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European Journal of Pharmacology 305 (1996) 201–205

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Positive allosteric action of eburnamonine on cardiac muscarinic acetylcholine receptors

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Received 2 November 1995; revised 20 February 1996; accepted 23 February 1996

Abstract

It was discovered recently that alcuronium and strychnine (which is a precursor of alcuronium) allosterically increase the affinity of cardiac muscarinic receptors for the antagonist, *N*-methylscopolamine. We have now investigated the effects of *l*-eburnamonine and vincamine, which are both closely related to strychnine. In experiments on rat heart atria, *l*-eburnamonine was found to increase the binding of [^{3}H]*N*-methylscopolamine with Ehlert's cooperativity coefficient $\alpha = 0.35$, which indicates that the strength of its allosteric action is close to that of alcuronium and strychnine ($\alpha = 0.31$ and 0.44, respectively). However, the affinity of *l*-eburnamonine for the cardiac muscarinic receptors is lower than the affinities of alcuronium and strychnine ($K_{AR} = 22.6$ μM, 0.15 μM, and 3.4 μM, respectively). In spite of its extremely close similarity to *l*-eburnamonine, vincamine has a negative allosteric effect on the binding of [^{3}H]*N*-methylscopolamine ($\alpha = 4.1$; $K_{AR} = 22.8$ μM). It is likely that a systematic investigation of the allosteric effects of the analogues of strychnine will not only yield new allosteric effectors on muscarinic receptors, but also clarify the structural features responsible for the direction (positive or negative) of their allosteric effect.

Keywords: Muscarinic receptor, heart; Muscarinic receptor, allosteric control; Allosteric interaction; Eburnamonine; Vincamine; Alcuronium; Strychnine

1. Introduction

It has been shown that the affinity of the M_2 and M_4 subtypes of muscarinic receptors for *N*-methylscopolamine can be increased by the neuromuscular blocking drug alcuronium (Tuček et al., 1990; Proška and Tuček, 1994; Jakubík and Tuček, 1994a,b; Jakubík et al., 1995), and that alcuronium also increases the affinity of cardiac muscarinic receptors for atropine and several other muscarinic receptor antagonists (Tuček et al., 1995; Hejnová et al., 1995). The *positive* allosteric action of alcuronium is in sharp contrast to the well known negative allosteric action of neuromuscular blockers (review Lee and El-Fakahany, 1991) and its discovery raised a number of questions (see review by Tuček and Proška, 1995), one of them being that of the existence of other compounds which would have the same positive effect as alcuronium. The discovery of such compounds among the drugs which are already available might help to clarify which features of the molecule of the allosteric modifier are responsible for its positive action and might give leads for the synthesis of new and more potent modifiers suitable for therapeutic use.

So far, attempts to increase the number of known substances with positive allosteric effects on muscarinic receptors have met with little success. Dong et al. (1995a) reported that the binding of [^{3}H]*N*-methylscopolamine to muscarinic M_3 receptors was increased by less than 20% by tetrandrine and fangchinoline and a positive allosteric effect of 9-methoxy-α-lapachone on muscarinic M_2 receptors was reported from the same laboratory (Dong et al., 1995b). Proška and Tuček (1995) and Lazareno and Birdsall (1995) described positive allosteric effects of strychnine on muscarinic M_2 and M_4 receptors. The discovery of the positive allosteric action of strychnine was not totally surprising. There is considerable similarity between the structures of strychnine and alcuronium (Fig. 1). In principle, strychnine dimerization is the basic step in the synthesis of alcuronium. It seems apparent that the main structural features responsible for the positive allosteric action of alcuronium on muscarinic M_2 and M_4 receptors

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PII S0014-2999(96)00169-0

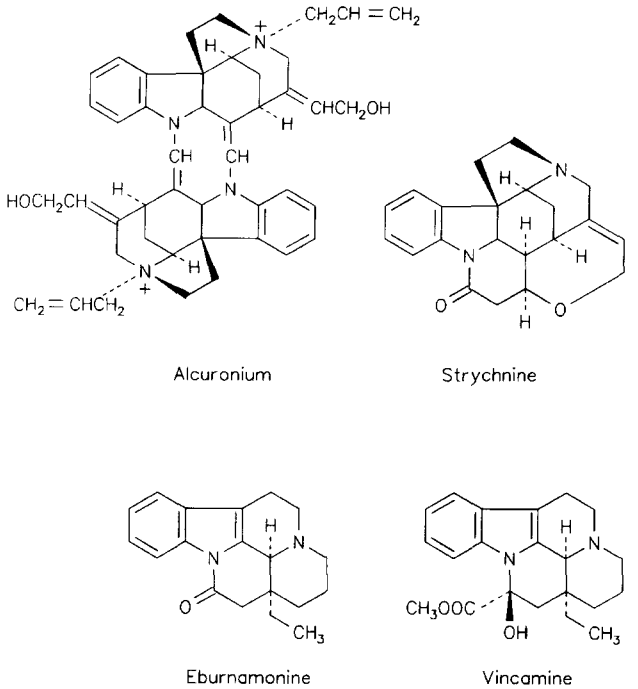

Fig. 1. Structural formulae of alcuronium, strychnine, *l*-eburnamonine and vincamine.

are already present in the strychnine molecule, although the potency of strychnine is less that that of alcuronium. We have now investigated two alkaloids from Vinca minor (Taylor, 1965) with structures similar to that of strychnine, namely vincamine and *l*-eburnamonine, and have found that, in spite of their close mutual similarity, these two compounds have diametrically opposite effects on the affinity of cardiac muscarinic receptors for their classical antagonist, *N*-methylscopolamine.

2. Materials and methods

2.1. Experimental

Adult male rats were killed by cervical dislocation and decapitation and their heart atria were homogenized with a polytron-type homogenizer in a medium consisting of 100 mM NaCl and 20 mM sodium Hepes (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonate, pH 7.4). The binding of the specific muscarinic antagonist, [^{3}H]*N*-methylscopolamine, to muscarinic receptors in the homogenate and changes of the binding in the presence of allosteric modifiers were measured as described (Proška and Tuček, 1994, 1995). Portions of the homogenate corresponding to 2 mg of original tissue were incubated for 5 h at 25°C in an incubation volume of 1.6 ml. The composition of the incubation medium corresponded to that of the homogenization medium but its pH was adjusted to 7.15 in experiments with *l*-eburnamonine and vincamine in order to increase their solubility. The medium was supplemented with 200 pM [^{3}H]*N*-methylscopolamine (a concentration close to one half of the K_d for the binding of [^{3}H]*N*-methylscopolamine), and with varying concentrations of the modifying drugs; 1 μM atropine was added to measure the non-specific binding of the radioligand. Under the conditions used, the K_d for the binding of [^{3}H]*N*-methylscopolamine was 416 pM at pH 7.4 and 430 pM at pH 7.15. The incubation was stopped by filtration through Whatman GF/B glass fibre filters and the bound radioactivity retained on filters was determined by liquid scintillation spectrometry. [^{3}H]*N*-Methylscopolamine (80.5 Ci/mmol) was obtained from DuPont-NEN (Bad Homburg, Germany), strychnine hemisulfate, *l*-eburnamonine and vincamine hydrochloride were from Sigma Chemical Co. (St. Louis, MO, USA) and alcuronium was from Hoffmann-La Roche (Basel, Switzerland).

2.2. Data treatment

The data were treated as described (Proška and Tuček, 1994, 1995; Jakubík and Tuček, 1994a,b; Jakubík et al., 1995). Values of the dissociation constants for the binding of allosteric modulators to muscarinic receptors (K_{AR}) and of the cooperativity coefficients α were computed according to Ehlert (1988) by fitting Ehlert's equation No. 6 to the data on the binding of [^{3}H]*N*-methylscopolamine in the presence of various concentrations of the modulators. Values of $\alpha < 1$ indicate positive cooperativity and $\alpha > 1$ indicate negative cooperativity between the binding of the allosteric modulator and of [^{3}H]*N*-methylscopolamine. Unless stated otherwise, the data are means ± S.E.M. of n observations.

3. Results

To investigate the effects of alcuronium, strychnine, *l*-eburnamonine and vincamine on the binding of [^{3}H]*N*-methylscopolamine to the muscarinic binding sites in the heart atria, identical portions of the homogenate were incubated in the presence of 200 pM [^{3}H]*N*-methylscopolamine and of 10^{-5}–10^{-9} M alcuronium, 10^{-4}–10^{-8} M strychnine, 10^{-4}–10^{-8} M eburnamonine, and 10^{-3}–$10^{-7.5}$ M vincamine. As can be seen from Fig. 2, the binding of [^{3}H]*N*-methylscopolamine was increased in the presence of alcuronium, strychnine and eburnamonine but diminished in the presence of vincamine. The inhibition of the binding by vincamine was not saturable, reaching less than 75% at 1 mM concentration. In view of this, and of our finding that vincamine slowed the association of [^{3}H]*N*-methylscopolamine with the receptors (data not shown), we conclude that vincamine inhibited binding allosterically rather than competitively.

The curves describing the binding of [^{3}H]*N*-methylscopolamine in the presence of alcuronium, strychnine and eburnamonine have an ascending and a descending part. It

has been shown in previous work (Proška and Tuček, 1994, 1995; Tuček and Proška, 1995) that the descending part of the curves is due to the extreme slowing of the binding of [^{3}H]*N*-methylscopolamine that occurs in the presence of high concentrations of alcuronium and strychnine. We have now found that the binding of [^{3}H]*N*-methylscopolamine in the presence of 10^{-4} M eburnamonine could be increased when the incubation was prolonged from 5 to 10 h (not shown), confirming that binding equilibrium had not been reached within 5 h at this high concentration of the allosteric effector.

The data in Fig. 2 and computed values in Table 1 indicate that the affinity of *l*-eburnamonine and vincamine for the receptors is 7–8 times lower than that of strychnine and more than 100 times lower than that of alcuronium.

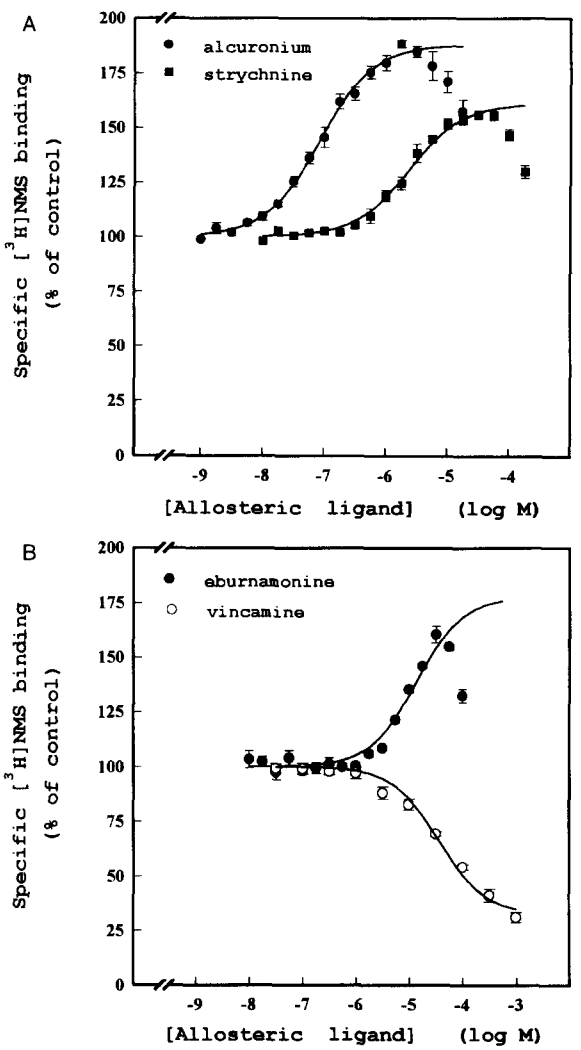

Fig. 2. Changes in the specific binding of [^{3}H]*N*-methylscopolamine to rat atrial membranes induced by (A) alcuronium and strychnine and (B) *l*-eburnamonine and vincamine. Abscissa: $\log_{10}$ of the concentration (M) of the drug. Ordinate: [^{3}H]*N*-methylscopolamine binding in the presence of the drug, expressed as per cent of the binding in the absence of the drug. Points are means ± S.E.M. of 3 experiments with incubation performed in duplicate. The lines correspond to Equation No. 6 in the study by Ehlert (1988) and to the values for the parameters in Table 1.

Table 1
Quantitative parameters of the binding and action of allosteric modulators

Modulator	*n*	K_{AR} (μM)	α
Alcuronium	15	0.15 ± 0.02	0.31 ± 0.01
Strychnine	10	3.36 ± 0.19	0.44 ± 0.06
l-Eburnamonine	4	22.57 ± 1.63	0.35 ± 0.01
Vincamine	4	22.78 ± 0.86	4.12 ± 0.23

K_{AR} = equilibrium dissociation constant for the binding of the modulator to the receptor; α = Ehlert's (Ehlert, 1988) cooperativity coefficient. Data are means ± S.E.M. of *n* observations.

The strength of the allosteric action expressed by Ehlert's (Ehlert, 1988) cooperativity coefficient (which indicates the magnitude of the change of the K_d for the binding of [^{3}H]*N*-methylscopolamine induced by the allosteric effector), however, was of the same order of magnitude for the positive effectors alcuronium, strychnine and *l*-eburnamonine. The affinities of eburnamonine and vincamine for muscarinic receptors were very similar to each other but the affinity of the receptors for [^{3}H]*N*-methylscopolamine was increased 3-fold by *l*-eburnamonine and diminished 4-fold by vincamine (Table 1).

4. Discussion

The finding that alcuronium, strychnine and *l*-eburnamonine have a positive allosteric effect on the binding of *N*-methylscopolamine to muscarinic M_2 receptors indicates that compounds closely related to strychnine by their chemical structure have a special relation to the allosteric binding site of muscarinic receptors and suggests that more substances displaying positive cooperativity on muscarinic receptors will be discovered in this group of compounds. Alcuronium, strychnine and *l*-eburnamonine have rigid skeletons and complex structures in which the easily accessible electron-rich indole moiety is prominent (Fig. 1). The indole benzene ring might be expected to interact both with the positive charges in the receptor's molecule and with the aromatic moieties of tyrosine and tryptophan which the receptor contains. The extracellularly located tryptophan residues in the molecule of the muscarinic M_1 receptor have indeed been found of importance in the interaction between the receptor and gallamine, which is another allosteric modifier with an aromatic ring in its structure (Matsui et al., 1995).

We do not know why alcuronium has a much higher potency than strychnine and *l*-eburnamonine but we suppose that one of three mechanisms may be responsible.

(a) Alcuronium is a 'dimeric compound' (applying Schwarting's (Schwarting, 1977) unrestrictive definition of such compounds) and may associate with two similar binding domains on the same receptor, which makes its association stronger than that of monomeric strychnine and *l*-eburnamonine. There is similarity between this view and

the model which Adams et al. (1985) proposed for the binding of diacridines and diquinolines to the α-adrenoceptors.

(b) Even if there is a single binding domain to which alcuronium, strychnine and *l*-eburnamonine all attach, it is conceivable that the binding of alcuronium is facilitated by its dimeric structure (the availability of two binding faces on a single molecule), which augments the probability that the ligand and the binding site of the receptor occur in positions suitable for mutual association.

(c) The two quaternary nitrogens of alcuronium may act as additional anchors in its binding to the receptors and be at least partly responsible for the high potency of alcuronium. Data obtained for chemically modified receptors (Jakubík and Tuček, 1995) and for receptor mutants (Leppik et al., 1994) suggest that the negatively charged aspartate residues in the vicinity of the orthosteric binding site are important for the binding and action of the allosteric ligands. Experiments with non-quaternary analogs of gallamine (Van Hijfte et al., 1995) have, howevere, indicated that the presence of the quaternary nitrogen in the molecules of the analogs is not crucial.

At present, no answer is available for the question of which features of an allosteric effector decide whether its allosteric action on a particular muscarinic receptor subtype is positive or negative. It is in relation to this question that the difference between the positive allosteric action of *l*-eburnamonine and the negative action of vincamine seems noteworthy and important. The only distinction between the structures of the two alkaloids is that the carbon atom in position 14 (see Windholz, 1983, for the numbering convention) is associated with a carbonyl oxygen in *l*-eburnamonine and with a hydroxyl plus a methoxycarbonyl group in vincamine. Investigation of other substituents located in the same position of the vincamine/eburnamonine molecule and fitting of such modified alkaloids to molecular models of muscarinic receptors should provide insight into the mechanism responsible for the change of affinity in either the positive or the negative direction.

l-Eburnamonine and vincamine and several of their derivatives (e.g. vinpocetine and vinconate) are being used clinically as vasodilators and it is believed that they preferentially improve cerebral blood flow (review, Spagnoli and Tognoni, 1983). Vincamine and the closely related drug, vinpocetine, have been suggested to increase the production of prostaglandins in smooth muscle (Machová et al., 1989). However, it was also proposed that *l*-eburnamonine (vinburnine) (Drago et al., 1990) and a chemically related drug, vinconate, (compound OM 853) (Koda et al., 1989) facilitate cholinergic neurotransmission in the brain. Vinconate was found to increase the release of acetylcholine, presumably via a modulatory action on presynaptic dopamine receptors (Iino et al., 1993) and to stimulate the hydrolysis of phosphatidylinositol via an unknown mechanism (Katsura et al., 1993). It seems possible that some of these effects may be due to allosteric interactions between *l*-eburnamonine, vincamine and related compounds and the G protein-coupled (muscarine and dopamine) receptors.

Acknowledgements

We thank Professor E.E. El-Fakahany for invaluable help in our work and Hoffmann-La Roche (Basel) for alcuronium. The study was supported by grant No. 309/96/1287 from the Grant Agency of the Czech Republic (S.T.) and by National Institutes of Health FIRC Award (E.E.E).

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