Relaxing Action of Sodium Nitroprusside Independent of Membrane Potential in the CCh-induced Contracture of the Guinea Pig Stomach Muscle

Yasuji SAKAMOTO

Department of Physiology, School of Medicine, Fukuoka University

Abstract

Carbachol (CCh, 10⁻⁶ M) induced biphasic contraction of longitudinal muscle of the guinea pig stomach, consisting of rapid phasic contraction and contracture. The contracture was almost completely inhibited by sodium nitroprusside (SNP, 10⁻⁶ M) and S-nitroso-N-acetyl penicillamine (SNAP, 10⁻⁶ M). A membrane permeable analogue of cyclic GMP, 8Br-cGMP (10⁻⁴ M), also inhibited the CCh-induced contracture. Although a heme-site inhibitor of nitric oxide-sensitive guanylyl cyclase, 1-H-[1, 2, 4]oxadiazolo-[4, 3-a]quinoxalin-1-one (ODQ ; 10⁻⁶ M), reduced the inhibitory action of SNP, it did not affect the inhibitory action of 8Br-cGMP, indicating that the effect of SNP was developed via cyclic GMP production in the presence of D600. Charybdotoxin (10⁻⁷ M), an inhibitor of Ca²⁺-activated K⁺ channel, did not influence on the CCh-induced contracture. On the other hand, CCh induced a depolarization of the longitudinal muscle cell membrane (from −60 mV to −45 mV) in the presence of 10⁻⁶ M D600, but SNP did not affect the depolarization. These results suggest that in the presence of D600 SNP induces relaxation of CCh-induced contracture of the longitudinal muscle of the guinea pig stomach via cyclic GMP but not membrane potential dependent mechanism

Key words: stomach muscle, relaxation, SNP, ODQ.

Introduction

In gastrointestinal smooth muscle, nitric oxide (NO)-releasing compounds such as sodium nitroprusside (SNP) and S-nitroso-N-acetylpenicillamine (SNAP) has been reported to greatly inhibit mechanical activity without much effect on the electrical activity (Ozaki et al. 1992). In rat circular muscle of the gastric funds, SNP significantly hyperpolarized the membrane in the resting state (Kitamura et al. 1993). Recently effects of NO donors have been reported to be mediated by cyclic GMP-dependent and -independent mechanisms (Knudsen et al. 1992; Takeuchi et al. 1996; Boltina et al. 1994). For example, NO caused the direct activation of calcium sensitive K⁺ channels in vascular smooth muscle (Boltina et al. 1994: Peng et al. 1997) and in human myometrium (Bradley et al., 1998). However, in the circular muscle of the

Correspondence to: Yasuji Sakamoto, Department of Physiology, School of Medicine, Fukuoka University, 814-0180 Fukuoka, Japan. Fax: 092-865-6032, Email: sakamoto@msat.fukuoka-u.ac.jp
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corpus of the guinea pig stomach, a significant hyperpolarization was not observed with an
inhibition of spontaneous phasic contraction in the resting state (Nasu and Sakamoto, 1991).
Thus a relaxing mechanism of NO donor, which is known to be a vasodilator, is still compli-
cated in various smooth muscle. Therefore in the present study the effects of SNP on the CCh-
induced contracture and depolarization of the longitudinal muscle cell membrane in the guinea
pig stomach were further investigated.

Materials and methods

Guinea pigs of both sexes weighing 250-300 g were stunned and bled. The stomach was
excised and a strip of longitudinal muscle (1 mm in width and 5 mm in length) was dissected
from the greater curvature aspect of the corpus. The mucous and submucosa were mechani-
cally removed from the muscle strip under a stereomicroscope. Longitudinal muscle strips
without their circular muscle layers were used. The preparations were suspended in an organ
bath (10 ml) containing Krebs bicarbonate solution, maintained at 35°C, and gassed with 3% CO2
in 97% O2 (pH of 7.4). The composition of Krebs bicarbonate solution used was (mM): NaCl
120, KCl 5.9, CaCl2 2.5, MgCl2 1.2, NaH2PO4 1.2, NaHCO3 15.5 and glucose 11.5. The initial
resting tension was set to 1 mN, then the preparations were equilibrated for 2 hrs to obtain a
steady tension before beginning the experiments. The contraction of the muscles was recorded
isometrically with a force displacement transducer (Shinkouseiki U2) linked to a penrecorder
(National Inc.). A concentration of 10^-6 M of carbachol (CCh) was used to induce the tonic
contraction (contracture), because concentrations of more than 10^-5 M CCh frequently produced
a transient inhibition prior to development of the contracture. This relaxation may be induced
by nitric oxide (NO) released from the tissue preparation, since the relaxation is inhibited by
application of L-NAME (data not shown). The membrane potential was observed using a
microelectrode technique from small strips of the longitudinal muscle to avoid the disturbance
due to the mechanical responses. The chemical agents used were carbachol (CCh : Sigma
Chem.), sodium nitroprusside (SNP : Sigma Chem.), S-nitroso-N-acetylpenicillamine (SNAP :
RBI), charybdotoxin (ChTx : RBI), iberiotoxin (RBI), SK & F 96365 (Calbiochem), 8-Bromo-
guanosine 3'5'-cyclic monophosphate (8Br-cGMP : Sigma Chem.), nifedipine (Sigma Chem.), D-
600 (RBI), 1H-][1,2,4]oxadiazolo[4, 3-a]quinoxalin-1-one (ODQ : Sigma Chem.), and Ng-
nitro-L-arginine methyl ester hydrochloride (L-NAME : Wako chem.). Drugs were adminis-
tered to the bathing fluid (10 ml). All concentrations in the text refer to final concentrations of
free base in the bathing fluid and were expressed in terms of molarity.

Results

Effect of SNP on CCh-induced tonic contracture of longitudinal muscle

Exogenously added carbachol (CCh, 10^-8 M) induced biphasic contraction of longitudinal
muscle of the guinea pig stomach, consisted of a rapid transient and a delayed tonic contraction
or contracture (inset of Fig. 1). The contracture was immediately and completely inhibited to
the initial level by application of SNP (10^-6 M). The inhibitory effect was dose dependent and
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ID$_{50}$ (the concentration of SNP causing 50% inhibition of the contracture) was $4 \times 10^{-7}$ M, as shown in Fig. 1. S-nitroso-N-acetyl-penicillamine (SNAP: $10^{-5}$ M) also induced an inhibition of the contracture (data not shown). A high K$^+$ concentration (30 mM)-induced contracture was also completely inhibited by D600 ($10^{-5}$ M) and significantly reduced by SNP ($10^{-5}$ M) but not completely, and finally the remaining one was fully inhibited by subsequent application of D600 (Fig. 2). The inhibitory effect of SNP on CCh-induced contracture was reconfirmed in another series of experiment, in which the preparations were treated with a low concentration of SNP ($10^{-7}$ M). Normalized dose-response curve of the CCh-induced contracture shifted to the right in the preparations treated with SNP (Fig. 3). The maximum amplitude of the contracture was slightly decreased in this preparation treated with SNP (data not shown).

Fig. 1. Inhibition of CCh ($10^{-6}$ M)-induced contracture by SNP. The inset shows the CCh-induced phasic contraction followed by tonic contraction (contracture). Each point represents the mean±S.E. of ten experiments. Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by $10^{-5}$ M SNP. Abscissa: log concentration (M) of SNP.

Fig. 2. Inhibition of high K$^+$-induced contracture by D600 and SNP. Complete and partial inhibition of 30 mM K$^+$-induced contracture by D600 ($10^{-4}$ M) (a) and SNP ($10^{-4}$ M) (b), respectively. Remaining contracture in the presence of SNP was blocked by subsequent application of D600 ($10^{-4}$ M).
Fig. 3. The normalized concentration-response curves of the CCh-induced contracture in the absence and presence of low concentrations of SNP (10^-7 M). Ordinate: amplitude of contracture (%) expressed as percentage of the maximum contracture induced by 10^-5 M CCh. Abscissa: log concentration (M) of CCh. Open and closed circles show mean values of the contractile responses in the Krebs solution without and with SNP, respectively. Each point represents the mean±S.E. of ten experiments.

Effect of a membrane permeable analogue of cyclic GMP, 8Br-cGMP on CCh-induced contracture

It is generally accepted that the nitric oxide (NO)-induced relaxation is mediated with cyclic GMP (Kuriyama et al. 1995). Therefore an effect of membrane permeable cyclic GMP, 8Br-cGMP (10^-4 M), was examined. Exogenously added 8Br-cGMP (10^-4 M) partially inhibited the CCh-induced contracture, but a subsequent application of SNP completely inhibited the remaining contracture (inset of Fig. 4). The relaxation phase was exponential and its time constant was 10.0±0.56 sec (n=10) and 60.2±5.3 sec (n=10) in SNP and 8Br-cGMP, respectively (Fig. 4).

Effect of guanylyl cyclase inhibitors on CCh-induced contracture

New potent selective inhibitor of soluble guanylyl cyclase, ODQ, has been identified (Garthwaite et al. 1995; Moro et al. 1996) and the effect of ODQ (10^-5 M) on the CCh-induced contracture was tested in the present experiment. The preparations were pretreated with ODQ to inhibit soluble guanylyl cyclase and then SNP was introduced. The pretreatment produced small contracture, but it was blocked by D600 (10^-6 M). Next, CCh (10^-5 M) was applied to the preparations to induce contracture of the muscle in the presence of ODQ and D600. Although SNP completely inhibited the contracture in the absence of ODQ (Fig. 5a), it did not inhibit the contracture in the presence of ODQ (Fig. 5b). However, subsequent application of 8Br-cGMP (10^-4 M) after SNP completely inhibited the contracture (Fig. 5b).

Effects of charybdotoxin (ChTx) on CCh-induced contracture

Effect of a calcium activated K^+ channel blocker, ChTx, on the CCh-induced contracture was studied to know whether membrane repolarization via ChTx-sensitive K^+ channel contrib-
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Fig. 4. Time course of relaxation of CCh (10^{-6} M)-induced contracture in the presence of after application of SNP (10^{-6} M: ■) or 8Br-cGMP (10^{-4} M: △). All experimental points are fitted to each theoretical exponential curve (n=10). Inset shows a typical recording. Note the longer time course of relaxation with 8Br-cGMP than SNP. Ordinate: amplitude of contracture (%), expressed as percentage (100%) of the amplitude at point of drugs application. Abscissa: sequential time after the application of drugs.

Fig. 5. Antagonistic action of 1H-[1, 2, 4]oxadiazolo[4, 3-a]quinoxalin-1-one (ODQ) on the relaxation induced by SNP. Upper trace shows full relaxation of the contracture by SNP (a). Lower trace shows disappearance of the relaxation by pretreatment of ODQ and reappearance of relaxation by the subsequent application of 8Br-cGMP (b). All horizontal bars show application time of drugs.
utes to relaxation of the muscle preparations. To remove a contribution of inhibition of Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channel or nonselective cation channel to relaxation, D600 (10\(^{-5}\) M) and SK & F96365 (10\(^{-5}\) M) was initially applied to the preparations. Both drugs partially inhibited the CCh-induced contracture, but did not influence on the inhibitory action of SNP at all (Fig. 6). Further, subsequent application of ChTx (10\(^{-7}\) M) (Fig. 6) or iberiotoxin (10\(^{-7}\) M: data not shown) did not affect the contracture. However, the contracture which persisted after D600- and ChTx-treatment was completely inhibited by SNP (10\(^{-5}\) M) or 8Br-cGMP (10\(^{-4}\) M) (Fig. 6).

**Effect of SNP on membrane potential of longitudinal muscle.**

Since it is difficult to record the electrical activity from the longitudinal muscle cells by conventional microelectrode technique, D600 (10\(^{-6}\) M) was used to reduce contractile movements of the tissue preparations throughout the experiments. When CCh (10\(^{-6}\) M) was applied to the preparation in the presence of D600, the membrane was depolarized from \(-60.4\pm2.3\) mV to \(-45.3\pm2.6\) mV (n=10) as shown in Fig. 7. Subsequent application of low (10\(^{-6}\) M) or high (10\(^{-5}\) M) concentration of SNP did not affect the membrane potential, although the duration of the slow wave was slightly reduced by SNP (upper trace of Fig. 7).

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**Fig. 6.** Effect of SK & F 96365 (10\(^{-5}\) M) and charybotoxin (10\(^{-7}\) M) on the inhibitory action of SNP (10\(^{-4}\) M) (a) and 8Br-cGMP (10\(^{-4}\) M) (b) applied during CCh-induced contracture. All horizontal bars above the recording indicate application time of various drugs. Upper and lower traces are obtained from the same preparation. Note there is no effect of charybotoxin on the relaxation.
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Discussion

In rat gastric funds, SNP or 8Br-cGMP activated guanylate cyclase and then induced an apamin-sensitive hyperpolarization with relaxation (Kitamura et al. 1993). In bovine tracheal smooth muscle cells, ChTx inhibits the relaxation induced by SNP via cyclic GMP (Hamaguchi et al. 1992). Recently, the ChTx-sensitive, cyclic GMP-insensitive, relaxation was obtained in myometrium (Bradley et al., 1998) and in coronary arteries of human (Bychkov et al. 1998). Above results indicate that NO donor-induced relaxation involves an activation of K⁺ channel via cyclic GMP or not via cyclic GMP. In the present experiments, however, ChTx or iberiotoxin did not affect the contracture at all. Apamin also did not affect the SNP-induced relaxation. In the histamine-induced contracture (We have observed that TEA directly blocked the CCh-induced contracture through receptor blocking), TEA did not affect the SNP-induced relaxation (unpublished observation). Furthermore, the CCh-induced depolarization of longitudinal muscle membrane was not influenced by SNP. Therefore, a contribution of Ca²⁺-activated K⁺ channel to the SNP-induced relaxation may be neglected. On the other hand, cyclic GMP-formation inhibitor, ODQ, inhibited the relaxing action of SNP and changed the relaxation phase. Relaxing phase was different greatly from that of SNP or 8Br-cGMP.
alone, that is, the time course of the relaxation during ODQ was not exponential (Fig. 5). The remaining contracture, which was not inhibited by the firstly applied SNP in the presence of ODQ, was fully inhibited by secondly applied 8Br-cGMP. Therefore, ODQ may inhibit the binding of NO to enzyme (to heme-site of guanylyl cyclase) (Garthwaite et al. 1995), resulting in an inhibition of cyclic GMP formation. Thus cyclic-GMP formation is necessary for SNP to inhibit the CCh-induced contracture.

A mechanism of difference in the time course of between SNP- and 8Br-cGMP-induced relaxation is unknown at the present time. However, a concentration of cyclic GMP immediately after application of SNP may be higher than that as 8Br-cGMP application. Other possibility of direct inhibition of Ca\(^{2+}\) influx by SNP may not be neglected (Clapp and Gurney, 1991), since in this study SNP significantly inhibited the high K\(^{+}\)-induced contracture (Fig. 2) (Rubiales et al., 1988). In addition, a reduction of CCh (10\(^{-5}\) M)-induced maximum contracture by a low concentration of SNP supports this argument.

A relaxing mechanism of cyclic GMP is still not clear in the present experiments. However, following mechanisms should also be considered: a stimulation of Ca\(^{2+}\) pump (Furukawa et al. 1988; Vrolix et al. 1988; Yoshida et al. 1991) or an activation of Ca\(^{2+}\) efflux (by Na\(^{+}\)-Ca\(^{2+}\) exchange) (Furukawa et al. 1991) of the plasma membrane, resulting in relaxation.

Finally contribution of Ca\(^{2+}\) pump of the sarcoplasmic reticulum membrane via cyclic GMP to the relaxation may not be neglected, since reuptake of Ca\(^{2+}\) to store sites was enhanced by SNP-treatment during Ca\(^{2+}\) loading in Ca\(^{2+}\)-free Krebs solution (unpublished observation).

Thus it is concluded that SNP induces relaxation of the CCh-induced contracture of the guinea-pig stomach muscle via cyclic GMP and the relaxation is independent of the membrane potential change in the presence of D600. A further investigation of the relaxation via cyclic GMP in this preparation is required.

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