Inotropic Effects of Gaseous Transmitters in Isolated Rat Heart Preparation

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We studied the effects of carbon monoxide and sodium hydrosulfide, hydrogen sulfide donor, on contractile activity of the left ventricle in Langendorf-perfused isolated rat heart. Carbon monoxide 5×10^{-5} M significantly accelerated sinus rhythm and left-ventricular pressure wave growth and decay. To the contrary, negative inotropic and chronotropic effects were observed at higher concentrations of carbon monoxide (10^{-4}, 3×10^{-4} M). Sodium hydrosulfide (10^{-4}-4×10^{-4} M) decreased all the parameters of left-ventricular contractive activity and reduced contraction rate. Carbon monoxide and hydrogen sulfide, which together with nitrogen oxide are qualified as a new class of gaseous signal compounds, may substantially modulate pumping function of the heart.

Key Words: *carbon monoxide; hydrogen sulfide; retrograde perfusion; inotropic effect; chronotropic effect*

Currently, the existence of a special group of gaseous compounds with signal functions is well established. This group includes nitrogen oxide (NO), carbon monooxide (CO), and hydrogen sulfide (H_2S) [1,2,6,13]. These three compounds induce pronounced relaxation of vascular smooth muscles. In addition, NO plays an important regulating role in the heart where it acts as a paracrine signal compound and to a certain extent as a transmitter that mediates parasympathetic influences [11]. Positive inotropic effect of low NO concentrations has been demonstrated, while high concentrations produce a negative inotropic effect [5]. Acceleration of automatic activity of heart pacemaker under the effect of NO has been described [6]. On the other hand, the data on cardiotropic effects of CO and H_2S are extremely fragmentary and controversial. Some investigators describe the positive inotropic effect of CO [9], whereas others report suppression of contractive activity under the influence of this compound [7]. Inhibition of L-type calcium current under the effect of CO was also described; it is usually coupled with negative inotropic effect [12]. Some studies suggest protective effects of these compounds under conditions of ischemia [7,9,10]. However, the mechanisms of these cardioprotective effects remain unclear.

In addition to recently discovered roles of CO and H_2S as physiological regulators, these gases in higher concentrations produce well-known toxic effects. Moreover, people virtually constantly contact with these gases, especially cigarette or hookah smokers [4]. In this connection, it is interesting to study the possibility of modulating mechanical activity of the myocardium and heart rhythm generation in the sinoatrial node of mammals by CO and H_2S.

This study is focused on the effects of CO and H_2S on parameters of contractive activity and left-ventricular contraction rate in Langendorf-perfused isolated rat heart.

MATERIALS AND METHODS

The experiments were carried out on 38 male outbred rats weighing 300-350 g. The animals were decapi-

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tated, the thorax was promptly opened, and the heart was isolated and connected to the perfusion apparatus via the aorta. Standard Langendorf perfusion was performed with Krebs–Henseleit solution containing (in mM) 118.0 NaCl, 4.7 KCl, 25.0 $NaHCO_3$, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, and 5.5 glucose and oxygenated with carbogen (95% O_2+5% CO_2), pH 7.3-7.4 at 37.5°C. Parameters of left-ventricular contractive activity were evaluated by measuring the left-ventricular pressure via a catheter attached to the pressure transducer passed through a cannula and aortic valve into the left ventricle. Signal from the transducer was registered by a computer using an LA-2 digitizer (ZAO Rudnev-Shilyaev) and Pulsar software (developed by A. A. Zakaryan, Department of Human and Animal Physiology, M.V.Lomonosov Moscow State University). The obtained pressure curves were used to calculate heart rate, pressure wave amplitude, and maximum rates of pressure rise (dP/dt_{max}) and drop (dP/dt_{min}).

For preparing CO stock solution, gaseous CO (99%) was bubbled through Krebs-Henseleit solution for 30 min immediately before use. CO solubility is 2.691 mg per 100 g distilled water [14], therefore CO concentration in stock solution was 0.96 mM. We considered it ~1 mM, therefore further we present rounded values of CO concentrations in working solutions. For evaluation of H_2S effects on electrical activity, sodium hydrosulfide (NaHS; Sigma) was used. In water solution it dissociates to Na^+ and HS^- ions; the latter interacts with the proton and forms H_2S water solution. The gas gradually leaves the solution; therefore, stock NaHS solution was prepared immediately before the experiment. During the experiment, CO and NaHS stock solutions were administered into the perfusion line using an LSP04-1A infuser (Longerpump) at a rate needed to obtain necessary final concentration in the perfusion solution.

The experimental data were processed statistically using Statistica 6.0 software. Assessment of differences between linked samples was carried out using the Wilcoxon test.

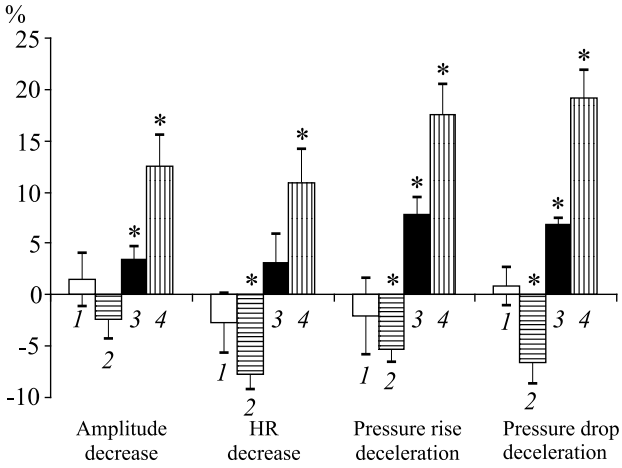

Fig. 1. Dependence of changes in isolated rat heart contractive activity on CO concentration. *1*) 10^{-5} M; *2*) 5×10^{-5} M; *3*) 10^{-4} M; *4*) 3×10^{-4} M. Ordinate: maximum intensity of changes produced by CO from the corresponding control values (%). *$p<0.05$ in comparison with the control (Wilcoxon test; n=8).

RESULTS

Four CO concentrations were tested: 10^{-5}, 5×10^{-5}, 10^{-4}, and 3×10^{-4} M. In a concentration of 10^{-5} M

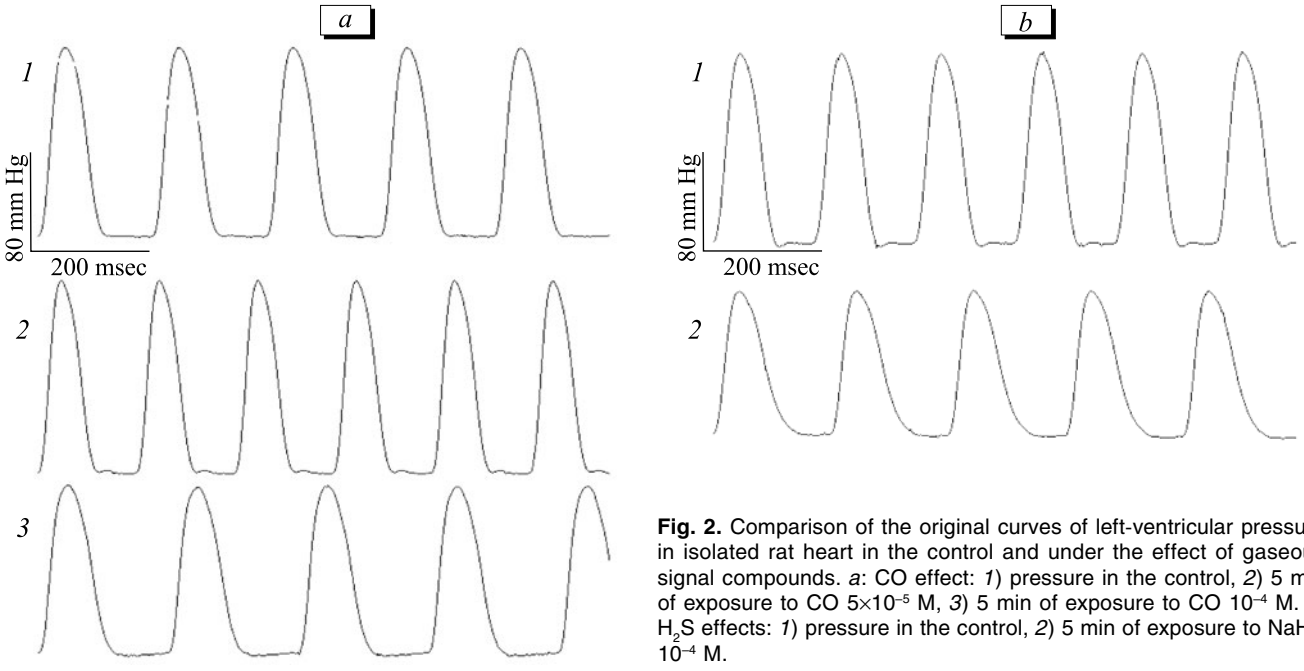

Fig. 2. Comparison of the original curves of left-ventricular pressure in isolated rat heart in the control and under the effect of gaseous signal compounds. *a*: CO effect: *1*) pressure in the control, *2*) 5 min of exposure to CO 5×10^{-5} M, *3*) 5 min of exposure to CO 10^{-4} M. *b*: H_2S effects: *1*) pressure in the control, *2*) 5 min of exposure to NaHS 10^{-4} M.

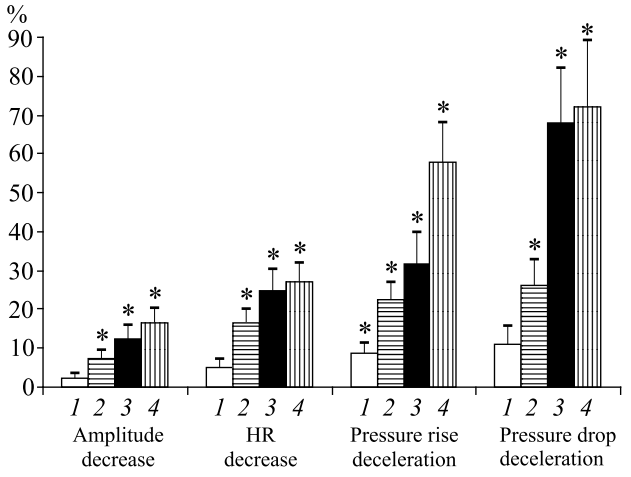

Fig. 3. Dependence of changes in isolated rat heart contractive activity on NaHS concentration. *1*) 5×10^{-5} M; *2*) 10^{-4} M; *3*) 2×10^{-4} M; *4*) 4×10^{-4} M. Ordinate: maximal intensity of changes produced by NaHS from the corresponding control values (%). *$p<0.05$ in comparison with the control (Wilcoxon test; $n=7$).

CO induced no significant changes in the analyzed parameters of isolated heart activity. Application of 5×10^{-5} M CO significantly accelerated heart rate and increased maximum rates of pressure rise and drop. Thus, in this concentration CO exerted stimulating influences on the heart with evident positive chronotropic and inotropic effects (Figs. 1 and 2, *a*). On the contrary, in higher concentrations CO decelerated heart rate and reduced all other parameters of contractive activity (Figs. 1 and 2, *a*).

Thus, effects of CO on mechanical activity of isolated rat heart depend on its concentration: it increases contractive activity in relatively low concentrations and suppresses heart function in concentrations of 10^{-4} M and higher, which can be associated not with regulatory, but toxic effects of CO in these concentrations.

In contrast to CO, H_2S donor NaHS produced similar effect in all tested concentrations: it decreased all parameters of contractive activity (Figs. 2, *b* and 3). In 3 experiments, extrasystoles and transient ventricular tachycardia were observed after application of 2×10^{-4} and 4×10^{-4} M NaHS, which attests to possible proarrhythmic effects of H_2S. These data agree with previously demonstrated shortening of action potential under the effect of H_2S following NaHS application in concentrations of 10^{-4} M and higher [3], since it is well known that shortening of action potentials in the myocardium weakens contractions.

Thus, we showed that gaseous signal compounds CO and H_2S induce substantial changes in contractive activity and contraction rate of isolated rat heart. It should be noted that H_2S in effective concentrations induces only negative inotropic and chronotropic influences, while CO in low concentrations increases contractive activity and accelerates heart rate. These data as well as the presence of the enzymes for synthesis of endogenous CO and H_2S in the myocardium [6,13] are the points toward inclusion of CO and H_2S in the group of important natural regulators of heart function.

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