated cardiac myocytes from adult rats. European Journal of Pharmacology 1995;286:19-30.

[3] Nováková M, Ela C, Bowen WD. Highly selective sigma receptor ligands elevate inositol 1,4,5-trisphosphate production in rat cardiac myocytes. European Journal of Pharmacology 1998; 353:315-327.

[4] Monassier L, Bousquet P. Sigma receptors: from discovery to highlights of their implications in the cardiovascular system. Fundamental and Clinical Pharmacology 2002;16(1):1-8.

[5] Di Salvo TG, O´Gara PT. Torsade de pointes caused by high-dose intravenous haloperidol in cardiac patients. Clinical Cardiology 1995;18(5):285-290.

[6] Nováková M, Moudr J, Bravený P. A modified perfusion system for pharmacological studies in isolated hearts. Biosignal 2000;16:162-164.

[7] Bébarová M, Matejovič P, Pásek M, Nováková M. Effect of haloperidol on transient outward potassium current in rat ventricular myocytes. European Journal of Pharmacology 2006; in press.

Address for correspondence

Marie Nováková, M.D., Ph.D.
Department of Physiology, Faculty of Medicine
Masaryk University Brno
Komenského náměstí 2
662 43 Brno
Czech Republic
majka@med.muni.cz