Pertussis toxin suppresses carbachol-evoked cardiodepression but does not modify cardiostimulation mediated through \( \beta_1 \)- and putative \( \beta_4 \)-adrenoceptors in mouse left atria: no evidence for \( \beta_2 \)- and \( \beta_3 \)-adrenoceptor function

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Abstract Activation of \( \beta_1 \)-, \( \beta_2 \)-, \( \beta_3 \)- and putative \( \beta_4 \)-adrenoceptors modifies cardiac function. These receptors are usually coupled to \( G_s \) protein, but \( \beta_2 \)- and \( \beta_3 \)-adrenoceptors could also couple to \( G_{i/o} \) proteins. The mouse heart is used increasingly for studies of genetically disrupted or overexpressed proteins, including \( \beta \)-adrenoceptor subtypes. We therefore investigated in contracting mouse left atria (2 Hz, 37°C) if inactivation of \( G_{i/o} \) proteins with pertussis toxin modifies or uncovers effects mediated through \( \beta \)-adrenoceptor subtypes. The negative inotropic effects of carbachol in atria exposed to catecholamine or high calcium (6.8 mmol/l) were assumed to be mediated through muscarinic receptors coupled to \( G_{i/o} \). We report conditions under which incubation of left atria with 200 ng/ml pertussis toxin for 24 h nearly abolished the carbachol responses. Although it has been reported that muscarinic receptor-mediated cardiodepression has an obligatory contribution of nitric oxide, the nitric oxide synthase inhibitor \( N^G \)-monomethyl-L-arginine (0.1–1 mmol/l) did not modify the negative inotropic effects of carbachol, inconsistent with an involvement of nitric oxide. The positive inotropic effects of \( \beta_1 \)-noradrenaline and \( \beta_2 \)-adrenaline, mediated through \( \beta_1 \)-adrenoceptors, were not affected by pertussis toxin. \( \beta_2 \)-Adrenaline did not cause positive inotropic effects attributable to \( \beta_1 \)-adrenoceptor-mediated, in the presence of CGP 20712A (300 nmol/l) to block \( \beta_1 \)-adrenoceptors, in control atria or atria pretreated with pertussis toxin. The positive inotropic effects of \( \beta_4 \)-CGP 12177 (1 \( \mu \)mol/l), a compound with agonist activity at the putative \( \beta_4 \)-adrenoceptor, were unaffected by pertussis toxin. The \( \beta_3 \)-adrenoceptor-selective agonist BRL 37344 (1 \( \mu \)mol/l), in the presence of \( \beta_4 \)-propranolol (200 nmol/l), did not cause positive or negative inotropic effects in control and pertussis toxin-treated atria. In left atria obtained from mice injected with 150 \( \mu \)g/kg i.p. pertussis toxin which abolished carbachol-evoked cardiodepression, the positive inotropic effects of \( \beta_1 \)-adrenaline were antagonised by CGP 20712A. The \( \beta_2 \)-adrenoceptor-selective antagonist ICI 118551 (50 nmol/l) did not cause additional blockade of the effects of high \( \beta_1 \)-adrenaline concentrations in the presence of CGP 20712A, ruling out the involvement of \( \beta_2 \)-adrenoceptors. The results with intraperareternal PTX validate our in vitro PTX method. We conclude that inhibition of murine \( G_{i/o} \) proteins does not alter atrial positive inotropic effects mediated through \( \beta_1 \)- and putative \( \beta_4 \)-adrenoceptors and does not reveal functional \( \beta_2 \)- and \( \beta_3 \)-adrenoceptors.

Key words Mouse left atrium · Pertussis toxin \( \beta_1 \)-, \( \beta_2 \)-, \( \beta_3 \)- and putative \( \beta_4 \)-adrenoceptors · Carbachol · Noradrenaline and adrenaline · \( \beta_4 \)-CGP 12177 · contractile force

Introduction

The rate and force of contraction of mammalian heart can be enhanced via \( \beta_1 \)-, \( \beta_2 \)- and putative \( \beta_4 \)-adrenoceptors by coupling to \( G_s \) protein (Kaumann and Molenaar 1997). There is controversy, however, about the role of \( \beta_3 \)-adrenoceptor-mediated effects, cardiodepression has been claimed (Gauthier et al. 1996, 1998) but not seen by others in human ventricular tissues (Molenaar et al. 1997) or myocytes (Harding 1997). Also puzzling, \( \beta_1 \)-adrenoceptor selective agonists fail to cause inotropic effects in human atrium (Kaumann et al. 1997) but increase L-type calcium current in human atrial myocytes (Skeberdis et al. 1999). Although the \( \beta_3 \)-adrenoceptor usually couples to \( G \) protein (Emorine et al. 1989) there is also evidence for coupling to pertussis toxin (PTX)-sensitive \( G_{i/o} \) protein (Chaudhry et al. 1992) and this has been invoked to account for car-
diodepression (Gauthier et al. 1996). Furthermore, reports have appeared for concurrent coupling of \( \beta_2 \)-adrenoceptors to \( G_{\text{i/o}} \) protein and \( G_i \) protein in ventricular myocytes from adult rats and \( G_i \)-mediated cardiostimulation appears to become only prominent after PTX treatment (Xiao and Lakatta 1995) but others have failed to confirm this (Laflamme and Becker 1998). It has also been reported in murine ventricular myocytes that \( \beta_2 \)-adrenoceptor \( G_i \)-signalling only becomes apparent after inhibition of \( \beta_2 \)-adrenoceptor \( G_{\text{i/o}} \)-signalling with PTX (Xiao et al. 1999).

Hearts of transgenic mice have become models for \( \beta \)-adrenoceptor research, either overexpressing receptors (\( \beta_1 \): Bertin et al. 1993; Zolk et al. 1998; \( \beta_2 \): Bond et al. 1995; Milano et al. 1994) or with disrupted receptor genes (\( \beta_1 \): Rohrer et al. 1996; \( \beta_2 \): Kaumann et al. 1998). We asked whether inactivation of \( G_{\text{i/o}} \) proteins by PTX modifies or uncovers the function of native \( \beta_1 \)-, \( \beta_2 \)-, \( \beta_3 \)- and putative \( \beta_2 \)-adrenoceptors in mouse atrium, a suitable tissue increasingly used from transgenic mice (Bond et al. 1995; Heubach et al. 1999; Kaumann et al. 1998; Zolk et al. 1998). We introduce a procedure consisting in a 24 h in vitro pretreatment of the atria with PTX, followed by pacing of the atria and assessment of inotropic responses to agonists under conditions of selective \( \beta \)-adrenoceptor subtype activation. Carbachol-evoked cardiodepression, mediated through muscarinic \( M_2 \) receptors, has been considered as being consequence of the activation of \( G_{\text{i/o}} \) proteins (for physiological evidence see Adamson et al. 1993; Fleming et al. 1988; Tucek et al. 1987).

Nitric oxide (NO), produced through activation of NO synthases, has been suggested by some authors to be implicated in the cardiodepressant effects caused by muscarinic receptor stimulation (Balligand et al. 1993; Han et al. 1998) but not been found to be involved in several species by others (Méry et al. 1996; Nawrath et al. 1995). Targeted disruption of the endothelial NO synthase gene (\( i \)-NOS) has been reported to abolish the carbachol-evoked attenuation of positive inotropic responses to \( \beta \)-adrenoceptor agonists (Han et al. 1998) but Eschenhagen et al. (1997) have previously reported unaltered chronotropic and inotropic responses to carbachol in atria and papillary muscles from these mice compared to wild-type mice. In view of the controversy about a possible mediation by NO in the negative inotropic effects of carbachol we used the NO synthase inhibitor \( \omega \)-nitro-L-arginine (L-NOARG; Kilpatrick and Cocks 1994).

The results show that when negative inotropic responses to carbachol are nearly abolished by PTX, positive inotropic responses mediated through \( \beta_1 \)-adrenoceptors and putative \( \beta_2 \)-adrenoceptors were not modified. No evidence for \( \beta_3 \)- and \( \beta_1 \)-adrenoceptor-mediated effects was detected either without or with PTX treatment. L-NOARG failed to affect the negative inotropic responses to carbachol.

**Methods**

*Isolated left atria.* Mice of either sex (BALB/c, weighing 15–30 g) aged 8–14 weeks were killed by dislocation of the neck in accordance with Home Office (United Kingdom) procedures. The hearts were immediately dissected and placed in oxygenated solution at normal temperature containing (mmol/l): NaCl 118, NaHCO\(_3\) 25, KCl 4, KH\(_2\)PO\(_4\) 1.2, MgCl\(_2\) 1, CaCl\(_2\) 1.8, glucose 10, Na-pyruvate 2, EDTA 0.04, cocaine 0.003, corticosterone 0.03, phenolamine 0.001 and ascorbic acid 0.2, equilibrated with 95% O\(_2\) and 5% CO\(_2\). The water was deionised and double-distilled. The left atria were carefully dissected at room temperature and set up in pairs in a 50 ml organ bath (Blinks 1965) to contract at 2 Hz at 37°C as described (Kaumann et al. 1998). The tissues were attached to a Swema 4-45 strain gauge transducers and force recorded on a 12-channel Watanabe polygraph. After determination of a length-force curve, the length of each atrium was set to obtain approximately 50% of the resting tension associated with maximum developed force.

**Pertussis toxin pretreatment.** Six atria were incubated in 50 ml flasks and shaken (220 rpm) for 24 h at 37°C without CO\(_2\) in 5 ml Dulbecco’s modified Eagle’s medium (DMEM) containing 10% foetal calf serum and 200 ng/ml PTX or vehicle. PTX-treated atria and vehicle-treated atria were studied as pairs set up in the same organ bath. The influence of 24 h incubation of atria in DMEM on agonist sensitivity in the absence of PTX was compared with that of atria previously dissected, also set up usually as pairs into the same organ bath.

To corroborate findings obtained from in vitro PTX pretreatment with in vivo PTX pretreatment, mice were injected with 150 µg/kg i.p. PTX or solvent and paired left atria from each mouse set up 24 h later.

**Concentration-effect curves to the catecholamines were cumulative.** \( \beta_2 \)-adrenoceptor-mediated effects were investigated with \( (-) \)-noradrenaline in the presence of ICI 118551 (50 nmol/l) to block \( \beta_2 \)-adrenoceptors (Lemoine et al. 1985). To detect possible \( \beta_2 \)-adrenoceptor-mediated effects, \( (-) \)-adrenaline concentration-effect curves were determined in the absence and presence of CGP 20712A (300 nmol/l) to block \( \beta_1 \)-adrenoceptors, and in the presence of both CGP 20712A (300 nmol/l) and ICI 118551 (50 nmol/l; Kaumann 1986).

**Concentration-effect curves to the catecholamines were cumulative.** \( \beta_2 \)-adrenoceptor-mediated effects were investigated with a single concentration of BRL 37344 (1 µmol/l; Gauthier et al. 1998) in the presence of (–)-propranolol (200 nmol/l) to block \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors (Kaumann and Molenaar 1996). Effects mediated through the putative \( \beta_2 \)-adrenoceptor were investigated with \( (-) \)-CGP 12177 (1 µmol/l) in the presence of (–)-propranolol (200 nmol/l) and L-NOARG. The tissues were also investigated in the presence of (–)-propranolol (200 nmol/l). The antagonists were present for at least 60 min before adding the agonists. L-NOARG was incubated for at least 30 min before the administration of an agonist.

After equilibrium effects to the highest used \( \beta \)-adrenoceptor concentration or single concentration of BRL 37344 and \( (-) \)-CGP 12177 were observed, \( (-) \)-isoprenaline (600 µmol/l) was administered to determine maximum positive inotropic effects. Thereafter a cumulative concentration-effect curve to carbachol was determined in the presence of the previously administered drugs. In the groups of the PTX-pretreated atria with \( (-) \)-noradrenaline and CaCl\(_2\) (4.8 mmol/l) the curve for carbachol was determined without previous administration of \( (-) \)-isoprenaline. All experiments were terminated by raising the CaCl\(_2\) concentration to 8.0 mmol/l.

Log EC\(_{50}\) values for the catecholamines or –log IC\(_{50}\) values for carbachol were estimated by interpolation from individual concentration-effect curves. The data are expressed as mean ± standard error. When agonist log concentration ratios in the presence and absence of an antagonist were estimated, its errors were calculated as described (Kaumann 1990). Significance between differences were assessed with the paired or unpaired Student’s \( t \) test at \( P<0.05 \).

**Drugs.** DMEM was from ICN (Zoetermeer, The Netherlands), foetal calf serum was from Gibco-BRL (Breda, The Netherlands), L-NOARG, corticosterone, \( \omega \)-isoprenaline hydrochloride, \( (-) \)-nor-