Ammonium Chloride-Induced Modification of the Effect of Sodium Nitroprusside on the Contractility of Guinea Pig Papillary Muscle

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The effective use of nitrates as antianginal drugs is traditionally believed to be due to their vasodilatory properties [2]. The possibility of a direct cardiotropic effect of nitric compounds is usually not taken into account. On the other hand, sodium nitroprusside (SNP) has been shown to be able to activate the slow inward calcium current in the fibers of the frog atrium [3]. Without question, the reversion of this effect of SNP by an increase of the intracellular pH (pH_i), is worthy of note. Taking into account the possible shift of pH_i in both the acid and alkaline directions during the development of ischemia or of myocardial hypoxia [7], it seemed of interest to study the changes of the myocardial response in the presence of SNP for an altered intracellular concentration of protons.

MATERIALS AND METHODS

The experiments were carried out on the papillary muscle (smaller that 1 mm in diameter and 2 to 4 mm long) obtained from the right ventricle of the guinea pig heart. The isolated muscles were perfused with oxygenated Krebs-Henseleit solution (pH 7.3-7.4) at a temperature of 25±0.5°C and at a stimulation frequency of 0.5 Hz.

The mechanical activity of the papillary muscles was recorded in the isometric mode with the aid of a 6MKhIS mechanotron. The curves of myocardial contraction were recorded on an N306 recorder and were also entered via a digital-to-analog converter into an SM-4 computer and later processed using designated software.

Before the start of the experiments the preparations were adapted in the test cell for 1 h. The changes of intracellular pH were caused by replacing 20 mM of NaCl in the perfusion fluid with an equimolar amount of NH_4Cl. Sodium nitroprusside (Sigma) was used in a concentration of 10 μM. All the solutions were prepared directly before the experiments. The solution containing SNP was protected from the light.

The experimental results were statistically processed on an SM-4 computer and on an IBM PC with the use of standard Statgraphics software.

RESULTS

As the first step we assessed the effect of changing the pH_i on the contractility of the guinea pig papillary muscle. A number of scientists previously showed that equimolar replacement of NaCl with NH_4Cl causes intracellular alkalosis, whereas the subsequent removal of NH_4Cl is accompanied by a short-term drop of pH_i, due to free exchange of NH_3 between the extracellular medium and the myoplasm [8,10].

We reproduced this methodological approach in the experiments on the isolated preparations of the

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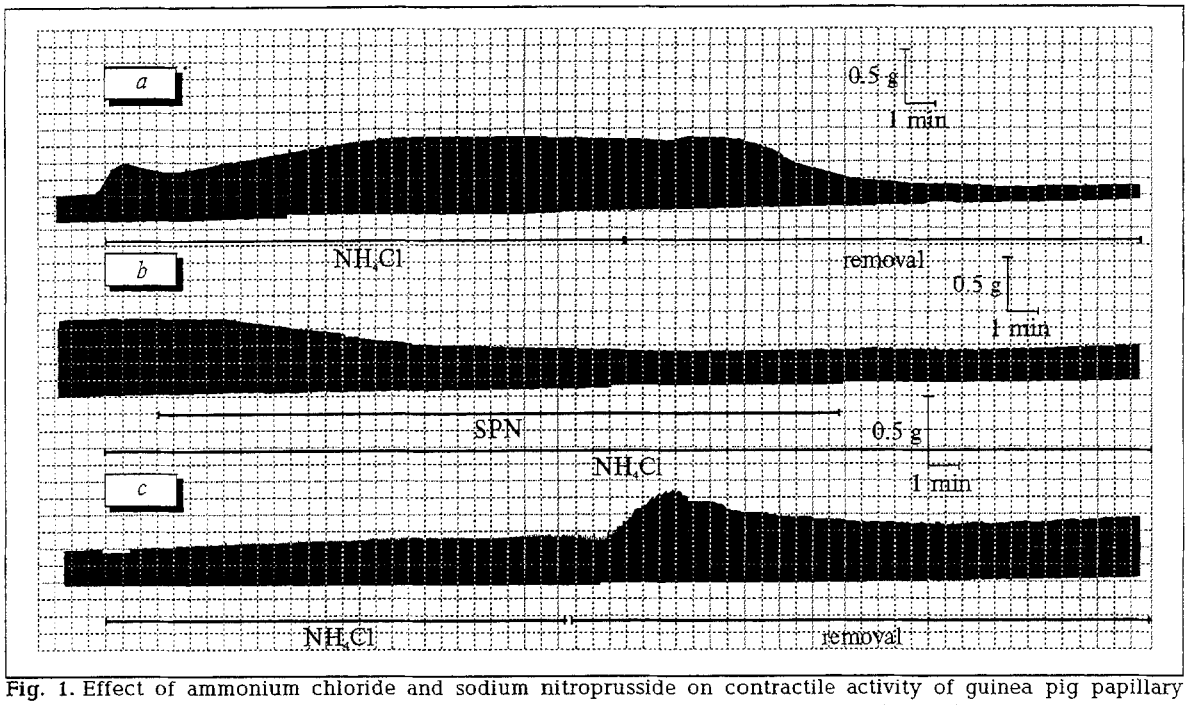

Fig. 1. Effect of ammonium chloride and sodium nitroprusside on contractile activity of guinea pig papillary muscle. Stimulation frequency 0.5 Hz, t=25°C, continuous recording. *a*) introduction and subsequent removal of NH_4Cl (20 mM); *b*) effect of sodium nitroprusside (10 mM) against the background of NH_4Cl (20 μM).

guinea pig heart. Introduction of NH_4Cl (20 mM) in the perfusion fluid was attended by a rapid (in the 1st-2nd min) increase of the amplitude of the contractile response (F_{max}) of the papillary muscle (Fig. 1, *a*). In the 15th min, F_{max} attained the steady-state level, exceeding the initial value by 90±32% (n=11, p<0.01). In the 10th-15th min, following the removal of ammonium chloride, F_{max} dropped to a new steady-state level, which constituted 50±14% of the baseline values (p<0.05). After that the contractility of the myocardium slowly (during 20 min) recovered to reach the initial level (Fig. 1, *a*). These results are in tune with reports of other scientists, who observed a similar dynamics of cardiac mechanical activity, as well as of the calcium current for ammonium chloride-induced changes of pH_i [1,3,10].

As expected, the experiments on the perfusion of isolated heart preparations with SNP (10 μM, n=5) showed that SNP exerted no marked effect upon the contractility of the intact papillary muscle. However, addition of the preparation after preliminary intracellular alkalinization caused by a 15-min exposure to ammonium chloride (20 mM) led to a rapid (in the 2nd min, on average) suppression of myocardial contractility (Fig. 1, *b*). Under the influence of SNP F_{max} dropped 30±17% (n=7, p<0.05) vs. the value of this parameter recorded after the 15-min exposure to NH_4Cl.

A 15-min removal of SNP under conditions of intracellular alkalinization was not accompanied by a recovery of myocardial contractility (Fig. 1, *b*). In most experiments of this series, in the first minutes following the removal of ammonium chloride from the perfusion fluid, not a suppression of myocardial contractility, but rather its stimulation was observed (Fig. 1, *c*). For instance, in the 2nd-3rd min following the removal of ammonium chloride F_{max} increased by 60±42% in the experimental series with SNP, whereas in the control series this parameter was reduced by 35±14% vs. the value recorded prior to the removal of ammonium chloride (p<0.05 in both cases).

In some experiments (n=5) for combined action of SNP and NH_4Cl contractile responses with a diverse amplitude of contraction (the phenomenon of mechanical alternation) developed. It was established that an increase of the stimulation frequency of the papillary muscle from 0.5 to 1.0 Hz was attended by an enhancement of these alternations (Fig. 2). It should be mentioned that neither SNP nor ammonium chloride produced such an effect in the experiment. Alternations could be eliminated only by removing both SNP and ammonium chloride. It should be emphasized that the rapid suppression of F_{max} and the development of mechanical alternations were also observed for intracellular alkalinization following the 15-min exposure to SNP (n=2).

These results provide evidence that modification of the intracellular pH can lead to the development of an atypical effect of nitrates. Alkaliniza-

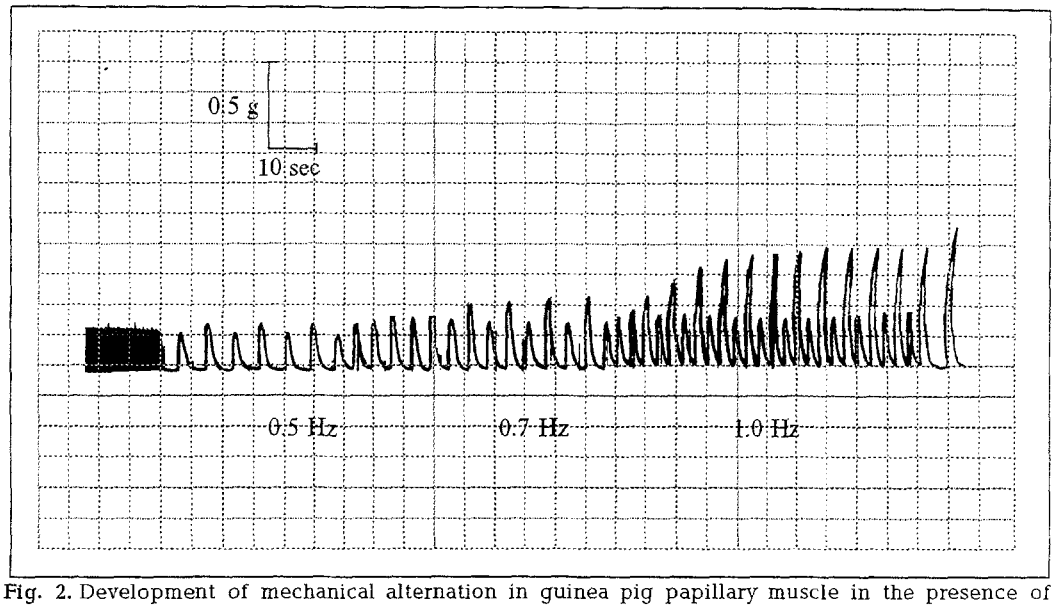

Fig. 2. Development of mechanical alternation in guinea pig papillary muscle in the presence of SNP (10 μM) against the background of NH_4Cl (20 mM) for stepwise increase of stimulation frequency from 0.5 to 0.7 and 1.0 Hz.

tion in the cells of the myocardium promotes a negative inotropic effect of SNP. The effect of nitrates is known to be realized via the activation of soluble guanylate cyclase and a rise of the intracellular level of cGMP [4,9]. When assessing the possible intracellular targets of cGMP in the myocardium [11,13,14], one may assume that cGMP-mediated inhibition of the inward slow calcium current (I_{Ca}) underlies the effect of SNP discovered by us. Evidently, different cGMP-dependent pathways of inhibition of I_{Ca} are possible [12], but it is probably the pH_i increase that somehow promotes the regulatory effects of cGMP not observed under control conditions.

Against the background of alkalinization the stabilization of the inhibiting effect of SNP, which does not disappear after removal of the preparation, is worthy of note. It may be speculated that an increase of pH_i hinders the hydrolysis of cGMP by inhibiting the activity of phosphodiesterase, or that a certain additional influence is needed to reverse the effect of cGMP, for example, an increase of the cAMP content, as in the situation characteristic of troponin I phosphorylation-dephosphorylation according to the data of Winegrad [14]. The enhanced contractility of the papillary muscle during simulation of intracellular acidification after the removal of SNP may be due to the activation of cGMP phosphodiesterase at acidic values of pH_i or be caused by some other processes.

Thus, the changes in the pH_i of cardiomyocytes may promote such atypical effects of SNP as suppression of contractility and provocation of mechanical instability in the myocardium. A noteworthy fact is that individual cases of disturbances of the cardiac rhythm have been described in the literature for administration of nitrates to patients [6]. Apparently, the changes in the ratio of cyclic nucleotides [5] and the shift of pH_i may be instrumental in determining this phenomenon. In view of the wide use of nitric compounds in the treatment of ischemic heart disease and the possibility of pH_i alteration during the development of a number of pathological states, further studies of the nature of the above phenomena are undoubtedly needed.

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