The Effects of Nitroglycol on Rat Isolated Cardiac Muscles

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Abstract: To elucidate the action of nitroglycol (Ng) on cardiac muscles, the contractile and chronotropic responses of the isolated rat cardiac muscles to Ng in a cumulative manner were investigated. Ng produced negative chronotropic and inotropic effects on spontaneously beating right atria in concentrations ranging from 10^{-7} to 3×10^{-4} M. Ng also produced dose-dependent negative inotropic effects on electrically driven left atrial muscles. On the other hand, in right ventricle muscles, Ng induced positive inotropic effects. These results suggest that Ng acts directly on the cardiac muscles as well as vascular smooth muscles in acute poisoning.

Key words: Nitroglycol, Isolated cardiac muscles, Rat, Inotropic effect, Chronotropic effect

Introduction

Nitroglycol (Ng) has been known to cause sudden death in workers in the dynamite industry^{1,2}. Workers had almost no symptoms during their work, but after the weekend, they often had a heart attack on the next Monday, so it is known as the Monday disease due to Ng. Other symptoms induced by Ng are headache, nausea, vomiting, lowering of blood pressure, increase in the pulse rate, and cyanosis^{3,4}. Heart attack similar to angina pectoris is thought to be a main cause of sudden death due to Ng, but the effects on heart function due to Ng seem contradictory. Suwa *et al.* reported that Ng had a direct action on heart function in Ng poisoned rabbit⁵. On the other hand, Clark reported that the repeated injection of Ng into the rat does not lead to any changes in the ECG, heart rate or resting blood pressure of the resting animal⁶. And there are as yet no reports on the effects of Ng on the cardiac muscles although vasodilatory effects on vascular smooth muscle due to Ng have been reported^{7,8}.

Therefore, the purpose of this study is to clarify the direct effects of Ng on isolated cardiac muscles.

Material and Methods

Animals

7 male Donryu strain rats (Body weight 500–600 g) were purchased from Charles River Japan. They were housed in wire-meshed cages (3 or 4 rats/cage) and allowed to eat foods pellets and to drink water ad libitum. They were maintained at a temperature of $24 \pm 1^\circ\text{C}$, with a relative humidity of $55 \pm 5\%$, under an automatic lighting schedule of a 12-h light (8:00–20:00)/12-h dark (20:00–8:00) cycle.

Preparation of heart muscle strips

7 male Donryu rats were anesthetized by sodium pentobarbital 25 mg/kg. The hearts were removed quickly and immersed in ice-cold Krebs-Hensleit buffer of the following millimolar composition: NaCl 118; KCl 4.6; MgSO_4 1.2; NaHCO_3 27.2; CaCl_2 1.75; KH_2PO_4 1.2; and glucose 11.1. After adhering tissues were removed, the right and left atria and strips of the right ventricles (approximately 2 mm wide and 25 mm long) were dissected from the hearts. The strips were mounted individually by thread under a resting tension of 1 g in 30 ml organ baths containing Krebs-Hensleit buffer (Fig. 1). The buffer was maintained 37°C

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and bubbled with 95% O₂/5% CO₂. The preparations were placed between a pair of platinum electrodes. The right atria were allowed to beat spontaneously, but the left atria and strips of the right ventricle were driven at 3.3 Hz (left atria) or 1 Hz (right ventricle) with square-wave pulses of 1 ms duration and of an intensity sufficient to elicit contraction (usually 1 V) with a stimulator (Nihon Koden SEN-6203). The mechanical activity was measured isometrically, with transducers (Nihon Koden, TB-612T), and recorded on a thermal array recorder (Nihon Koden, RTA-1200). The contractile rate was also continuously monitored on a tachygraph unit (Nihon Koden, AT-601G). Contractions in spontaneously beating right atria were used to trigger a tacograph. The preparations were allowed to equilibrate for at least 1 hr before the administration of drugs. During the equilibration period, the tissues were washed with fresh Krebs-Henseleit buffer. Ng was added to the organ bath in a cumulative manner in concentrations ranging from 10^{-7} to 3×10^{-4} M.

Ng synthesis

Ng was synthesized by adding ethylene glycol to a mixed solution of conc. H₂SO₄ and conc. HNO₃ (1:1), and purified by washing with deionized water⁹⁾.

Statistics

Values are expressed as the means $\pm$ SD. Comparisons were made by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test, where comparisons with a common control were made. The level of statistically significant difference was $p < 0.05$.

Results

In the right atria, Ng produced a slight but significant negative chronotropic response (Fig. 2). The mean heart rate was 236.1 ± 19.8 bpm before Ng application. There were significant difference in the concentrations greater than 10^{-4} M Ng as compared with the control values. HR decreased 1.5 ± 1.7 , $2.2 \pm 1.9\%$ with 10^{-4} , 3×10^{-3} M Ng, respectively. Ng exerted significant negative inotropic effects in a dose-dependent manner in the right atria (Fig. 3). Contractility decreased 15.5 ± 6.1 , $16.9 \pm 7.1\%$ with 10^{-4} , 3×10^{-3} M Ng as compared with the control values, respectively. Ng also had negative inotropic effects on the left atria in a dose-dependent manner, though there were no significant differences in these changes (Fig. 4).

On the other hand, Ng produced a positive inotropic

Fig. 1. Experimental apparatus

response in the strips of right ventricle (Fig. 5). Contractility increased $15.2 \pm 11.8\%$ in the concentration of 3×10^{-6} M Ng as compared with the control values and this change was significantly different from the control.

Discussion

This study showed that Ng had negative inotropic effects on the atria and had positive inotropic effects on the right ventricle in contrast to the rat isolated cardiac muscle preparations.

The fall in blood pressure is one of the characteristic symptoms in Ng poisoning¹⁰⁾. A previous study⁸⁾ showed that this effect is caused by the dilating action of Ng on the vascular smooth muscles. Ng also had direct dilating effects on the cardiac atrial muscles as well as on the vascular smooth muscles in this study. Ng as well as nitroglycerin is known to be a nitrovasodilator. These compounds can give rise to nitric oxide which can activate guanylate cyclase and then lead to the relaxation of smooth muscles. One of the mechanism of action due to Ng in cardiac atria may be the same, that is, it is suggested that nitric oxide released from Ng may cause the relaxation of cardiac atria.

Clark⁶⁾ reported that the repeated injection of Ng into the rat does not lead to any changes in the ECG, heart rate or resting blood pressure of resting animals. Minami also showed no special findings concerning heart rate or arrhythmia in rats which were injected subcutaneously with 65 mg/kg of Ng¹¹⁾.

Fig. 2. Effects of increasing concentrations of nitroglycol on the rate of spontaneous beating of the right atrium of rat

%changes in heart rate are given as the means $\pm$ S.D (n=7). *p<0.05, ***p<0.001 for the significant differences from the control, respectively (analysis of variance with Tukey-Kramer multiple comparison test).

Fig. 4. Effects of increasing concentrations of nitroglycol on the contractility of the left atrium of rat

%changes in contractility are given as the means $\pm$ S.D (n=7).

Nevertheless, Suwa *et al.* reported some changes such as depression of ST, and negative, low, and biphasic T on the electrocardiographic patterns and irregular pulse in rabbits injected with Ng subcutaneously⁵. This study showed that Ng itself had a slight but significant negative effect on the pace maker cell due to acute, cumulative Ng administration. This direct negative effect on the right atria was consistent with Suwa's report.

The positive inotropic effect observed in the strips of right

Fig. 3. Effects of increasing concentrations of nitroglycol on the contractility of the right atrium of rat

% changes in contractility are given as the means $\pm$ S.D (n=7). *p<0.05, ***p<0.001 for the significant differences from the control, respectively (analysis of variance with Tukey-Kramer multiple comparison test).

Fig. 5. Effects of increasing concentrations of nitroglycol on the contractility of the strip of right ventricle of rat

% changes in contractility are given as the means $\pm$ S.D (n=7). *p<0.05 for the significant differences from the control (analysis of variance with Tukey-Kramer multiple comparison test).

ventricles had not been previously reported. In the cardiac muscle, adrenergic beta-1 receptor is dominant and its activation leads to a positive inotropic effect, though there are alpha and beta adrenergic receptors in¹². The result observed here due to Ng would be partly induced by beta-1 receptor activation (data not shown). Further experiment

with various antagonists will be needed to analyze the difference between the inotropic effects in atria and those in ventricle reported here.

Johansson *et al.*⁷⁾ concluded that long term Ng treatment induces tolerance at the cellular level, resulting in a decrease in blood pressure and a reflexogenic increase in heart rate, and also interferes with catecholamine formation and possibly receptor functions. Since it is suggested that Ng may act on receptor functions, long term Ng exposure experiments would be necessary to clarify these effects.

Clark reported that Ng did not affect cardiac function, but did increase cardiac sensitivity to exogenous epinephrine but this study showed that Ng directly affected cardiac function, that is, Ng has dilatory effects on the cardiac atria as well as on smooth muscles and there was a slight but significant effect on the pace maker cells. These effects of Ng may reflect its relation to cardiac insufficiency reported in human cases. Parmeggiani¹³⁾ suggested that the mechanism of death appears to be sudden arrhythmia rather than coronary spasm. It would be important to investigate the effects of Ng on cardiac function. Further long term experiments would be necessary to clarify in detail because this study was based on acute, single exposure to Ng in isolated cardiac muscles.

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