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Short communication

Potentiation by alcuronium of the antimuscarinic effect of *N*-methylscopolamine in guinea pig left atria

Andrea Maaß, Evi Kostenis, Klaus Mohr *

Department of Pharmacology and Toxicology, Institute of Pharmacy, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany

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Abstract

Alcuronium is known to stabilize allosterically the binding of the muscarinic antagonist *N*-methylscopolamine to muscarinic M_2 receptors and thus to elevate the equilibrium binding of *N*-methylscopolamine in homogenized cardiac tissue. In order to check for a functional consequence of this effect, the action of alcuronium alone and in combination with *N*-methylscopolamine was determined in contracting guinea pig left auricles with oxotremorine-M as the negative inotropic agonist. For sake of comparison, the allosteric modulator W84 = hexane-1,6-bis(dimethyl-3'-phthalimidopropyl-ammonium bromide) was included. Alcuronium displayed a weak antimuscarinic action ($pA_2 = 5.7$). In conjunction with 10^{-7} M *N*-methylscopolamine, alcuronium ($\geq 10^{-6}$ M) induced a more pronounced antimuscarinic effect than expected for a combination of competitive antagonists. The extent of overadditivity with combinations of W84 and 10^{-7} M *N*-methylscopolamine was smaller. In conclusion, alcuronium potentiates the antimuscarinic effect of *N*-methylscopolamine in contracting cardiac preparations with high effectivity.

Keywords: Muscarinic receptor; Allosteric modulation; Alcuronium; Heart

1. Introduction

Compounds from various pharmacological groups have been demonstrated in vitro to retard allosterically the dissociation of radiolabelled antagonists such as [^{3}H]*N*-methylscopolamine from muscarinic M_2 receptors (for review Lee and El-Fakahany, 1991). The compounds also inhibit the association of ligands, and for a number of allosteric modulators antimuscarinic properties have been described demonstrating an inhibition of agonist binding. Information concerning the functional consequences of the allosteric stabilization of antagonist binding is rather scarce. Phthalimidopropyl substituted alkane-bis-ammonium compounds and structurally closely related agents have been shown to induce in conjunction with conventional antagonists such as atropine a stronger antimuscarinic effect than expected for a combination of competitive antagonists (Lüllmann et al., 1969; Mitchelson, 1975; Mohr et al., 1993; Christopoulos and Mitchelson, 1994). In contrast, other allosteric modulators such as gallamine and methoctramine do not elicit an overadditive action (Clark and Mitchelson, 1976; Melchiorre et al., 1987).

Among the allosteric modulators, alcuronium is outstanding. Recently, Proška and Tuček (1994) demonstrated in homogenates of rat heart atria that alcuronium inhibits the dissociation of *N*-methylscopolamine with higher potency than the association of this ligand, thus increasing the equilibrium binding of *N*-methylscopolamine. Furthermore, alcuronium is the most potent allosteric modulator at muscarinic M_2 receptors known to date (Tränkle et al., 1994). Accordingly, alcuronium should be a useful tool to test whether the allosteric stabilization of *N*-methylscopolamine binding may be related to an augmentation of the action of this antagonist. Therefore, the effects of alcuronium alone and in combination with *N*-methylscopolamine were determined in isolated guinea pig left atria exposed to oxotremorine-M. This agonist was chosen, because the tritiated form [^{3}H]oxotremorine-M is a commonly used radioligand. For sake of comparison, the phthalimidopropyl substituted alkane-bis-ammonium compound

* Corresponding author. Tel. 0049-228-739103, fax 0049-228-739215.

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W84 = hexane-1,6-bis(dimethyl-3'-phthalimidopropyl-ammonium bromide) was included the antimuscarinic effects of which have not been before investigated with oxotremorine-M as the agonist.

2. Materials and methods

Isolated guinea pig left auricles were suspended in 20 ml of oxygenated Tyrode's solution (in mM: NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, $MgCl_2$ 1.05, NaH_2PO_4 0.21, dextrose 5.5; 95% O_2-5% CO_2, pH 7.3, 32°C). The atria were preloaded with 5 mN and electrically stimulated via contact electrodes at 3 Hz (rectangular pulses of 5 ms duration, 1.5-fold over the stimulation threshold of ~1 V). After 1 h of equilibration (force of contraction ~7 mN) oxotremorine-M was applied in cumulative fashion with each concentration being present for 10 min. During the subsequent wash out period of 30 min duration the force of contraction regained the control level. One of the antagonists contained in the combination to be finally applied was administered for 1 h and the concentration-effect curve of oxotremorine-M was determined. After a wash out period of 30 min, the combination of antagonists was applied for 1 h and the effect of oxotremorine-M was measured again. An equilibration period of 1 h is sufficient to warrant the equilibrium binding of 10^{-7} M *N*-methylscopolamine in beating guinea pig atria (Lüllmann et al., 1988). For evaluation of the concentration-effect curve of oxotremorine-M, the force of contraction observed under oxotremorine-M at the end of each 10 min incubation period was expressed in percent of the pre-oxotremorine-M value. A curve was fitted to the data points by means of non-linear regression analysis (software Inplot from GraphPad, San Diego, CA, USA). As a measure of potency served the concentration of oxotremorine-M which reduced contraction force to 50% of the predrug value. Under control conditions the EC_{50} amounted to (means ± S.E.M.) 20 ± 1 nM (slope factor −1.63 ± 0.01, $n = 14$) in the series of experiments with alcuronium and to 13 ± 2 nM (slope factor −1.65 ± 0.06, $n = 23$) in the W84-series. The test compounds induced a parallel rightward shift of the concentration-effect curves of oxotremorine-M. Under control conditions, a time-dependent shift of the concentration-effect curves did not occur. The antimuscarinic action was quantified as the dose ratio $DR = EC_{50,\text{test compound}}/EC_{50,\text{control}}$. For a combination of competitive antagonists the expected dose ratio can be calculated as $DR_{\text{expected}} = DR_{\text{antagonist A}} + DR_{\text{antagonist B}} - 1$ (e.g. Lüllmann et al., 1969; Clark and Mitchelson, 1976).

Alcuronium was generously provided by Hoffmann-La Roche (Basle, Switzerland). *N*-methylscopolamine and oxotremorine-M were purchased from Sigma (Deisenhofen, Germany). W84 was synthesized by Dr. Joachim Pfeffer (Kiel, Germany).

3. Results

Alcuronium antagonized the negative inotropic action of oxotremorine-M in the beating guinea pig left atria. A plot of the dose ratios determined in the presence of 10^{-6} M–10^{-4} M alcuronium according to Arunlakshana-Schild (Fig. 1, upper panel) yielded a straight line with a slope of 1.08 ± 0.08 (mean ±

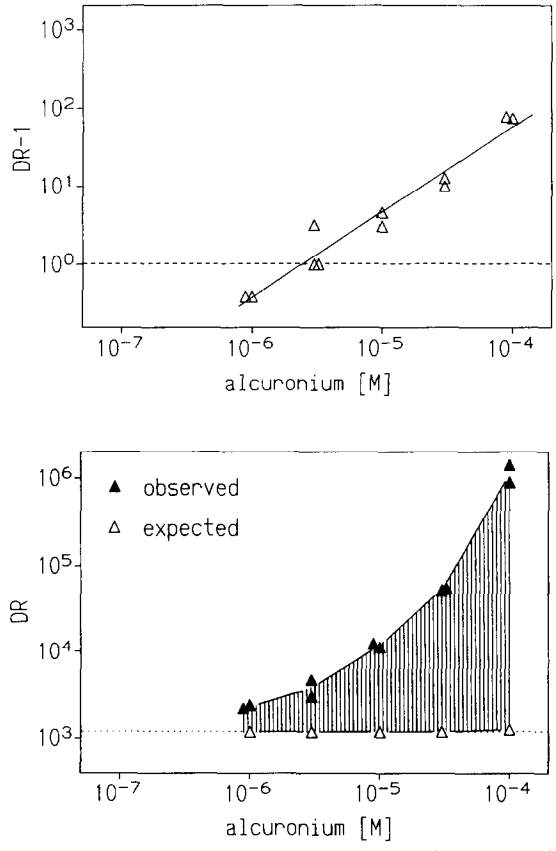

Fig. 1. Antimuscarinic effects of alcuronium alone (upper panel) and in combination with 10^{-7} M *N*-methylscopolamine (lower panel) in contracting guinea pig left atria with oxotremorine-M as the negative inotropic muscarinic agonist. Upper panel: Arunlakshana-Schild plot of the shift induced by alcuronium of the concentration-effect curve of oxotremorine-M. $DR = EC_{50,\text{alcuronium}}/EC_{50,\text{control}}$. EC_{50}: concentration of oxotremorine-M reducing the force of contraction to 50% of the control level. Points represent results obtained in single preparations. The line was fitted by linear regression analysis. Lower panel: shift of the oxotremorine-M concentration-effect curve induced by combinations of alcuronium with 10^{-7} M *N*-methylscopolamine. The dotted line indicates the DR under the influence of 10^{-7} M *N*-methylscopolamine alone. The expected shift of the combination ($DR = DR_{N\text{-methylscopolamine}} + DR_{\text{alcuronium}} - 1$) is illustrated by the open triangles, the observed shift is indicated by the filled triangles (points representing results obtained in single preparations). The shaded area illustrates the extent of overadditivity.

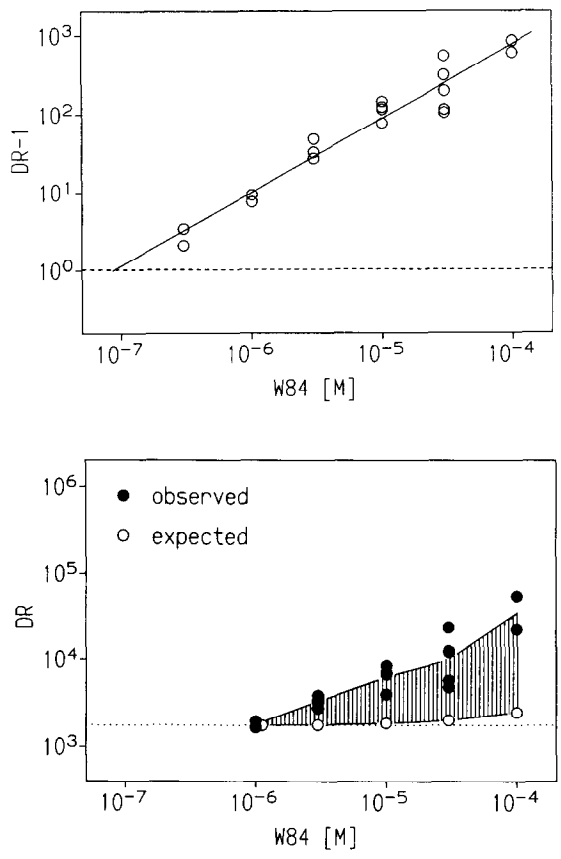

Fig. 2. Antimuscarinic effects of W84 alone (upper panel) and in combination with 10^{-7} M *N*-methylscopolamine (lower panel). Upper panel: Arunlakshana-Schild plot of the shift induced by W84 of the concentration-effect curve of oxotremorine-M. Lower panel: shift of the oxotremorine-M concentration-effect curve induced by combinations of W84 and 10^{-7} M *N*-methylscopolamine. Dotted line: shift induced by 10^{-7} M *N*-methylscopolamine alone. Open circles: expected shift for the combination; closed circles: observed shift. Shaded area: extent of overadditivity.

S.E.M.), which was not significantly different from unity – as if the action were competitive ($P = 0.32$, one sample *t*-test). With a slope constrained to unity a pA_2-value of 5.66 ± 0.02 (mean ± S.E.M.) was found. This value is in keeping with the $pA_2 = 5.9$ reported by Schaer (1972) and the K_d-value of 6 μM (corresponding to $pA_2 = 5.2$) found by Nedoma et al. (1985).

The antimuscarinic effect of W84 is illustrated in Fig. 2 (upper panel). In the Arunlakshana-Schild plot the data points could be connected by a line with a slope of (mean ± S.E.M.) 0.94 ± 0.06 (not significantly different from unity, $P = 0.35$, one sample *t*-test). A $pA_2 = 6.94 \pm 0.04$ (mean ± S.E.M.) can be derived from the data. With carbachol as the agonist, a flattening of the line had been observed with various alkane-bis-ammonium compounds at concentrations $\geq 10^{-4}$ M (Lüllmann et al., 1969; Mitchelson, 1975). This unusual behaviour was a first indicator of an allosteric mode of action. It cannot be excluded that a similar phenomenon would have occurred with oxotremorine-M as agonist at W84 concentrations exceeding 10^{-4} M.

In the combination experiments, *N*-methylscopolamine was applied as a conventional competitive antagonist. The concentration of 10^{-7} M *N*-methylscopolamine displaced the concentration effect curve of oxotremorine-M to the right by a factor of DR = 1177 ± 113 (mean ± S.D., $n = 3$) in the alcuronium experiments and DR = 1740 ± 417 (mean ± S.D., $n = 5$) in the W84 experiments (dotted lines in Fig. 1 and Fig. 2, lower panels). There is no significant difference between the DR-values ($P = 0.07$, unpaired t-test).

When alcuronium was combined with *N*-methylscopolamine, the observed antimuscarinic effect exceeded the effect which would have been expected in case of a combination of competitive antagonists. The extent of the overadditivity (shaded area in Fig. 1, lower panel) increased with increasing concentrations of alcuronium. A comparison with the respective results obtained under the influence of W84 plus *N*-methylscopolamine (Fig. 2, lower panel) reveals that the overadditive effect was more pronounced with alcuronium than with W84.

4. Discussion

The combination of alcuronium with the conventional antagonist *N*-methylscopolamine displays a higher antimuscarinic effect in beating guinea pig left atria than expected on the basis of the individual antimuscarinic effects of both components. Even at 10^{-6} M alcuronium, a concentration well below the pA_2, an overadditive action was seen. Since alcuronium has been shown by Proška and Tuček (1994) to elevate the binding of *N*-methylscopolamine in cardiac membranes, an augmentation of the binding of this antagonist in the contracting auricles is the most likely explanation for the potentiation of the effect of *N*-methylscopolamine.

To the best of our knowledge, overadditive actions have been reported so far only for the phthalimidopropyl-substituted alkane-bis-ammonium compounds W84 (Lüllmann et al., 1969) and the heptamethonium analogue (Mitchelson, 1975; Christopoulos and Mitchelson, 1994) as well as for some structurally closely related bisquaternary agents (e.g. Mohr et al., 1993). In cardiac homogenates these compounds inhibit the dissociation of radiolabelled conventional antagonists with rather high potency (Tränkle et al., 1994). For W84, the stabilization of the binding of [^{3}H]*N*-methylscopolamine has also been demonstrated in beating guinea pig left atria, yet the allosteric potency of W84 is about tenfold lower than in cardiac homogenates (Lüß and Mohr, 1992). In contrast, the

dissociation of the agonist [^{3}H]oxotremorine-M from cardiac membranes is only marginally affected even at high concentrations of W84 (Jepsen, 1988). Probably, compounds such as W84 shift in the beating atria the interplay at the receptor between agonist and antagonist in favour of antagonist binding. As a consequence, the action of the antagonist would be augmented.

With regard to the molecular structure, alcuronium and the alkane-bis-ammonium compounds have in common the bisquaternary nature, but otherwise the molecular properties are rather different. The results obtained with alcuronium reveal that the capability to potentiate the action of *N*-methylscopolamine is not confined to alkane-bis-ammonium compounds or structurally closely related molecules. Thus, this finding supports the notion that the potentiation of antagonist action encountered in intact cardiac preparations is a functional consequence of the allosteric stabilization of antagonist binding to muscarinic M_2 receptors.

However, at present it cannot be explained why some of the allosteric modulators do not augment antagonist action, although antagonist dissociation is retarded in cardiac homogenates. Possible reasons may be: (1) the inhibition of antagonist association by the compound occurs at far lower concentrations than the allosteric inhibition of antagonist dissociation so that antagonist binding is not effectively promoted; (2) the binding of the agonist is allosterically affected by the compound to the same extent as the binding of the antagonist with the interplay between agonist and antagonist thus being unaltered; (3) the allosteric potency of the compound is especially sensitive to the composition of the incubation medium and is attenuated to a major extent in 'physiological' media such as Tyrode's solution.

It shall be mentioned that plasma concentrations of about 1 μM are encountered when alcuronium is applied as a neuromuscular blocking agent during surgical anaesthesia (Raaflaub and Frey, 1972). At this concentration the potentiation of the antimuscarinic effect of *N*-methylscopolamine started to occur under the experimental conditions of this study. Yet, it has to remain open, whether a similar interaction would occur with the therapeutically applied antimuscarinic agents such as atropine and whether this effect would be of any clinical relevance.

Nevertheless, with regard to the allosteric modulation of muscarinic M_2 receptors alcuronium appears to be a valuable research tool to gain more insight into the mode of action of allosteric modulators at muscarinic acetylcholine receptors and into the functional consequences of the allosteric effect.

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