The inhibition of \( \alpha_1 \)-adrenoceptor-mediated contractions of rabbit pulmonary artery by \( \text{Ca}^{2+} \)-withdrawal, pertussis toxin and N-ethylmaleimide is dependent on agonist intrinsic efficacy*

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Summary. Contractions were induced in rings of rabbit pulmonary artery with the preferential \( \alpha_1 \)-adrenoceptor agonists, whereas St 587, clonidine and B-HT 920 were partial agonists (intrinsic activities 0.62, 0.38 and 0.42, respectively). Experiments with \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptor antagonists indicated that the receptors involved are of the \( \alpha_1 \) type only. Removal of extracellular \( \text{Ca}^{2+} \) inhibited maximal contractions to phenylephrine and methoxamine by 30% and 49%, respectively. The remaining contraction components of the full agonists were abolished by the "intracellular \( \text{Ca}^{2+} \) antagonist" TMB-8 [(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate]. Contractions to St 587, clonidine and B-HT 920 were virtually abolished in \( \text{Ca}^{2+} \)-free medium. Pretreatment of the donor rabbits with pertussis toxin (2.5 \( \mu \)g/kg i.v., 5—6 days before sacrifice) attenuated the efficacies of the full agonists, phenylephrine and methoxamine by only 24% and 17%, respectively, whereas maximal contractions to the partial agonists, St 587, clonidine and B-HT 920, were inhibited by 46%, 61% and 75%, respectively. Also the sulfhydryl reagent, N-ethylmaleimide (10 \( \mu \)M), reduced contractile efficacies of phenylephrine and methoxamine to a lesser degree than those of St 587, clonidine and B-HT 920. When agonists were used at equieffective concentrations (i.e. \( \text{EC}_{50} \)-\( \text{EC}_{95} \) for phenylephrine and methoxamine, \( \text{EC}_{70} \)-\( \text{EC}_{95} \) for St 587 and \( \text{EC}_{95} \) for clonidine and B-HT 920) the degree of inhibition by removal of extracellular \( \text{Ca}^{2+} \) and pertussis toxin and N-ethylmaleimide was similar for all agonists. These data suggest that a unitary \( \alpha_1 \)-receptor may stimulate contractions via two different mechanisms. At a low degree of receptor stimulation, contractions are mediated by a pertussis toxin- and N-ethylmaleimide-sensitive influx of extracellular \( \text{Ca}^{2+} \). At a higher degree of receptor stimulation, an additional mechanism is activated which is insensitive to the two G protein inhibitors and mediated by \( \text{Ca}^{2+} \) mobilization from intracellular sites.

Key words: Phenylephrine — Methoxamine — St 587 — Clonidine — B-HT 920 — Postsynaptic \( \alpha_1 \)-adrenoceptor — Pertussis toxin

Introduction

In whole animals, both \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptor agonists can produce vasopressor responses (Timmermans and van Zwieten 1980a, b). In the resistance arteries responsible for these effects, the two types of receptors can be clearly distinguished with selective antagonists (Timmermans and van Zwieten 1980a, b). In addition, there are obvious differences in the agonist intrinsic efficacy of the two different mechanisms. Experiments with \( \text{Ca}^{2+} \)-antagonists indicated that the vasopressor effect mediated by postsynaptic \( \alpha_1 \)-adrenoceptors is not directly dependent on the influx of extracellular \( \text{Ca}^{2+} \). On the other hand, \( \text{Ca}^{2+} \) seems indispensable for vasoconstriction of resistance arteries initiated at postsynaptic \( \alpha_2 \)-adrenoceptors (van Meel et al. 1981, 1982).

Also for large arteries, such as the rat aorta or the rabbit pulmonary artery, it has been reported that contractions in response to preferential \( \alpha_1 \)-adrenoceptor agonists were largely independent of the extracellular \( \text{Ca}^{2+} \) concentration, whereas preferential \( \alpha_2 \)-adrenoceptor agonists required extracellular \( \text{Ca}^{2+} \) to elicit contractions (Godfraind et al. 1982; Holck et al. 1983; Haeusler et al. 1986). Accordingly, \( \text{Ca}^{2+} \) entry blockers had little effect on methoxamine (\( \alpha_1 \)) contractions in this tissue, but antagonized the concentration-response curve of clonidine (\( \alpha_2 \)) in a non-competitive fashion (Holck et al. 1983). Preliminary data also suggested differential sensitivities to pertussis toxin (PTX) of contractions induced by \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptor agonists (Haeusler et al. 1987). However, receptor characterization experiments have indicated that — in contrast to the microvasculature — contractions of rabbit pulmonary artery are mediated only by \( \alpha_1 \)-adrenoceptors (Docherty and Starke 1981; Holck et al. 1983; Docherty 1987). The present paper provides evidence that these \( \alpha_1 \)-adrenoceptors can induce contractions via two different mechanisms. The differential activation of the two mechanisms seems to depend merely on the degree of receptor stimulation.

Materials and methods

Dissection procedure and set-up of tissues. Pulmonary artery segments were removed from rabbits of either sex (1.5—
2 kg). The rabbits were stunned by a blow on the head and killed by exsanguination. The chest was opened and the heart and the lungs were dissected out. The pulmonary artery was exposed by blunt dissection along the adventitia. The main lobar vessels distal to the first major branch of the pulmonary artery were excised and placed in chilled modified Krebs’ solution (composition see below). The preparations were cleared of adhering connective tissue and cut into rings of 3 mm width. These rings were suspended between two stirrup-shaped stainless steel hooks in 5 ml organ baths. The incubation medium was modified Krebs’ solution of the following composition (mM): Na+ 145.0, K+ 5.95, Ca2+ 1.7, Mg2+ 1.2, Cl− 128.15, HCO3− 25.0, H2PO4− 1.2, SO42− 1.2, glucose 10.6 and EDTA 0.025. The solution was aerated continuously with 95% O2, 5% CO2, temperature was maintained at 37°C. Isometric tension of the vascular rings was recorded with a force transducer coupled to a DC-amplifier and a pen recorder; the initial load was adjusted to 15 mN. The endothelium of the rings was not removed. Before the actual experiment, preparations were allowed to stabilize for about 1 h. Then they were constricted once with phenylephrine (1 μM). This initial contraction was generally smaller than all the following responses. After an appropriate wash-out period, cumulative concentration-response curves for the following agonists were generated according to the method of van Rossum (1963): phenylephrine (10 nM–10 μM), methoxamine (0.1–300 μM), St 587 (30 nM–10 μM), clonidine (10 nM–10 μM) and B-HT 920 (1–100 μM). When the maximum contraction to a drug had been reached, tissues were washed at 6 min intervals with fresh Krebs’ solution until tension had returned to its initial value. Each ring was exposed to two different adrenoceptor agonists. The order of exposures was randomized and had no effect on contractile responses. Repeated exposure of a vascular ring to the same agonist also yielded reproducible responses.

Experiments with nominally Ca2+-free solution. In these experiments, a concentration-response curve with each agonist was first obtained in normal Krebs’ solution (control). Then the tissues were incubated in nominally Ca2+-free Krebs’ solution (without a chelating agent) for 10 min. The organ bath was filled with fresh Ca2+-free solution at 0 min, 3 min, 6 min and 9 min; at 10 min addition of increasing concentrations of the agonist was started. In other experiments the Ca2+-free solution contained the “intracellular Ca2+ antagonist” TMB-8 (100 μM) (Chiou and Malagodi 1975; Brand and Felber 1984).

Treatment of tissues with receptor antagonists and N-ethylmaleimide (NEM). In experiments with antagonists or NEM, response of a tissue was tested first in normal Krebs’ solution (control) and then in the presence of the antagonist or inhibitor. The effects of the α1-adrenoceptor antagonist, prazosin, and the α2-adrenoceptor antagonist, yohimbine, were only tested qualitatively as pA2 values for both agonists have been reported previously in the same tissue (Holck et al. 1983; Docherty 1987). Prazosin and yohimbine were tested against the aforementioned concentrations of all agonists. We also performed some experiments with the β-adrenoceptor antagonist propranolol. The tissues were always equilibrated for 30 min with a concentration of an antagonist before determining contractile responses of an agonist.

NEM (10 μM), a sulfhydryl alkylating agent that interacts with G proteins of the G1 type (Jakobs et al. 1982; Harden et al. 1982; Uj 1984) was added to the organ bath 15 min before generating the second concentration-response curve with one of the agonists.

Drugs and solutions. Phenylephrine HCl, clonidine HCl, propranolol HCl, N-ethylmaleimide (NEM) and TMB-8 [8- (N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate] were from Sigma (Munich, FRG). Methoxamine HCl was obtained from Burroughs Welcome (London, UK). B-HT 920 [2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo(4,5-d) azepine HCl] was donated by Dr. W. Eisert, Thomas (Berach, FRG). St 587 [2-(2-chloro-trifluoromethyl-phenyl)-imidazolidine nitrate] was from Boehringer Ingelheim (Ingelheim, FRG). Prazosin HCl was obtained from Pfizer (Karlsruhe, FRG); yohimbine HCl was from Kali-Chemie (Hannover, FRG). The α1-adrenoceptor agonists and antagonists were dissolved in 1 mM HCl containing 1 mg/ml ascorbic acid; NEM was dissolved in distilled water. Pertussis toxin (PTX) was purchased from List Biological Laboratories (Campbell, CA, USA) (Lot No. PT-42). The toxin was dissolved in 0.1 M sodium phosphate (pH 7.0) with 0.5 M sodium chloride, stored at 4°C and diluted immediately before use as described above.

Statistics. Results are expressed as mean values ± SEM. The determination of pD2 (−log EC50 values) was as described by van Rossum (1963). Statistical differences between mean values were determined by analysis of variance followed by the Fisher least significant difference test for comparison of different means (Snedecor and Cochran 1967). P values of less than 0.05 were considered significant.

Results

Effects of different α-adrenoceptor agonists

Under control conditions, the α1-adrenoceptor agonists, phenylephrine, methoxamine and St 587, as well as the α2-adrenoceptor agonists, clonidine and B-HT 920, elicited concentration-dependent contractions in rings of rabbit pulmonary artery; pD2 values for the five agonists were 7.38 ± 0.06, 6.00 ± 0.08, 6.41 ± 0.05, 6.73 ± 0.08 and 4.87 ± 0.05, respectively. Phenylephrine induced a maximum tension of 33.6 ± 1.8 mN. The same maximum contractions could be achieved with noradrenaline (not shown), and phenylephrine was considered a full agonist (intrinsic ac-