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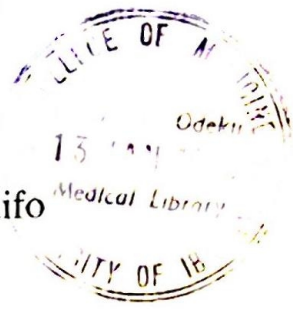

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Chloroquine interacts with muscarinic receptors and inhibits cholinergic effects in the heart

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Summary

Binding and electrophysiological studies were carried out in order to investigate the mechanisms underlying the cardiac anticholinergic action of chloroquine. Muscarinic receptors of guinea-pig heart homogenates were labelled with tritiated quinuclidinyl-benzilate ([^{3}H]-QNB) in the absence and in the presence of chloroquine. Chloroquine (10^{-5}–10^{-4} M) produced a shift of the QNB saturation binding curve to the right. Increasing the chloroquine concentration in the presence of a constant QNB concentration produced a progressive decrease in QNB binding, but the QNB–chloroquine competition curves were shallow (Hill coefficients = 0.65). Chloroquine also shifted the QNB–carbachol competition curve to the right without changing its Hill slope. In electrophysiological experiments, chloroquine inhibited the negative chronotropic effect of carbachol in Langendorff-perfused hearts. This inhibitory effect of chloroquine was obtained at concentrations lower than those expected to produce significant binding to muscarinic receptors, and was not completely reversible. It is concluded that the binding of chloroquine to cardiac muscarinic receptors is complex and that in addition to the interaction at the receptor, other mechanisms are involved in the inhibition of the muscarinic agonist-induced negative chronotropic effect.

Résumé

Des expériences de liaison aux récepteurs ainsi que d'électrophysiologie ont été menées afin d'investiguer les mécanismes de l'action anticholinergique cardiaque de la chloroquine. Les récepteurs muscariniques présents dans des homogénats de coeur de cobaye ont été marqués à l'aide du quinuclidinylbenzilate tritié ([^{3}H]QNB) en présence ou en absence de chloroquine. La chloroquine (10^{-4}–10^{-5} M) a produit un déplacement vers la droite de la courbe de saturation en QNB. L'augmentation de la concentration de chloroquine en présence d'une concentration constante de QNB a causé une diminution progressive de la liaison de QNB, mais les courbes de compétition ont une faible pente (coefficient de Hill = 0.65). La chloroquine a également produit un déplacement vers la droite de la courbe de compétition QNB–carbachol, sans en modifier la pente. Dans les études électrophysiologiques, la chloroquine a inhibé l'effet chronotrope négatif du carbachol sur des coeurs perfusés selon la technique de Langendorff. Cet effet inhibiteur de la chloroquine a été obtenu à des concentrations inférieures à celles nécessaires pour produire une liaison considérable aux récepteurs, et n'était pas complètement réversible. Il est conclu que la liaison de la chloroquine aux récepteurs muscariniques cardiaques est complexe et que, en plus de l'intéraction au niveau du récepteur, des méchanismes supplémentaires sont en jeu au cours de l'inhibition de l'effet chronotrope négatif des agonistes muscariniques.

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Introduction

Chloroquine is a 4-aminoquinoline derivative which exerts a variety of pharmacological effects, the best known of which are its antimalarial and anti-inflammatory actions. Chloroquine has a cardiac anti-arrhythmic action [1],

which is probably related to its local anaesthetic properties. Chloroquine has also been shown to increase the heart rate as well as electrocardiographic voltages in the frog [2]. In a recent study in which clinical doses (225 or 600 mg base) of chloroquine were administered orally to healthy volunteers, we have shown that the drug causes (1) an increase in resting heart rate, (2) a decrease in the influences of deep breathing, of postural change, as well as of the Valsalva manoeuvre on heart rate, and (3) a decrease in the mean beat-to-beat variation of the cardiac cycle (R–R interval) [3]. Similar effects are known to be produced by atropine, suggesting that the effects of chloroquine are due to an antimuscarinic action. Anticholinergic actions of chloroquine have been reported [4–6], and some evidence has been provided for the binding of chloroquine to muscarinic receptors [7,8]. However, none of the previous studies used cardiac tissues. As muscarinic receptors in various organs can be of different pharmacological subclasses, the present study was undertaken to assess the interaction of chloroquine with cardiac muscarinic receptors (predominantly of m-2 type).

Materials and methods

Hearts obtained from guinea-pigs (weighing 400–700 g) were used in the study. Hearts destined for radioligand studies were Langendorff-perfused with Tyrode's solution for 1 min to remove blood from the coronary system and were processed as described below. Hearts destined for electrophysiological studies were perfused for at least 1 h before the experiment. They were immersed in a bath filled with Tyrode's solution.

Binding studies were performed on homogenates obtained from whole hearts. The protocol followed is practically the same as the one used in previous studies [9,10]. Homogenates (100 µl or c. 50 µg protein) were incubated in Tyrode's solution containing quinuclidinyl-benzilate (QNB) (total volume of incubation: 1.2 ml). The incubation was carried out at 37°C for 90 min, in the presence or in the absence of chloroquine. Each experiment was done in duplicate. The separation of bound from unbound radioactive ligand was achieved by rapid filtration through glass fibre filters (GF/C or GF/B, Whatman) followed by washing twice with 5 ml ice-cold Tyrode's solution. The washed filters were placed in vials to which 10 ml of scintillation fluid (Dimilume-30 + Soluene-350, Packard) were added. Radioactivity was counted in a β-counter after 12–24 h at 45% efficiency. Muscarinic-specific binding defined as the atropine-displaceable binding was calculated as difference between the total binding and the non-specific binding remaining in the presence of 10^{-6} M atropine.

For electrophysiological experiments, electrocardiograms (ECGs) of isolated hearts were recorded with two electrodes attached to the walls of the incubation bath. The signal from the electrocardiograph (EK-8, Burdick) was amplified and filtered (frequency band: 10–100 Hz; Bio-Amplifier 631, Phipps and Bird) before being displayed on a pen recorder (Biocorder 5–200, Science Instruments). The muscarinic agonist-induced negative chronotropic effect was determined by measuring R–R intervals at different times following the administration of carbachol, in the absence or in the presence of chloroquine. The experiments were carried out at 25–26°C. This temperature is lower than the one used in binding studies due to a high frequency of occurrence of arrhythmias at 37°C, especially during muscarinic agonist application.

The composition of the Tyrode's solution was (in mM): NaCl 127, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, glucose 5, and either $NaHCO_3$ 23 (for ECG studies) or tris-hydroxymethylaminomethane–HCl 10 (for binding studies). When $NaHCO_3$ was used, the solution was bubbled with carbogen (95% O_2 + 5% CO_2) to give a pH of 7.5 at 25°C. Chloroquine, quinine hydrochloride and carbamylcholine chloride (carbachol) were from Sigma. Atropine sulphate was from Boehringer. Tritiated QNB ([^{3}H]QNB; specific activity 43.3 Ci/mmol) was from Amersham.

Results

Evidence for the interaction of chloroquine with muscarinic receptors is provided in Fig. 1. Figure 1a illustrates the results of an experiment in which muscarinic receptors were labelled with increasing concentrations of QNB in the absence or in the presence of chloroquine. In the absence of chloroquine, QNB bound

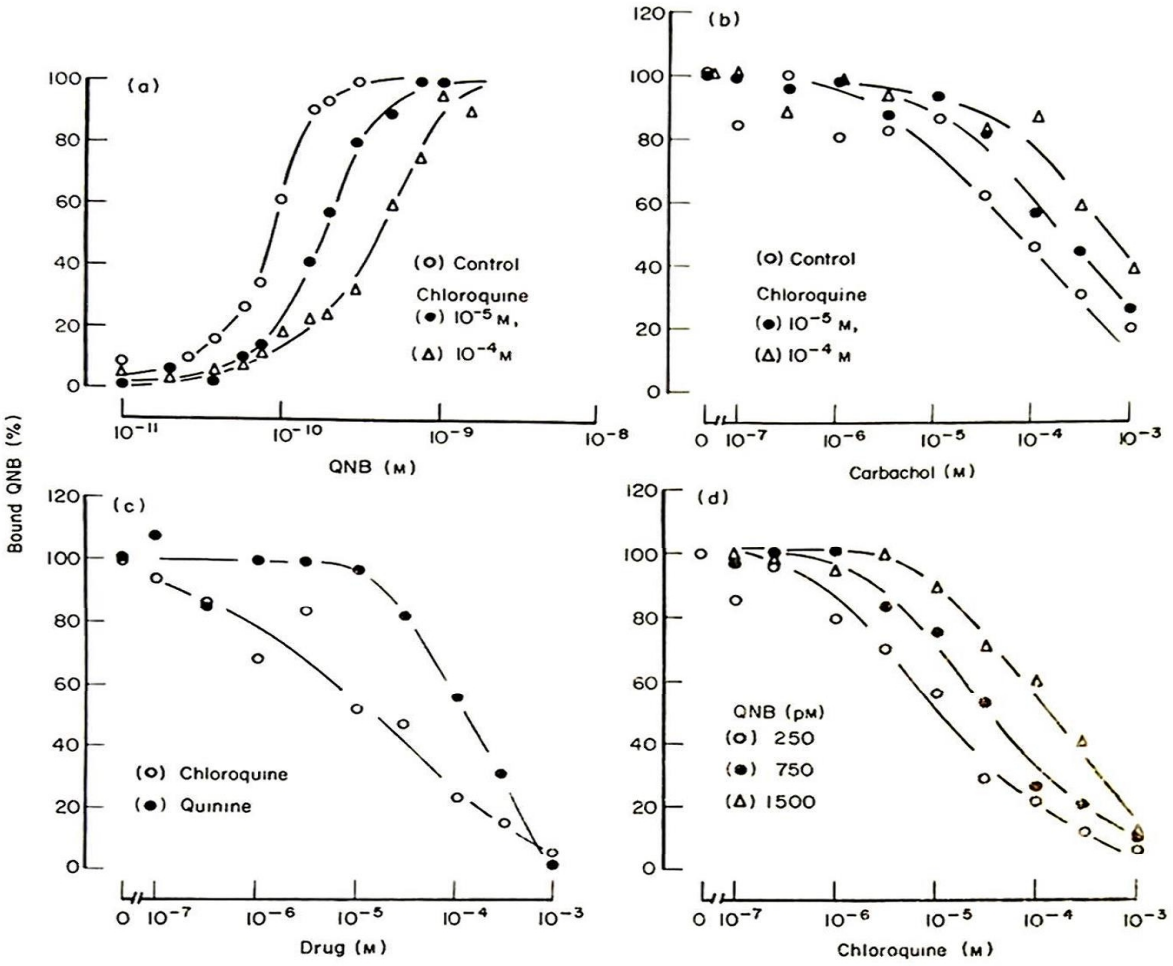

Fig. 1. Binding of chloroquine to muscarinic receptors. (a) Shift of QNB saturation curve by chloroquine. Labelling of muscarinic receptors was done using increasing QNB concentrations in the absence and in the presence of chloroquine. (b) Shift of QNB–carbachol competition curve by chloroquine. Various carbachol concentrations were used to decrease QNB binding, in the absence and in the presence of chloroquine. The QNB concentration used to label the receptors was 750 pM. (c) Inhibition of muscarinic-specific QNB binding with increasing chloroquine or quinine concentrations in the presence of a constant QNB concentration (750 pM). (d) Inhibition of QNB binding with increasing chloroquine concentrations as in (c), but for three different QNB concentrations.

with an apparent dissociation constant of 94 pM. In the presence of 10^{-5} or 10^{-4} M chloroquine, the saturation curve was shifted to the right without an apparent change in the maximal binding capacity. The dissociation constant for the chloroquine–muscarinic receptor interaction could be roughly estimated from the magnitude of the shift produced by chloroquine in two such experiments: (K_I = (concentration of chloroquine)/(EC_{50} ratio − 1), where K_I is the apparent dissociation constant and EC_{50} is the QNB concentration producing 50% binding) and was found to be 2.0×10^{-5} M.

The binding of chloroquine to muscarinic receptors was also revealed by its ability to influence the QNB–carbachol competition (Fig. 1b). Carbachol decreased the QNB binding in a concentration-dependent way, with a half-

maximum inhibitory concentration (IC_{50}) of 6.5×10^{-5} M in the presence of 750 pM QNB. The competition curve was shifted to the right in the presence of chloroquine. The Hill coefficient for carbachol inhibition of QNB binding was low ($n_H = 0.62$) and was not changed in the presence of chloroquine.

In the experiment of Fig. 1c the receptors were labelled with a constant QNB concentration in the presence of varying concentrations of chloroquine or quinine. Quinine, another quinoline-containing antimalarial drug, was included in this experiment for comparison with chloroqine. Both chloroquine and quinine produced a concentration-dependent decrease in the amount of QNB bound. However, whereas the inhibition of QNB binding by quinine occurred over 2 logarithmic units of concentration, the decrease of QNB binding by chloroquine occurred over more than 4 units, with an IC_{50} of about 1.4×10^{-5} M. The Hill coefficients for the inhibition of QNB binding were 0.57 and 1.5 for chloroquine and quinine, respectively. The pseudo K_I for chloroquine calculated from these results is 10^{-5} M, i.e. close to the value obtained from saturation experiments. The same experimental protocol was repeated for chloroquine using three different QNB concentrations (Fig. 1d). Even with high QNB concentrations chloroquine was able to completely inhibit QNB binding. In all cases the competition curves were shallow, with Hill coefficients of about 0.65.

In order to test for a functional implication of this binding of chloroquine on receptors, the influence of chloroquine on muscarinic agonist-induced negative chronotropism was studied. Figure 2a shows an experiment in which the time course of carbachol-induced change in R–R interval, before and after addition of chloroquine was studied. In the absence of chloroquine, carbachol (10^{-6} M) produced an increase in the duration of the cardiac cycle (i.e. a slowing of heart rate). The negative chronotropic effect of carbachol reached a maximum before decaying to a steady value within 10–15 min. This fade of the negative chronotropic response to carbachol is probably due to a desensitization process [11,12] since it could be shown in other experiments that such a fade was accompanied by a decreased responsiveness to further carbachol application after short (<10 min) washout periods. However, recovery from this desensitization process reached steady-state within 15 min of washout, and most (>90%) of the responsiveness to carbachol was recovered (K. Mubagwa, unpublished data). In the experiment of Fig. 2a, after washout of carbachol, the heart was perfused with 10^{-6} M chloroquine for 15 min. Chloroquine alone produced an increase of the R–R interval from 0.43 to 0.77 sec at the concentration used. In the presence of chloroquine, carbachol had no effect on the cardiac cycle duration, suggesting that chloroquine antagonizes the cardiac muscarinic receptor-mediated response. It is to be noted that this inhibitory effect of chloroquine on the muscarinic response was maximal at a concentration lower than the estimated K_I, i.e. at a concentration which produces binding to less than 50% of muscarinic receptors. After chloroquine washout, further application of carbachol produced a negative chronotropic effect, although the magnitude of the response was small compared to the effect obtained before chloroquine administration (Fig. 2a). The fact that some responsiveness to carbachol was recovered after chloroquine washout also suggests that the lack of carbachol response in the presence of chloroquine was not due to a persistent desensitization following the control carbachol application. Washing chloroquine for longer (30–60 min) or increasing carbachol concentration to 10^{-5}–10^{-4} M did not restore the original responsiveness of the preparation (data not shown). Figure 2b presents the results of an experiment in which carbachol was applied in the presence of various chloroquine concentrations. Inhibition of the chronotropic response to carbachol was obtained with concentration as low as 10^{-9} M chloroquine. Complete dose–response studies were hampered by the lack of reversibility of chloroquine action and by the fact that even in preparations not exposed to chloroquine the responsiveness to carbachol tended to decrease with time, although to a much lower degree than in preparations exposed to chloroquine. However, in four out of eight preparations, 10^{-6} M chloroquine was able to completely inhibit the carbachol-induced increase in R–R interval.

Discussion

The results confirm that chloroquine interacts

with muscarinic receptors as previously shown in neuroblastoma-glioma cells [7], and recently in undifferentiated chick preparations [8]. The pseudo K_I of chloroquine for cardiac muscarinic receptors as roughly estimated from experiments described in the present study is $1–2 \times 10^{-5}$ M, i.e. close to the value (0.6×10^{-5} M) obtained earlier [8], and indicates that chloroquine interacts with muscarinic receptors with relatively low affinity. One feature of the chloroquine interaction with cardiac muscarinic receptors obtained in the present study, however, is different from the results obtained earlier. The inhibition by chloroquine of the binding to cardiac muscarinic receptors occurred at concentrations ranging over more than 3–4 log units (Fig. 1c and d). This was also reflected in the low slopes obtained in Hill plots ($n_H = 0.57–0.60$). Such a result is not consistent with a simple competitive antagonism by chloroquine for binding to a single population of homogeneous sites. The low Hill slope of chloroquine binding resembles the one obtained with agonists, which show shallow competition curves as a result of their binding to different agonist-affinity classes of muscarinic receptors [13,14]. Thus the results with chloroquine could also be explained by assuming that this drug interacts with a heterogeneous population of sites. Alternatively, it is possible that chloroquine, besides acting as a competitive antagonist, also binds to a secondary site on the muscarinic receptor, which exerts co-operativity with the binding to the primary site. A similar situation is obtained for the interaction of muscarinic receptors with gallamine and pancuronium [15] or with verapamil [16]. It is also to be noted that chloroquine has been shown to inhibit the response mediated by other receptors [4,6]. It is thus very unlikely that the interaction of chloroquine with muscarinic receptors is selective.

The electrophysiological results show that carbachol's effect on heart rate was decreased or suppressed in the presence of chloroquine. The diminished response to carbachol in the presence of chloroquine is not due to a persistent desensitization from a previous carbachol

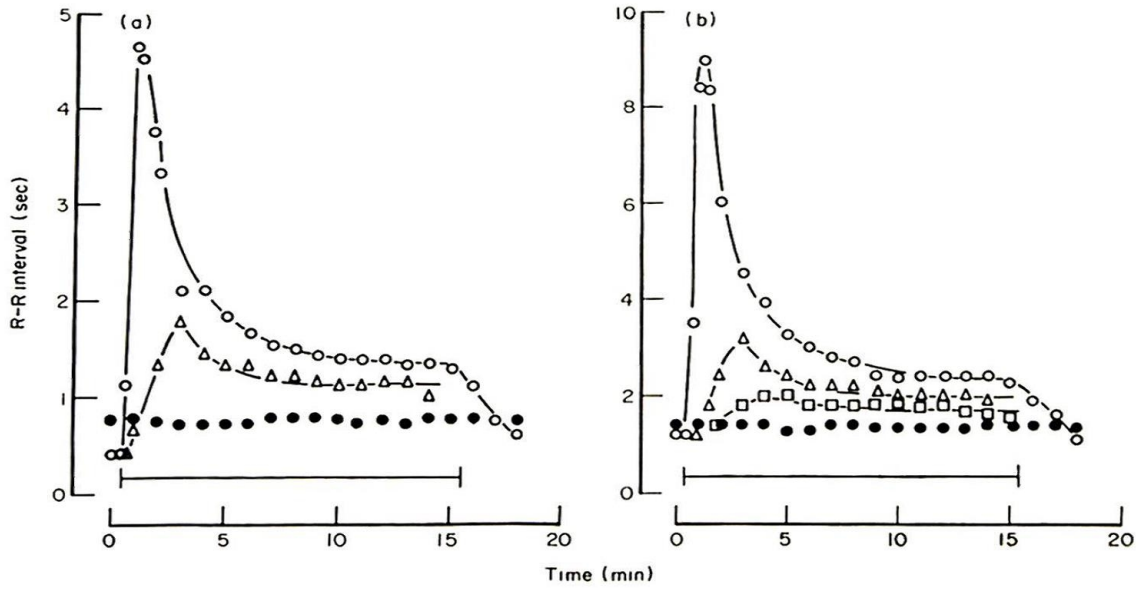

Fig. 2. Inhibition by chloroquine of the negative chronotropic effect of carbachol (10^{-6} M). (a) Time-course of the effect of carbachol on R–R interval in the absence (○), in the presence (●), and after washout (△) of 10^{-6} M chloroquine. (b) Time-course of the effect of carbachol on R–R interval obtained in the absence (○), and in the presence of various concentrations of chloroquine [(△) 10^{-9} M, (□) 10^{-7} M, (●) 10^{-6} M]. In (a) and (b), the duration of exposure to carbachol is indicated by a horizontal line below the data points.

application because: (1) carbachol was applied at a time when the recovery from desensitization should have been complete (i.e. after 15 min of carbachol washout in the absence of chloroquine and a further 15 min washout in its presence); and (2) there was a partial recovery of the responsiveness to carbachol after chloroquine washout. Thus, our data indicate that chloroquine antagonizes the negative chronotropic action of carbachol. However, this antagonistic effect was obtained even at chloroquine concentrations which do not produce significant binding to muscarinic receptors. The discrepancy between the relatively low affinity of chloroquine for muscarinic receptors and its high potency to inhibit the negative chronotropic action of carbachol is too large to be accounted for by the difference in the temperatures used in the two sets of experiments and suggests that mechanisms other than competition for binding to the receptors are involved. A possible mechanism for the antagonism to the chronotropic action of carbachol is a direct blocking effect of chloroquine on the receptor-to-effector coupling mechanisms or on the effector system itself. That is, chloroquine might have an inhibitory effect on the GTP-binding protein (G_k) or on the membrane lipid metabolism which couple [17–20] the agonist–receptor interaction to the activation of K^+ channels, or a direct blocking effect on these channels. It is to be noted that a discrepancy has also been observed between the effects of quinidine on isolated rabbit atrial muscle and those on ventricular muscle and has been tentatively explained by a blocking action of quinidine on muscarinic-sensitive K^+ channels (present in atrial but not in ventricular muscle) [21]. Recently, it has been shown that quinidine, besides its well-known muscarinic antagonist properties [9,22], is also capable of directly blocking the muscarinic-activated channels of atrial tissues [23]. Whether chloroquine, which is structurally related to quinidine, has similar effects on the muscarinic-activated channel needs to be investigated.

Acknowledgments

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References

1. Burrel ZL, Martinez AC. Chloroquine and hydroxychloroquine in the treatment of cardiac arrhythmias. N Engl J Med 1959;258:798–800.
2. Chinyanga HM, Fletcher JJ, Vartanian GA. Increase of electrocardiogram voltage and contractile force of the frog's heart induced by chloroquine. West Afr J Pharmacol Drug Res 1976;3:90–101.
3. Mubagwa K, Adler J. Muscarinic antagonist action of clinical doses of chloroquine in healthy volunteers. J Auton Nerv Syst 1988;24:147–55.
4. Olatunde IA. Quantitation of the degree of antagonism of chloroquine to histamine, acetylcholine and serotonin (PA_2 values). Arch Int Pharmacodyn 1970;185:66–70.
5. Minker E, Kadar T, Matejka Z. Effect of chloroquine and mepacrine on the spontaneous and evoked movements of the rat portal vein. Acta Physiol Acad Sci Hung 1980;55:71–80.
6. Lot TY, Bennet T. Comparison of the effects of chloroquine, quinacrine and quinidine on autonomic neuroeffector mechanisms. Med Biol 1982;60:307–15.
7. Gossuin A, Maloteaux JM, Trouet A, Laduron P. Differentiation between ligand trapping into intact cells and binding on muscarinic receptors. Biochem Biophys Acta 1984;804:100–6.
8. Schmidt H, Oettling G. Chloroquine is a muscarinic antagonist. Binding and dose–response studies with chick embryo cells. Eur J Pharmacol 1987;133:83–8.
9. Fields JZ, Roeske WR, Morkin E, Yamamura H. Cardiac muscarinic cholinergic receptors: biochemical identification and characterization. J Biol Chem 1978;253:3251–8.
10. Mubagwa K, Carmeliet E. Interaction of AQA 39, D600, verapamil and dilitiazem with cardiac cholinergic effects. Arch Int Pharmacodyn Ther 1987;286:71–84.
11. Jalife J, Hamilton AJ, Moe GK. Desensitization of the cholinergic receptor of the sinoatrial cells of the kitten. Am J Physiol 1980;234:H439–48.
12. Carmeliet E, Mubagwa K. Desensitization of the acetylcholine-induced increase of potassium conductance in rabbit cardiac Purkinje fibres. J Physiol (Lond) 1986;371:239–55.
13. Birdsall NJM, Hume EC. Biochemical studies on acetylcholine receptors. J Neurochem 1976; 27:7–16.
14. Birdsall NJM, Hume EC, Burgen ASV. The

binding of agonists to brain muscarinic receptors. Mol Pharmacol 1978;14:723–6.
15. Dunlap J, Brown JH. Heterogeneity of binding sites on cardiac muscarinic receptors induced by the neuromuscular blocking agents gallamine and pancuronium. Mol Pharmacol 1983;24:15–22.
16. Waelbroek M, Robbereecht P, De Neef P, Christophe J. Effects of verapamil on the binding properties of rat heart muscarinic receptors: evidence for an allosteric site. Biochem Biophys Res Commun 1984;121:340–5.
17. Breitwieser GE, Szabo G. Uncoupling of cardiac muscarinic and β-adrenergic receptors from ion channels by a guanine nucleotide analogue. Nature 1985;317:538–40.
18. Pfaffinger PJ, Martin JM, Hunter DD, Nathanson NN, Hille B. GTP-binding proteins couple cardiac muscarinic receptors to a K channel. Nature 1985;317:536–8.
19. Sorota S, Tsuji Y, Tajima T, Pappano AJ. Pertussis toxin treatment blocks hyperpolarization by muscarinic agonists in chick atrium. Circ Res 1985;57:748–58.
20. Kim D, Lewis DL, Graziadei L, Neer EJ, Bar-Sagi D, Clapham DE. G-protein βγ-subunits activate the cardiac muscarinic K^+-channel via phospholipase A_2. Nature 1989;337:557–60.
21. Nawrath H, Sacks U, Zong X-G. Antimuscarinic action of quinidine on the heart? A study in myocardial preparations from cat hearts. Br J Pharmacol 1984;81:103–11.
22. Mirro MJ, Manalan AS, Bailey JC, Watanabe AM. Anticholinergic effects of disopyramide and quinidine on guinea pig myocardium. Mediation by direct muscarinic blockade. Circ Res 1980;47:855–65.
23. Nakajima T, Kurachi Y, Ito H, Takikawa R, Sugimoto T. Anti-cholinergic effects of quinidine, disopyramide, and procainamide in isolated atrial myocytes: mediation by different molecular mechanisms. Circ Res 1989;64:297–303.

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