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Short communication

Effects of inhibitors of cGMP-dependent protein kinase in atrial heart and aortic smooth muscle from rats

Dörte Bäumner, Hermann Nawrath *

Pharmakologisches Institut der Universität Mainz, Obere Zahlbacher Straße 67, D-55101 Mainz, Germany

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Abstract

Several activators of cGMP-dependent protein kinase (protein kinase G) such as 8-Br-cGMP reduced force of contraction in rat left atria. Inhibitors of protein kinase G antagonized the negative inotropic effect of 8-Br-cGMP but not of acetylcholine in atria. However, the acetylcholine-induced relaxation in aortic rings was significantly inhibited by protein kinase G inhibition. It is concluded that the reduction by 8-Br-cGMP of force of contraction in atria is related to activation of protein kinase G. In response to acetylcholine, activation of protein kinase G is probably a major step in smooth muscle relaxation but is not involved in the reduction of force of contraction in atria.

Keywords: Atrium, rat; Contraction, force of; Aortic ring; Relaxation; cGMP-dependent protein kinase; Acetylcholine

1. Introduction

Acetylcholine exerts a negative inotropic effect in atrial preparations which involves opening of potassium channels and inhibition of adenylate cyclase (Hartzell, 1988). Acetylcholine also increases guanosine-3′,5′-cyclic monophosphate (cGMP) levels in the heart (George et al., 1970), and 8-Br-cGMP has been shown to induce a negative inotropic effect (Nawrath, 1976). However, up to now it was not possible to clearly decide to what extent, if at all, cGMP contributes to the effects of acetylcholine in the heart (Hartzell, 1988).

Recently, cGMP-dependent protein kinase (protein kinase G) has been shown to be present in rat cardiac myocytes (Méry et al., 1991). The availability of specific activators and inhibitors of protein kinase G may help to define more precisely (1) whether the effect of cGMP derivatives on the heart is due to activation of protein kinase G and (2) whether cGMP is involved in the inotropic response to acetylcholine. To answer these questions, we studied the interaction of activators and inhibitors of protein kinase G and the influence of inhibition of protein kinase G on the effect of acetylcholine in isolated rat left atria. For comparison, the effects of acetylcholine on aortic smooth muscle and its interaction with an inhibitor of protein kinase G were investigated as well.

2. Materials and methods

Left atria and thoracic aorta with intact endothelium were isolated for recording isometric tension as described previously (Jahnel et al., 1994). Atria were electrically driven by square-wave pulses (Grass S4; 1 ms duration; voltage 20% above threshold) at 0.5 Hz.

The Tyrode's solution, containing (mmol/l) NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.6, EDTA 0.05, was equilibrated with 95% O_2 and 5% CO_2 at 37°C (pH 7.4). The effects of drugs were investigated by exposing tissues to either single or to cumulatively increasing concentrations after establishment of a stable response.

2.1. Chemicals

Activators of protein kinase G: 8-bromoguanosine-3′,5′-cyclic monophosphate (8-Br-cGMP) (Sigma, Mu-

* Corresponding author. Tel. 06131 177298, fax 06131 176611.

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nich, Germany); 8-(4-chlorophenylthio)-guanosine-3′,5′-cyclic monophosphate (8-pCPT-cGMP), β-phenyl-1,N^2-ethenoguanosine-3′,5′-cyclic monophosphate (PET-cGMP), Sp-8-bromoguanosine-3′,5′-cyclic monophosphorothioate (Sp-8-Br-cGMPS), Sp-8-(4-chlorophenylthio)-guanosine-3′,5′-cyclic monophosphorothioate (Sp-8-pCPT-cGMPS), and inhibitors of protein kinase G: Rp-8-bromoguanosine-3′,5′-cyclic monophosphorothioate (Rp-8-Br-cGMPS), Rp-8-(4-chlorophenylthio)-guanosine-3′,5′-cyclic monophosphorothioate (Rp-8-pCPT-cGMPS) (Biolog, Bremen, Germany); phenylephrine (Sigma, Munich, Germany). Acetylcholine and all other chemicals were obtained from Merck, Darmstadt (Germany).

2.2. Evaluation of results

Results are expressed as means ± standard error of means (S.E.M.). Peak levels of phasic contractions in atria or tonic tension in aortic rings precontracted with phenylephrine 1 μmol/l were evaluated and are given as percentage of control values. Student's paired two-tailed *t*-test was used to determine the significance of differences between means. Where appropriate, a two-way analysis of variance (repeated measurements design) was used. $P < 0.05$ was taken as being statistically significant. The differences are marked by one, two or three asterisks corresponding to $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

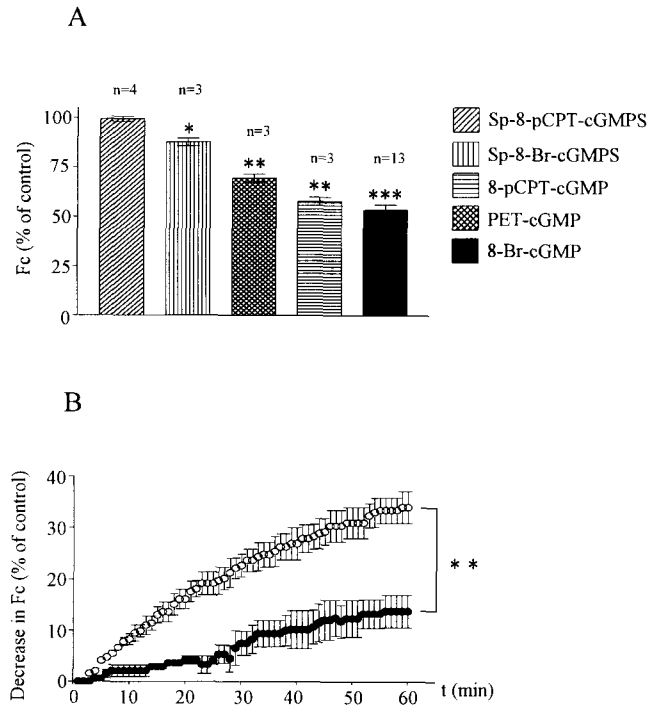

Fig. 1. (A) Influence of several activators of protein kinase G, 100 μmol/l each, on force of contraction of rat left atria at 0.5 Hz. (B) Influence of Rp-8-Br-cGMPS, an inhibitor of protein kinase G, on the negative inotropic effect of 8-Br-cGMP in rat left atria at 0.5 Hz ($n = 3$). (○) 8-Br-cGMP 10 μmol/l; (●) 8-Br-cGMP 10 μmol/l in the presence of Rp-8-Br-cGMPS 100 μmol/l. Data represent means ± S.E.M. (unpaired data). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

3. Results

Fig. 1A shows the effects of several cGMP derivatives on force of contraction in rat left atria. Steady-state effects were reached within 60 min. All these derivatives are assumed to activate protein kinase G (Jastorff, 1992; Genieser/Biolog, personal communication). Whereas force of contraction remained virtually unchanged after the addition of Sp-8-pCPT-cGMPS 100 μmol/l (99.4 ± 1.3% of control values), it was decreased by different amounts by the other derivatives of cGMP tested. Force of contraction was decreased to 87.8 ± 2.0% of control by Sp-8-Br-cGMPS, to 69.6 ± 2.1% by PET-cGMP, to 58.0 ± 2.1% by 8-pCPT-cGMP, and to 53.6 ± 2.5% by 8-Br-cGMP, at 100 μmol/l each.

Fig. 1B shows the development of the negative inotropic response to 8-Br-cGMP 10 μmol/l in rat left atria. After preincubation (45 min) of the preparations with Rp-8-Br-cGMPS 100 μmol/l, an inhibitor of protein kinase G, the maximal inotropic effect of 8-Br-cGMP was reduced by about 60%. In another series of experiments, the effect of 8-Br-cGMP 100 μmol/l, in the presence of Rp-8-Br-cGMPS 100 μmol/l, was diminished by about 25% (not shown). Similar results were obtained with Rp-8-pCPT-cGMPS 100 μmol/l, also an inhibitor of protein kinase G (not shown). Both inhibitors did not change force of contraction when given alone.

Acetylcholine decreased force of contraction by up to about 58% of control in a concentration-dependent manner (Fig. 2A). To evaluate whether protein kinase G is involved in the negative inotropic action of acetylcholine, the concentration-response relationships of acetylcholine were repeated in the presence of Rp-8-Br-cGMPS 100 μmol/l. The inhibitor of protein kinase G was applied 45 min before acetylcholine was added. Despite the presence of the inhibitor of protein kinase G, which inhibited the effects of 8-Br-cGMP, the effect of acetylcholine remained unchanged.

Fig. 2B shows that acetylcholine induced (10 μmol/l) relaxation of rat aortic rings to 51.2 ± 8.6% of the phenylephrine-induced contracture. After washout, the aortic rings were again precontracted with phenylephrine 1 μmol/l, and then incubated with Rp-8-Br-cGMPS 100 μmol/l for 30 min. During this period, the contracture increased to 108.5 ± 1.2% of control values (not shown). Additional application of acetylcholine

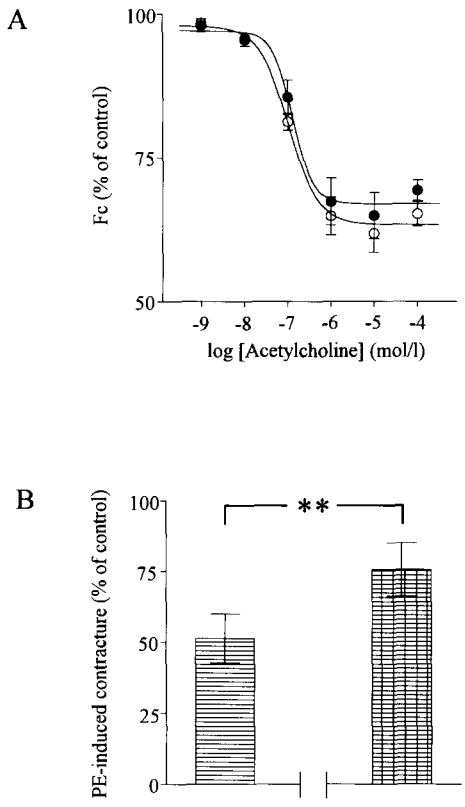

Fig. 2. (A) Influence of Rp-8-Br-cGMPS, an inhibitor of protein kinase G, on acetylcholine-evoked negative inotropic effects in rat left atria at 0.5 Hz ($n = 3$). (○) Acetylcholine; (●) acetylcholine in the presence of Rp-8-Br-cGMPS 100 μmol/l. (B) Influence of Rp-8-Br-cGMPS on acetylcholine-induced relaxation in rat aortic rings precontracted with phenylephrine 1 μmol/l ($n = 4$). Left column: acetylcholine 10 μmol/l; right column: acetylcholine 10 μmol/l in the presence of Rp-8-Br-cGMPS 100 μmol/l (** $P < 0.01$). Data represent means ± S.E.M. (paired data).

now produced a relaxation to only 75.5 ± 9.4% of control.

4. Discussion

Several activators of protein kinase G reduced force of contraction in rat left atria, as shown previously for 8-Br-cGMP (Nawrath, 1976), by different amounts. The reason for the lower potency of Sp-8-pCPT-cGMPS and Sp-8-Br-cGMPS to decrease force of contraction in rat left atria remains unclear and contrasts with biochemical data indicating potent and selective stimulation of protein kinase G (Genieser/Biolog, personal communication).

We have demonstrated that specific inhibitors of protein kinase G, Rp-8-Br-cGMPS and Rp-8-pCPT-cGMPS, antagonize the effects of 8-Br-cGMP, the antagonism being more pronounced at low than at high concentrations of 8-Br-cGMP. This can be taken as evidence that the negative inotropic response to 8-Br-cGMP in rat left atria is, indeed, related to protein kinase G activation. Rp-8-Br-cGMPS did not antagonize the negative inotropic response to acetylcholine in left atria. By contrast, it antagonized the relaxing effect of acetylcholine in aortic strips. This discrepancy brings up again the question about which functional differences exist between the modulatory role of cGMP in the control of myocardial contractility and smooth muscle tone. Protein kinase G seems therefore not to be involved in the acetylcholine-evoked negative inotropic effect in left atria, although an increase, albeit small, in cGMP levels has been observed. A possible explanation is that the increase in cGMP content in response to acetylcholine is too small to induce a negative inotropic effect. Alternatively, the cGMP-protein kinase G signal pathway may be less efficient in heart than in smooth muscle. The prominent effect of acetylcholine in atria may rather be on potassium channels (Ten Eick et al., 1976), and it has been shown earlier that 8-Br-cGMP does not mimic the effect of acetylcholine on ^{42}K efflux (Nawrath, 1977).

By stimulating endothelial cells, acetylcholine uses the nitric oxide pathway, resulting in an increase of cGMP levels to induce vasodilation (Moncada et al., 1991). Our own results and the findings of Nakazawa and Imai (1994) indicate that cGMP mediates relaxation, at least partly, via activation of protein kinase G.

Acknowledgement

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