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# EFFECTS OF SIGMA RECEPTOR LIGAND BD737 IN RAT ISOLATED HEARTS

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A b s t r a c t

The present study was focused on possible effects of highly selective, high affinity sigma receptor ligand BD737 in rat isolated hearts perfused according to Langendorff. Electrogram recorded by touch-less method and left ventricular pressure acquired by balloon method were followed during acute exposure of isolated rat heart to nanomolar concentrations of the drug. Ventricular ectopic beats and periods of bigeminy were present in all experiments. Moreover, heart rate fluctuations were observed. In analogy to the positive inotropic effect previously described in isolated rat cardiomyocytes, BD737 exerted a slight insignificant increase of systolic pressure in our experimental model. In conclusion, the inotropic and chronotropic effects of sigma ligand BD737 in nanomolar concentration were confirmed in isolated rat hearts.

K e y w o r d s

Isolated heart, Sigma receptor, Electrogram, Arrhythmias, Rat

## INTRODUCTION

The technique for studying completely isolated mammalian heart introduced by Oscar Langendorff in 1895 (*1*) has been widely used and modified ever since. The technique is appropriate for hearts of homoiotermic animals, e.g. with a coronary vascular system. The principle is to perfuse the heart by oxygenated solution 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.

During last century, this technique became a fundamental for numerous other methods employed in basic cardiovascular research, such as perfusion of multicellular heart preparations or obtaining cardiomyocytes by enzymatic digestion. Nevertheless, original concept of isolated heart perfusion still represents golden standard in physiological studies focused on basic parameters of cardiac performance (such as electrogram and pressure developed by left ventricle) or on testing of various drugs in pharmacology.

In pharmacological experiments, not only newly developed compounds are examined but also routinely clinically used preparations with side-effects reported are tested in order to explain these adverse effects. Scores of such drugs bind to various receptors in the central nervous system and often also in some peripheral tissues. An example of such binding site is sigma receptor. Although it was originally reported in the central nervous system, it is present in many peripheral tissues, including the heart muscle. The list of compounds that bind with high affinity to sigma receptors includes benzomorphans, morphinans, butyrophenones, phenothiazines, phenylpiperidines, guanidines and steroids, or better neurosteroids. Many of them are drugs used in everyday clinical practice for pain management (e.g. pentazocine, morphine) or in psychiatry (haloperidol). Their cardiovascular adverse side effects have been reported repeatedly and are studied intensely (for review – see *2*). However, for better understanding the mechanisms of sigma receptor signaling it is necessary to use highly selective compounds which bind exclusively to sigma receptor or even to one of its subtype (up to now, three subtypes were reported) since many of above listed drugs show certain affinity to various other receptor groups. A representative of such highly selective, high affinity ligand of sigma receptor is 1S,2 R-cis-N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)-cyclohexylamine (BD737)(*3*).

The aim of this paper was to examine the effects of highly selective, high affinity sigma receptor ligand BD737 on electrical and mechanical responses of isolated rat hearts.

## MATERIALS AND METHODS

All experiments followed the guidelines for animal treatment approved by local authorities and followed the EU law.

In the study the hearts of 12 adult male Wistar rats were included, divided into two equal groups. The body weight of animals varied from 300 to 390 (average, 330±50) grams. Only male animals were used since binding to sigma receptors is modified by progesterone and its derivatives and thus female animals are not suitable for this kind of study.

The rats under deep ether anesthesia were sacrificed by cervical dislocation. The chest was quickly opened, the heart with sufficiently long piece of aorta cut out and placed in a preparation bowl with a cold (5°C) Krebs-Henseleit solution of the following composition: NaCl, 118 mM; NaHCO3, 24 mM; KCl, 4.2 mM; KH2PO4, 1.2 mM; MgCl2, 1.2 mM; CaCl2, 1.2 mM; glucose, 5.5m M and Taurine, 10m M. Then, the aorta was cannulated and the heart fixed on the perfusion set-up. The hearts were perfused with Krebs-Henseleit solution of above mentioned composition at constant perfusion pressure of 80mmHg. The solution was oxygenated with 95% $O_2$ and 5% $CO_2$.

An improved perfusion apparatus for small animal hearts based on Langendorff technique (*Fig. 1*) was reported by Curtis et al. in 1986 (*4*) and it has been modified in our laboratory for employing several drugs or several concentrations of one drug (*5*). 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 perfusion 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.

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 (*6*) from the thermostatically controlled bath in which the heart is immersed.

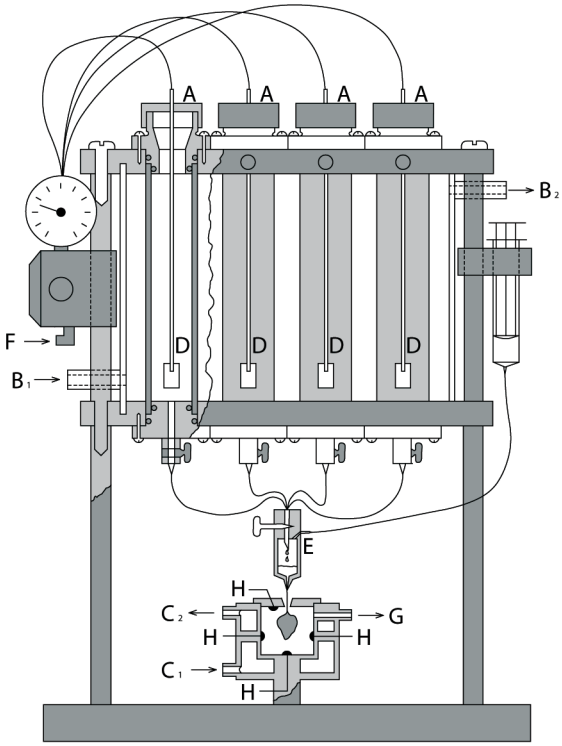


*Fig. 1*
Langendorff perfusion set for small animal heart adapted for pharmacological studies. A – reservoirs, B – connection to thermostat, C – connection to thermostat, D – bubbling stones, E – bubble trapper, F – pressure-keeping system, G – overflow, H – electrodes for touchless electrogram recording.

All experiments were carried out at the temperature 37°C. The hearts were allowed to beat spontaneously. After 30 minutes of stabilization, BD737 in concentration of either 10 or 100nM was administered to the perfusate for 30 minutes.

## RESULTS

The effects of BD737 on electrical and mechanical properties of rat myocardium were previously studied in isolated cardiomyocytes (*7*, *8*). In analogy to the positive inotropic effect which was observed in that experimental model (measured as an amplitude of stimulated twitch of isolated ventricular cardiac cells using video motion analyzer), BD737 at the concentration of 10nM exerted increase in systolic pressure measured by balloon method in the left ventricle. However, this increase

was only slight and insignificant – it increased from 95mmHg (at the end of control perfusion) to 120 mmHg on average. The diastolic pressure was not changed at this concentration of the tested drug. At the concentration of 100nM, increase in systolic pressure (up to 105mmHg) in all examined hearts was accompanied by slight increase in diastolic pressure in half of the hearts; others showed no change in diastolic pressure (see *Table 1*).

The most typical feature of the BD737 effect in both models (isolated cells as well as isolated organ) is a loss of electrical stability. In spontaneously beating hearts in our experiments, the heart rate fluctuated at the rate of approximately 2–3 cycles per minute in both concentrations of BD737. The heart rate at the beginning of experiment was 320±12 bpm and during fluctuations it was changing by 20%. This effect was observable in all hearts included in our study. Moreover, in the present study ventricular ectopic beats and periods of bigeminy were present in all examined hearts (for original recording see *Fig. 2*). Most of them appeared in the first 10 minutes of perfusion with the tested drug (*Fig. 3*). When evaluated according to Lambeth convention, all hearts were classified by number 2 (*9*).

## DISCUSSION

In cardiac tissue modulation of contractility by sigma receptor ligands was reported first in neonatal cultured cardiac cells (*10*) and later also in adult rat cardiomyocytes (*7*, *8*). In cultured newborn rat cardiomyocytes, the exposure to sigma ligands in nanomolar concentration range exerted specific changes in contractility, $[Ca^{2+}]_i$ transients and beating rates. The effects described in that study were unique, because they were induced in vitro (i.e. direct effect) by sigma ligands in nanomolar concentrations. Another important finding in this paper was the significantly increased incidence of irregular rate of contractions in as low as nanomolar concentrations of sigma ligands. However, there were two major limitations in this study. First, the preparation of cultured newborn cardiac myocytes is not very suitable for this type of investigation: it is very unstable and the results greatly depend on the stage of development of the cardiac cells in the culture. The second limitation was the use of specific sigma ligands which, at that time, did not exhibit sufficiently high affinity.

*Table 1*
Overview of measured parameters. Summary data from all experiments.

| | Increase in systolic pressure | Increase in diastolic pressure | Heart rate fluctuation | Bigeminy | Premature ventricular beats | Lambeth score |
|---|---|---|---|---|---|---|
| **10 nM** | 25% | - | + | + | + | 2 |
| **100 nM** | 12% | +/- | + | + | + | 2 |

In agreement with the study on newborn cultured rat cardiomyocytes *Nováková* et al. (*7*) found sigma binding sites in the membranes of adult rat ventricular cardiomyocytes. It was found that sigma ligands haloperidol, (+)-3-PPP and (+)-pentazocine caused significant increase in the amplitude of cell contraction already in nanomolar concentrations. These changes of contractility were accompanied by corresponding changes of $[Ca^{2+}]_i$ transients monitored by indo-1. The importance of these results was in demonstrating the effects of sigma ligands at binding affinity concentrations in contrast to previous studies which reported the effects only at concentrations by two to three orders of magnitude higher.

In order to explain the mechanism of the effect of sigma ligands on cardiac myocytes, the changes in cardiac contractility under the effect of highly selective, high affinity sigma receptor ligands 1S, 2R-cis-N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl) – cyclohexylamine (BD737) and N-[2-(3,4-dichlorophenyl)ethyl] – N,N',N' – trimethylethylenediamine (BD1047) in nanomolar concentrations were later studied by the same research group (*8*). These ligands caused potentiation of electrically-evoked amplitudes of contraction and of $Ca^{2+}$ transient. In addition, both ligands caused a dose-dependent increase of incidence of spontaneous twitches. These effects were markedly inhibited in presence of a phospholipase C inhibitor, neomycin, or after depletion of reticular $Ca^{2+}$ stores by thapsigargin or caffeine. Both substances caused an increase in the intracellular concentration of $IP_3$ determined by the $IP_3$ binding protein assay. It was suggested that the effects of these sigma ligands on contractility and spontaneous contractions were mediated by activation of phospholipase C and elevation of intracellular $IP_3$ level. These data brought evidence in support of the idea that the sigma-effects are due to binding to specific receptors and not by a cross-talk between the different receptor systems.

In the present study, the effects of this highly specific, high affinity sigma receptor ligand BD737 in rat isolated hearts were studied. Two concentrations were used: the concentration of 10nM is very close to binding constant for cardiac sigma receptor as reported previously (*7*). The most important finding of our experiments is electrical instability of the preparation. The heart rate fluctuations were observed at the rate of approximately 2–3 cycles per minute in both concentrations of BD737. This rate is characteristic for $IP_3$ production and $[Ca^{2+}]_i$ oscillations described previously in isolated rat cardiomyocytes (*8*). The rhythm irregularities – present as ectopic ventricular beats and frequent bigeminy – can be considered a sign of electrical dysbalance, most probably caused by the effect of sigma signaling on various ionic channels and – consequently – electrical currents on the membrane of cardiomyocyte.

The positive inotropic effect of sigma ligand – previously observed in rat isolated cardiomyocytes (where increase in the amplitude of stimulated twitch under the effect of sigma ligand reached values as high as 100% in comparison with control) – was diminished in isolated rat hearts. Most probably the whole organ can handle calcium changes – which represent the underlying mechanism of sigma signaling

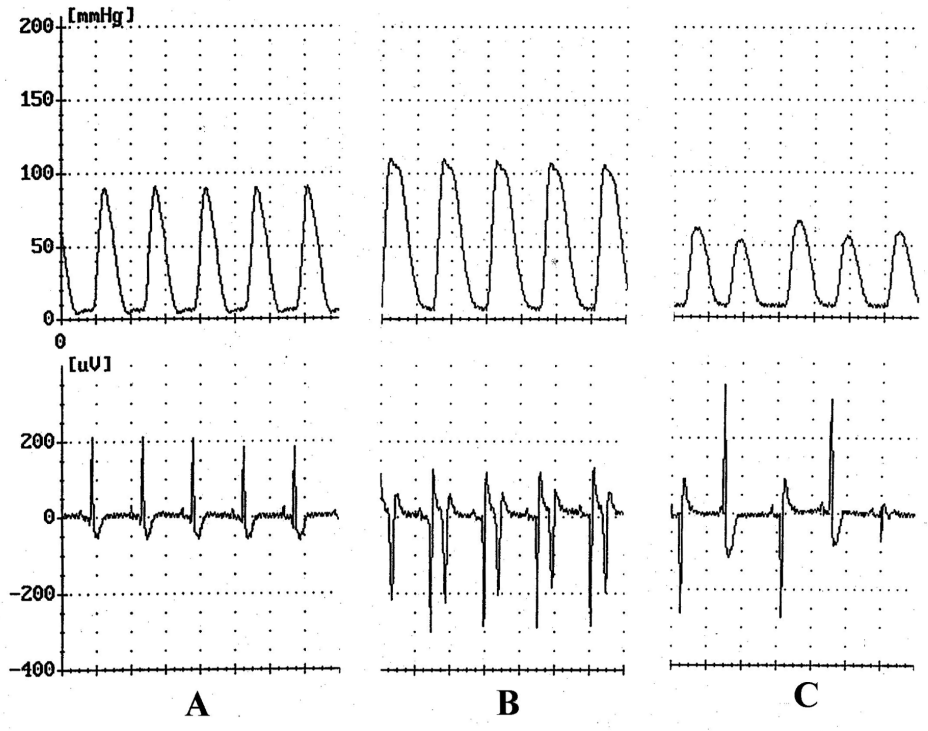


*Fig. 2*
Original recording of electrogram and left-ventricular pressure in rat isolated heart. Top – left-ventricular pressure curve, lower trace – electrogram (bipolar lead). A - control, B and C – effect of sigma ligand BD737, 10nM. Note the typical irregularities of rhythm (bigeminy – B, ectopic ventricular beats – C).

generally – better than isolated cardiac cells. Nevertheless, the increase in cytoplasmic calcium availability which follows binding of sigma ligand to its receptor in cardiac muscle triggers changes which lead to electrical instability and occurrence of spontaneous twitches in isolated cardiomyocytes on one side and various arrhythmias and heart rate fluctuations in isolated heart on the other side.

In conclusion, the inotropic and chronotropic effects of sigma ligand in nanomolar concentrations were confirmed in rat isolated hearts perfused according to Langendorff. However, more detailed examinations are needed in order to explain the underlying mechanisms for electrical changes caused by binding of sigma ligands to their cardiac receptors.

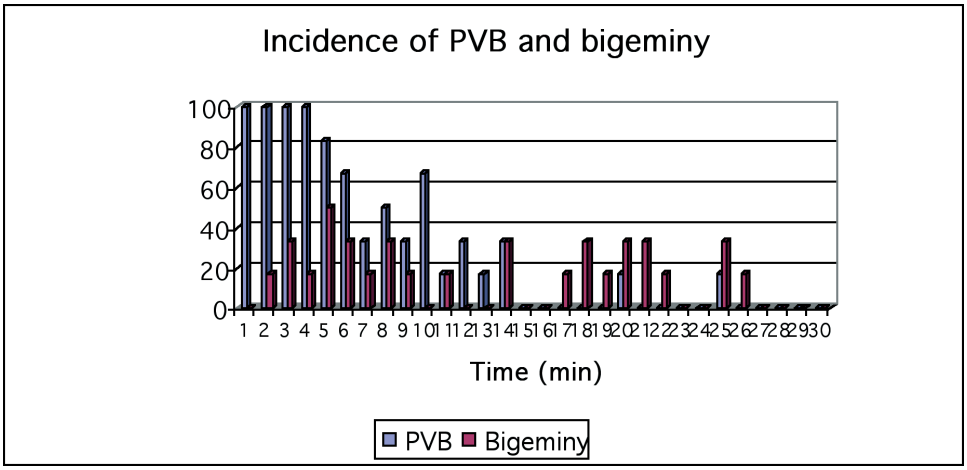


*Fig. 3*
Incidence of premature ventricular beats (PVB) and bigeminy during 30 minutes of exposure to 10nM BD737. Summary data from six hearts expressed in %.

A c k n o w l e d g e m e n t s

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