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Deferoxamine blocks interactions of fluoride and carbachol in isolated mammalian cardiac preparations

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Abstract

In papillary muscles, carbachol reduced the positive inotropic effects of isoprenaline (10 nmol/l). The negative inotropic effects of carbachol in isoprenaline-stimulated guinea pig papillary muscles were attenuated by additionally applied sodium fluoride (3 mmol/l). These effects of sodium fluoride were blocked by deferoxamine (200 μmol/l). In guinea pig left atria, sodium fluoride alone greatly reduced force of contraction. These effects in atria were blocked by 200 μmol/l deferoxamine, and positive inotropic effects of sodium fluoride were observed. It is suggested that the cardiac effects of muscarinic M_2 receptor agonists in the ventricle involve, at least in part, the activation of phosphatases which are blocked by fluoride and reactivated by deferoxamine.

Keywords: Sodium fluoride; Deferoxamine; Carbachol; Inotropy

1. Introduction

The positive inotropic and lusitropic effects of sympathetic stimuli are attenuated by parasympathetic stimuli (Löffelholz and Pappano, 1985; Hartzell, 1988; Pappano, 1991). The negative inotropic effects of acetylcholine (via muscarinic M_2 receptors) in the mammalian ventricle are strongly enhanced in the presence of cAMP-increasing agents (Endoh, 1987). Acetylcholine can inhibit adenylyl cyclase activity in broken cardiac cell preparations (Watanabe and Besch, 1975a). It is, however, controversial whether the negative inotropic effects of muscarinic M_2 receptor agonists are solely due to reductions in the isoprenaline-stimulated cardiac cAMP content in intact preparations. In spontaneously beating guinea pig ventricles, rat ventricles, rat cardiomyocytes, rabbit and dog ventricular preparations, acetylcholine derivatives reduce the isoprenaline-stimulated cAMP content (Endoh, 1987; Endoh et al., 1991; George et al., 1991). Others have failed to detect a reduction of isoprenaline-stimulated cAMP content by muscarinic M_2 receptor agonists in guinea pig and chicken cardiac ventricular preparations (Watanabe and Besch, 1975b; Biegon et al., 1980; Lindemann and Watanabe, 1985; Schmied and Korth, 1990; Neumann et al., 1994).

There are convincing evidences that acetylcholine reduces isoprenaline-stimulated protein phosphorylation in cardiac preparations (England, 1976; Iwasa and Hosey, 1983; Hosey et al., 1984; Lindemann and Watanabe, 1985; George et al., 1991; Gupta et al., 1994). The negative inotropic effect of muscarinic M_2 receptor activation was accompanied by increased protein phosphatase activity in guinea pig ventricular preparations (Ahmad et al., 1989). Moreover, we have shown that sodium fluoride, an inhibitor of protein phosphatases (for brief, phosphatases), attenuates the negative inotropic effects of muscarinic M_2 receptor activation (Neumann et al., 1995). Deferoxamine can block the effects of fluoride on K^+ channels (Dunne et al., 1989). Moreover, the positive inotropic effects of sodium fluoride in the rabbit atrium are blocked by deferoxamine (Hattori et al., 1995).

Therefore, the present study focused on the influence of deferoxamine on the interaction between sodium fluoride and muscarinic M_2 (by carbachol) receptor stimulation (in the presence of isoprenaline) in the mammalian heart.

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2. Materials and methods

2.1. Determination of inotropic response

Contraction experiments were performed as described previously (Neumann et al., 1995). Briefly, left atria, right atria or papillary muscles were isolated from the hearts of reserpinized (5 mg/kg, 16 h before the animals were killed) guinea pigs and rats. The bathing solution contained (in mmol/l) 119.8 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 0.42 NaH_2PO_4, 22.6 $NaHCO_3$, 0.05 Na_2EDTA, 0.28 ascorbic acid, 5.05 glucose, continuously gassed with 95% O_2 and 5% CO_2 and was maintained at 35°C and pH 7.4. Isometric force of contraction was measured after each muscle was preloaded to an optimal length. Preparations (left atrium, papillary muscle) were electrically stimulated at 1 Hz with rectangular pulses of 5 ms duration (Grass stimulator SD9; Grass, Quincy, MA, USA), the voltage being about 10–20% greater than threshold. The right atria were beating spontaneously. Preparations were allowed to equilibrate for 30 min. Thereafter, the drugs were added. The order and times of addition are shown in Figs. 1 and 2. As indicated in the appropriate legends, compounds were added alone or cumulatively.

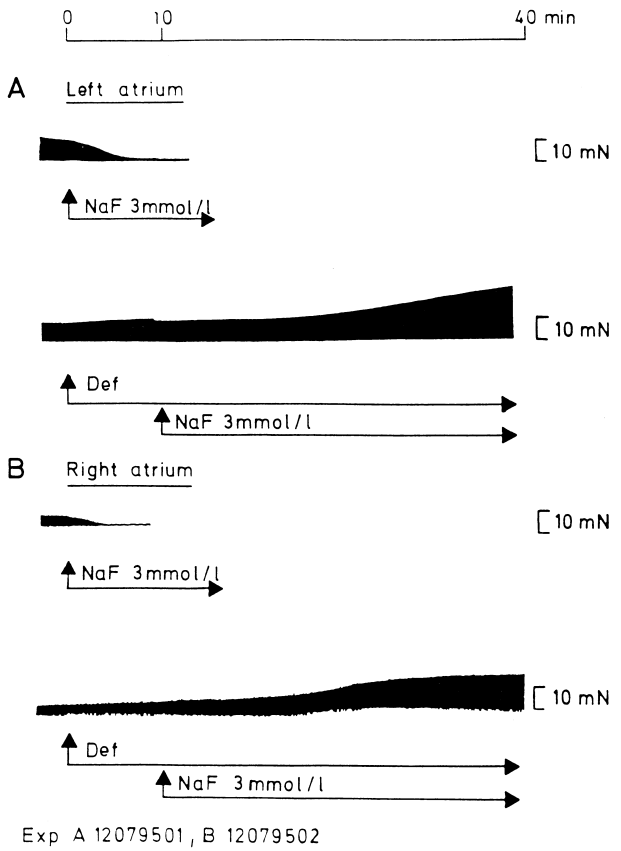

Fig. 1. Original recordings depict the influence of sodium fluoride or sodium fluoride plus deferoxamine on force of contraction in guinea-pig atria. Isolated electrically driven left atria (A) or spontaneously beating right atria (B) were treated with sodium fluoride alone (NaF, top panels) or sodium fluoride plus deferoxamine (Def, 200 μmol/l, bottom panels). Ordinates indicate force in milli Newton (mN). See time bar for incubation times.

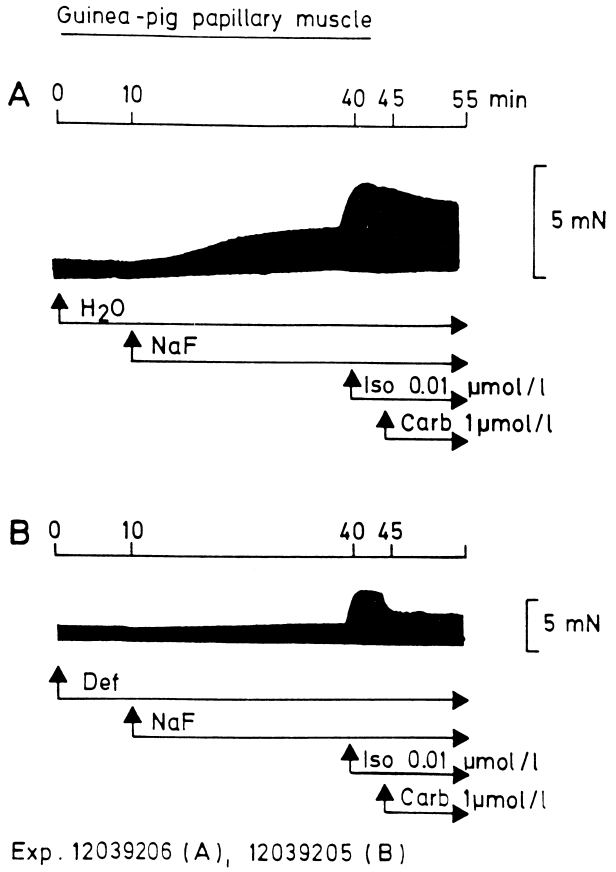

Fig. 2. Original recordings depict the influence of sodium fluoride (A) or sodium fluoride plus deferoxamine (B) on the negative inotropic effect of carbachol. Isolated electrically driven guinea-pig papillary muscles were treated with sodium fluoride (NaF, 3 mmol/l, A) or sodium fluoride plus deferoxamine (Def, 200 μmol/l, B). Thereafter, isoprenaline (Iso) and carbachol (Carb) were applied at the times indicated (compare time bar). Ordinates indicate force in milli Newton (mN).

2.2. Protein phosphatase assay

Assays for protein phosphatase activity were performed exactly as described (Neumann et al., 1995). Phosphatase activity was measured at 30°C, using [^{32}P]phosphorylase a as the substrate. The 50-μl incubation mixture contained (in mmol/l) 20.0 Tris–HCl (pH 7.0), 5.0 caffeine, 0.1 EDTA and 0.1% β-mercaptoethanol (v/v). The addition of homogenate initiated the reaction. The reaction was terminated 10 min later by the addition of trichloroacetic acid. Samples were centrifuged and the radioactivity in the supernatant was determined. Homogenates were prepared as described (Neumann et al., 1995). Briefly, guinea pig ventricles were freeze-clamped. Powdered tissue was homogenized in 10 ml medium containing (in mmol/l) 4.0 EDTA and 0.1% β-mercaptoethanol (v/v). The tissue was thawed and homogenized 3 times for 30 s each with a Polytron PT-10 (Kinematica, Lucerne, Switzerland), and

the sample was centrifuged for 20 min at 14 000 × *g*. The resulting supernatant was termed the homogenate.

2.3. Chemicals

The compounds used were sodium fluoride (Merck, Darmstadt, FRG), (±)-isoprenaline hydrochloride (Boehringer Ingelheim, Ingelheim, FRG), carbamylcholine chloride (from Sigma, München, FRG) and deferoxamine mesylate (CIBA, Basel, Switzerland). All other chemicals were of analytical grade or the best commercial grade available. Deionized and twice-distilled water was used throughout.

2.4. Statistics

Data are given as means ± S.E.M. The significance of differences was tested by using Student's *t*-test for paired and unpaired observations as appropriate. A $P < 0.05$ was regarded as significant.

3. Results

Fig. 1 shows that 3 mmol/l sodium fluoride exerted a negative inotropic effect in guinea pig left atria (A) and right atria (B); 3 mmol/l sodium chloride was ineffective. This effect of sodium fluoride on the atria was blocked by the preincubation with deferoxamine (200 μmol/l). The same results were obtained in four other preparations (data not shown). In rat left atria, sodium fluoride (3 mmol/l) exerted a positive inotropic effect. Sodium fluoride (3 mmol/l) exhibited a positive inotropic effect in guinea pig papillary muscles (Fig. 2A). Sodium chloride (3 mmol/l) did not exert a positive inotropic effect in guinea pig papillary muscles. Sodium fluoride (3 mmol/l) attenuated the negative inotropic effect of carbachol in the presence of isoprenaline (Fig. 2A). In contrast, 3 mmol/l sodium chloride failed to affect the effect of carbachol (data not shown). In rat papillary muscles, carbachol alone (1 μmol/l) exerted a negative inotropic effect which was not attenuated by the prestimulation with 3 mmol/l sodium fluoride. It should be noted that the preincubation with deferoxamine (200 μmol/l) abolished the action of sodium fluoride on the negative inotropic effect of carbachol in guinea pig papillary muscles (Fig. 2B). The time-dependent effect of carbachol on isoprenaline-stimulated guinea pig papillary muscles is shown in Fig. 3. The concentration-response curve of carbachol on the isoprenaline-stimulated force of contraction in papillary muscles is shown in Fig. 4. The negative inotropic effect of carbachol was greatly reduced by sodium fluoride (3 mmol/l) and was restored by the additional application of deferoxamine (200 μmol/l). Sodium fluoride (3 mmol/l) inhibited phosphatase activity in guinea pig ventricular homogenates to 46 ± 4% of control (= solvent). Deferoxamine (200 μmol/l) alone did not affect phosphatase activity. In the presence of deferoxamine (200 μmol/l), sodium fluoride (3 mmol/l) inhibited phosphatase activity to only 76 ± 6% of control ($n = 3$). Deferoxamine (200 μmol/l) alone

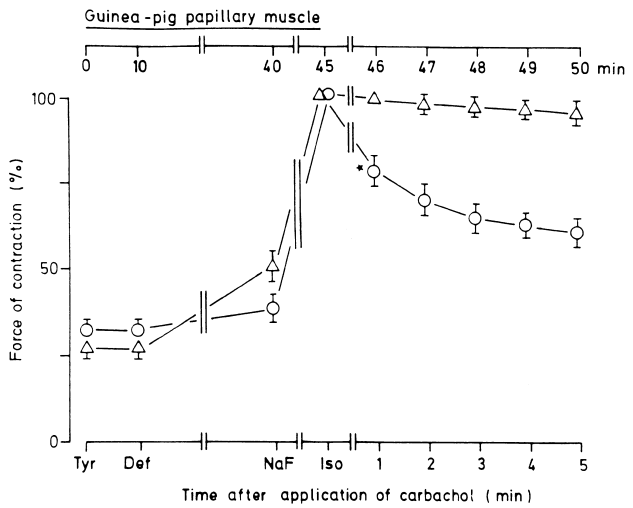

Fig. 3. Effects of carbachol (1 μmol/l) on isoprenaline (Iso, 10 nmol/l)-stimulated force of contraction in the presence of sodium fluoride (NaF, 3 mmol/l, triangles, $n = 8$) or sodium fluoride plus deferoxamine (Def, 200 μmol/l, circles, $n = 7$). The incubation times and concentrations of compounds were the same as in Fig. 2. Pre-drug (Tyr) values for force of contraction amounted to 1.45 ± 0.18 mN ($n = 15$). * Denotes the first significant difference between triangles and circles. Ordinate: force of contraction in isolated guinea-pig papillary muscles in percent of the effect of isoprenaline (Iso, 5 min).

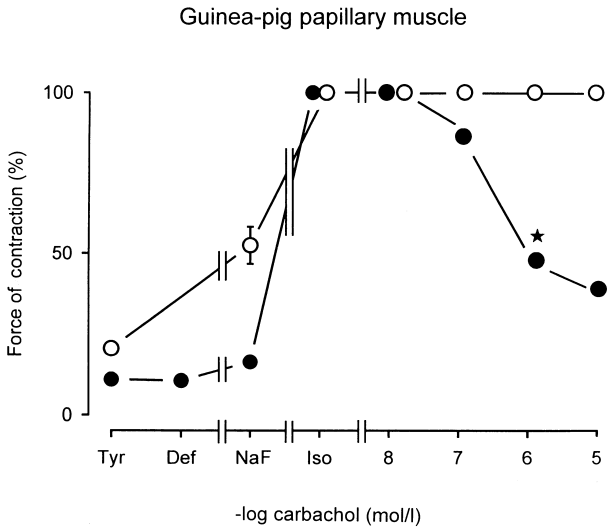

Fig. 4. Concentration-dependent effects of carbachol on isoprenaline (Iso, 10 nmol/l)-stimulated force of contraction in the presence of sodium fluoride (NaF, 3 mol/l, open circles, $n = 9$) or sodium fluoride plus deferoxamine (Def, 200 μmol/l, closed circles, $n = 7$). The effects of various concentrations of carbachol (cumulatively applied) were measured 5 min after drug addition. Pre-drug (Tyr) values for force of contraction amounted to 1.24 ± 0.16 mN ($n = 16$). * Denotes the first significant difference between open and closed circles. Ordinate: force of contraction in isolated guinea-pig papillary muscles in percent of the effect of isoprenaline (Iso, 5 min). Abscissa: concentration of carbachol.

hardly affected the force of contraction in atrial (Fig. 1) or ventricular preparations (Figs. 2 and 4). The negative inotropic effects of carbachol in guinea pig atria (carbachol alone) or in isoprenaline-stimulated guinea pig papillary muscles were not affected by the pretreatment of the preparations with deferoxamine (200 μmol/l) alone (data not shown).

4. Discussion

We have shown that sodium fluoride can attenuate the negative inotropic effects of muscarinic M_2 receptor agonism in isoprenaline-stimulated guinea pig papillary muscles (Neumann et al., 1995). We now present the evidence that this effect of carbachol can be restored by deferoxamine, which apparently complexes and thus inactivates fluoride. We shall discuss the ventricular and atrial actions of acetylcholine, sodium fluoride and deferoxamine separately.

4.1. Ventricular effects

It has been noted before that sodium fluoride exerts a positive inotropic effect in the ventricle. We have provided evidence that this effect is mediated at least in part by the inhibition of phosphatase activity (Neumann et al., 1995). This inhibition would increase the phosphorylation state of regulatory proteins and thereby initiate a positive inotropic effect. We cannot exclude the possibility that the actions of fluoride in the ventricle are due to additional mechanisms. For instance, sodium fluoride activates phospholipase C in hepatocytes (Blackmore et al., 1985), inhibits and stimulates the Ca^{2+} pump of the sarcoplasmic reticulum (Narayanan et al., 1991), and inhibits the sarcolemmal Na^+,K^+-ATPase (for instance: Murphy and Hoover, 1992). Moreover, pretreatment with pertussis toxin abolishes the inotropic, electrophysiological, and biochemical effects of muscarinic M_2 receptor agonists in cardiac preparations (Hazeki and Ui, 1981; Endoh et al., 1985; Pfaffinger et al., 1985; Kurachi et al., 1986; Neumann et al., 1994). Sodium fluoride can interact with pertussis toxin-sensitive GTP-binding proteins (Katada et al., 1984), possibly inhibiting their function. Thus, additional mechanism(s) besides phosphatase inhibition may contribute to the positive inotropic effect of sodium fluoride in the ventricle.

Muscarinic M_2 receptor agonists reduced the force of contraction in the guinea pig ventricle after β-adrenergic stimulation. This is accompanied by a reduction of the phosphorylation state of regulatory proteins like Phospholamban and the inhibitory subunit of Troponin in the ventricle (England, 1976; George et al., 1991; Gupta et al., 1994; Neumann et al., 1994). Similar phosphorylation data for the atria are apparently lacking.

The dephosphorylation of functional proteins in the ventricular myocardium could be partly due to increased phosphatase activity. Muscarinic M_2 receptor agonists can increase phosphatase activity in the preparations from guinea pig cardiac ventricles (Ahmad et al., 1989). Cardiac phosphatases can be divided into type 1, 2A, 2B and 2C. Sodium fluoride inhibits types 1, 2A and 2B but not 2C phosphatase activity (for review, Shenolikar and Nairn, 1991). Deferoxamine attenuated the ability of fluoride to inhibit type 1 and/or type 2A phosphatases, which were measured under the same conditions as used in our study. This supports our hypothesis that the action of acetylcholine in the ventricle is mediated, at least in part, by the activation of the protein phosphatases. This activation could be attenuated by fluoride and restored by deferoxamine.

4.2. Atrial effects

In contrast to its action in the guinea pig ventricle, carbachol alone (independent of β-adrenergic prestimulation) exerted a negative inotropic effect in guinea pig atria, most probably due to direct opening of a K^+ channel mediated by a pertussis toxin sensitive G-protein (Kurachi et al., 1986). Likewise, fluoride decreased the force of contraction substantially in guinea pig atrium (Fig. 1). To the best of our knowledge, this observation has not been reported before. In contrast, fluoride has a positive inotropic effect in rat atria (McIvor and Cummings, 1987) and rabbit left atria (Hattori et al., 1995), indicating that there is a profound species difference in signal transduction. Other investigators have observed that fluoride can stimulate the K_{ATP} channels in the insulinoma cell line RINm5F and that this effect is blocked by deferoxamine (Dunne et al., 1989). In a similar way, deferoxamine reversed the effect of sodium fluoride causing a positive inotropic effect in guinea pig atrium. It can be speculated that the K^+ channel opening effect of sodium fluoride in guinea pig atria is blocked by deferoxamine. How does the positive inotropic effect of fluoride in the presence of deferoxamine come about? A stimulatory effect of sodium fluoride on the formation of inositol phosphates has been reported in various tissues including the atrium (hepatocytes: Blackmore et al., 1985; bronchial smooth muscle: Hall et al., 1990; rabbit left atria: Hattori et al., 1995). Therefore, the stimulation of phosphoinositide hydrolysis induced by fluoride may be partly responsible for its positive inotropic effect in the presence of deferoxamine. It is also conceivable that the opposite effect of fluoride (positive inotropic effect in the ventricle, negative inotropic effect in the atrium) is due to the different expression of phosphatases in the atrium and ventricle. However, it is much more likely that fluoride has a dual effect in the atrium. When fluoride alone is applied to the atrial muscle, an increase in sarcolemmal K^+ conductance induced by fluoride (which does not occur in ventricular muscle) might mask the potential positive inotropic effect of the compound that is elicited by the inhibition of phosphatases and/or stimulation of phosphoinositide hydrolysis.

Diffusional barriers for fluoride may also play a role. The K^+ channels are located in the sarcolemma, whereas other functionally important targets, like phosphatases, are known to be situated, e.g., in the sarcoplasmic reticulum. Thus, sarcolemmal effects might overcome the intracellular effects of sodium fluoride in the atria.

4.3. Mechanism of action of deferoxamine

Deferoxamine molecules form stable 1:1 complexes with iron ions (ferrioxamine) and aluminium ions with high affinity (aluminoxamine, Yokel, 1994). Hence, it is conceivable that the interaction between sodium fluoride and deferoxamine is due to the complexation of deferoxamine with iron ions or aluminium ions. However, based on the published literature, it is likely that the complex with aluminium ions mediates the interaction. Among the 14 cations tested for their ability to complex fluoride in water, aluminium was the most effective (for review see Yokel, 1994). Fluoride forms numerous complexes with aluminium (Yokel, 1994). These complexes can act as high-affinity structural analogs of inorganic phosphate, affecting many biological processes including G-protein activation, protein phosphatases and protein kinase C activation (see above). Hence, we speculate that the effects of deferoxamine in the ventricle and the atrium may be due to complexation of aluminium fluoride. Therefore, the next step(s) in the signal cascade distal to aluminium fluoride may be blocked due to the inhibition of phosphatases in the ventricle and possibly, the opening of K^+ channels in the guinea pig atrium. We speculate that the sarcolemmal effects of sodium fluoride (atria) are more sensitive to deferoxamine than the intracellular effects (atria and ventricle). However, the exact biochemical basis for the differential effects in the atria and ventricles needs to be elucidated.

In summary, sodium fluoride attenuated the negative inotropic effects of muscarinic M_2 receptor agonism in the presence of β-adrenoceptor stimulation in the mammalian ventricle. This effect of sodium fluoride was reduced by deferoxamine.

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