Vol. 127, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1985 Pages 326-332

INTERACTIONS OF QUINIDINE AND LIDOCAINE WITH RAT BRAIN AND HEART MUSCARINIC RECEPTORS

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Received December 18, 1984

We have studied the effect of quinidine and lidocaine on binding to rat brain and cardiac muscarinic receptors. Both drugs had a higher affinity to brain stem and cardiac receptors, as compared with cerebral cortex, coinciding with the distribution of high-affinity agonist binding sites in the above tissues. The effects of the drugs on muscarinic antagonist and agonist binding did not fit simple competition to one receptor site, suggesting either preferential binding to high affinity agonist binding sites, or allosteric interactions. Batrachotoxin, which opens voltage sensitive sodium channels, had an opposite effect on agonist binding The possibility of allosteric interactions between the muscarinic receptors and a site analogous to the sodium channel is discussed.

A variety of antiarrhythmic drugs possess antimuscarinic activity and can block muscarinic antagonist binding to heart or brain muscarinic receptors. Among these drugs are quinidine (1, 2), lidocaine (3), procainamide (1, 3), disopyramide (1), amiodarone and bretylium (4, 5). These drugs have a relatively low potency as compared with that of classical muscarinic antagonists such as atropine and acopolamine, and may affect other membrane functions, at concentrations where they exert antimuscarinic activity. Thus lidocaine, quinidine, procainamide (6) and amiodarone(7) can act as blockers of voltage sensitive sodium channels. Local anesthetics (8) or amiodarone (9) can also interfere with α and β adrenergic receptor functions. In spite of this low specificity, or possibly because of it, some of the antiarrhythmic drugs show and apparent selectivity in their effect on binding to the muscarinic receptors. This is manifested by higher affinity of amiodarone (4) and bretylium (5) towards cardiac receptors as compared with their affinity towards cerebral cortex receptors.

In the present work we have examined whether the antiarrhythmic drugs quinidine and lidocaine also show apparent selectivity towards cardiac muscari-

0006-291X/85 $1.50

nic receptors as do amiodarone and bretylium. The close association between the antiarrhythmic activity of quinidine and lidocaine and the blockade of voltage sensitive sodium channels (6), prompted us to study the effects of the neurotoxin batrachotoxin (BTX) on muscarinic receptors. The latter is an activator of the sodium channels (10) and its activity and binding are blocked completely by lidocaine and quinidine (11, 12).

MATERIALS AND METHODS

Full details on the preparation of [^{3}H]-4NMPB (69.7 Ci/mmole) and on binding assays at 25°C using the filtration method have been given elsewhere (13). Tissue homogenates were prepared from rat brain stem, cerebral cortex, atrium and ventricle as described (4). Binding in the presence of 50 μM atropine was considered as non-specific binding. Binding of agonists as well as of quinidine sulfate (Teva, Jerusalem, Israel) and lidocaine hydrochloride (Sigma, St. Louis, USA) was inferred from their ability to inhibit specific binding of [^{3}H]-4NMPB.

Batrachotoxin was kindly supplied by Dr. J. Daly, Laboratory of Bioorganic Chemistry, NIADDK, National Institute of Health, Bethesda, MD. Tissue homogenates were preincubated with the toxin at 36°C for 40 min in Krebs solution and then used for binding assays at 25°C. All binding assays were performed in triplicate samples. The competition curves were analyzed by a nonlinear least-square curve fitting procedure using a model for two binding sites (14). Theoretical competition curves were fitted to the experimental data points using the nonlinear least square regression computer program BMDPAR (November 1978 revision), developed at the Health Science Computing Facility (University of California, Los Angeles, CA), as described in previous reports (14).

RESULTS AND DISCUSSION

The effects of quinidine and lidocaine on muscarinic receptors present in brain and cardiac tissues were examined by means of competition binding experiments, using the specific muscarinic antagonist [^{3}H]-4NMPB. Typical results for concentration dependent inhibition of [^{3}H]-4NMPB binding by quinidine and lidocaine are shown in Fig. 1. In agreement with previous results (1-3), both drugs inhibit muscarinic antagonist binding, quinidine being a more potent inhibitor than lidocaine (IC_{50} values for quinidine were 4-40 μM and for lidocaine 160-800 μM). For either drug, the IC_{50} values recorded in brain stem, atria and ventricle preparations were 4-10 times lower than those recorded in the cerebral cortex preparation (see Fig. 1). Since the binding of [^{3}H]-4NMPB in these preparations is to an apparent homogeneous population of sites (13, 15), the results suggest that quinidine and lidocaine are more potent inhibitors of muscarinic receptors

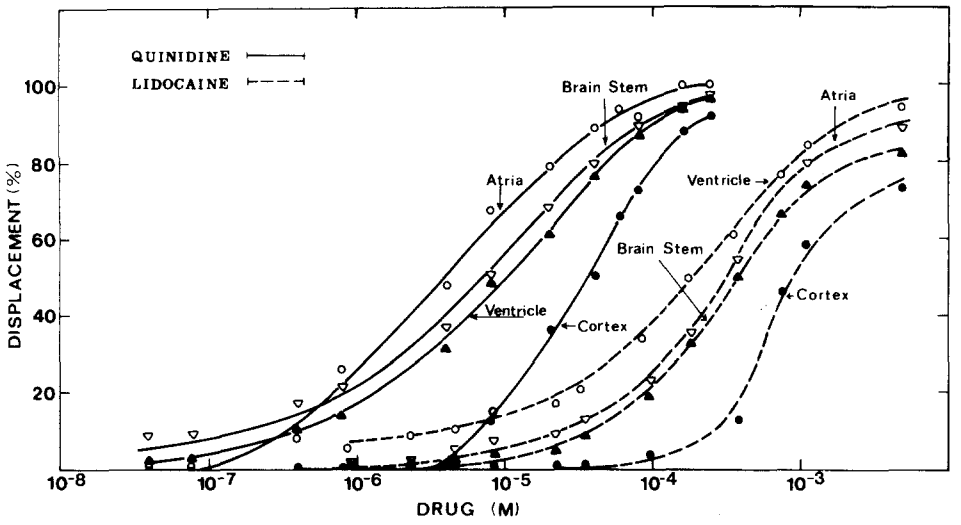

FIGURE 1. Inhibition of [^{3}H]-4NMPB binding to muscarinic receptors of various tissues by quinidine and lidocaine. Binding was determined as described in Methods and in the presence of 2.0 nM [^{3}H]-4NMPB. The IC_{50} values (3 experiments) for quinidine are (μM): cortex - 40±0.5, brain stem - 8±0.8, atrium - 4±0.8, and ventricle - 8±0.6. The IC_{50} values for lidocaine (n=3) are (μM): cortex - 800±50, brain stem - 460±40, atrium - 300±40 and ventricel - 160±20.

in brain stem and cardiac tissues than in the cerebral cortex. Such selectivity could represent binding of quinidine and lidocaine to the high affinity muscarinic agonist binding sites, since these are present at high levels in the heart and the brain stem (2, 15). Alternatively, the drugs could interact in the brain stem and cardiac tissues with a site distinct from the muscarinic recognition site, thereby gaining higher potency in displacing [^{3}H]-4NMPB from the muscarinic receptors.

In both cases (preferential binding to one class of sites, or allosteric interactions) the binding isotherms of the labeled ligand [^{3}H]-4NMPB in the presence of a constant concentration of the inhibitor are expected not only to shift to the right, but also to alter their shape (16). On the other hand, a simple competition on a single site would yield only a shift to the right. A change in the binding pattern of [^{3}H]-4NMPB in the presence of quinidine or lidocaine (Fig. 2) was indeed observed in the atria and brain stem, but not in the cortex. Thus, in both atria and brain stem, addition of the inhibitors induced a perturbation in the system, resulting in apparent heterogeneity of [^{3}H]-4NMPB binding; one fraction of the binding sites binds [^{3}H]-4NMPB with a

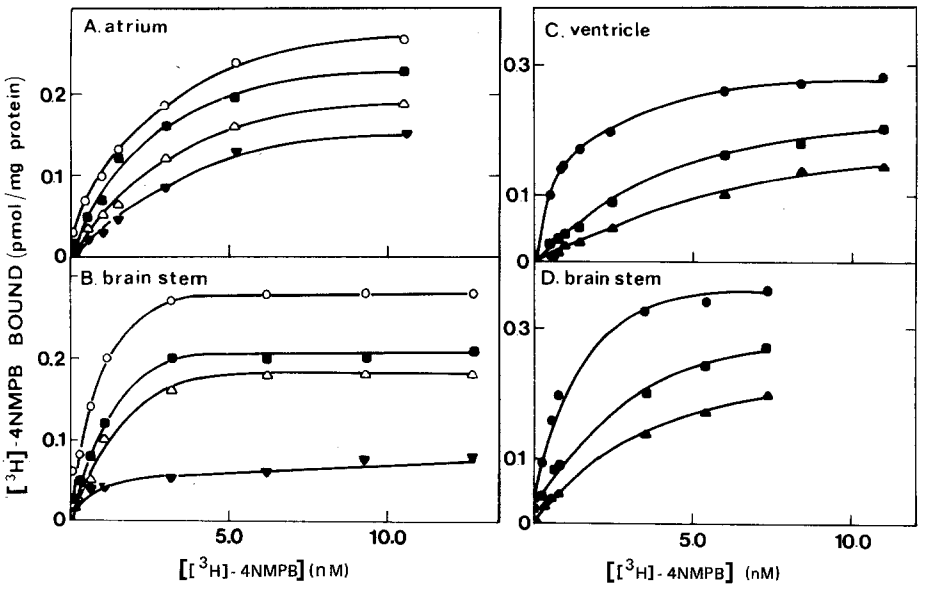

FIGURE 2. Binding curves of [^{3}H]-4NMPB in the absence and in the presence of quinidine and lidocaine. Data represent specific binding of [^{3}H]-4NMPB as a function of its concentration.
A and B: Binding in the absence (o) and in the presence of 0.8 (■), 4 (△) and 20(▼) μM quinidine.
C and D: Binding in the absence (●) and in the presence of 89(■) and 660 (▲) μM lidocaine.

reduced apparent affinity, as reflected by the rightward shift of the binding curve. The second fraction of sites, which is more strongly inhibited, does not bind the labeled antagonist at the concentration range of 0.1-1.2 nM, as reflected by the apparent reduction in the maximal binding capacity. In separate control experiments we have verified that this effect occurs only in the presence of the inhibitors. Preincubation of membranes with quinidine or lidocaine, followed by three washes with a ligand-free buffer, resulted in a complete recovery of [^{3}H]-4NMPB binding sites, ruling out the possibility of irreversible inhibition. These results also indicate that higher concentrations of [^{3}H]-4NMPB would overcome the inhibition by quinidine or lidocaine. However, high non-specific binding at concentrations higher than 20 nM [^{3}H]-4NMPB precludes reliable determinations at these higher [^{3}H]-4NMPB concentrations.

In order to verify the selectivity of lidocaine and quinidine to tissues enriched with high affinity agonist binding sites, we examined the effects of the inhibitors in these tissues on agonist binding to the muscarinic sites. The

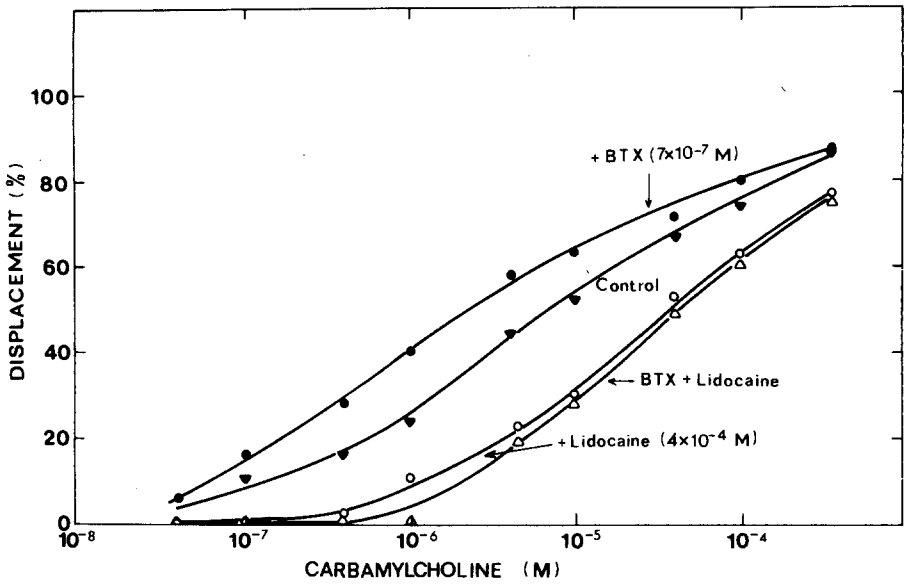

FIGURE 3. The effect of lidocaine and BTX on binding of cabamylcholine to brain stem muscarinic receptors. Binding of [^{3}H]-4NMPB (2 nM) was measured in the presence of various concentrations of carbamylcholine in the absence (control) and in the presence of the indicated concentrations of the drugs. Data presented are corrected for the inhibitory effect of $4x10^{-4}$ M lidocaine on [^{3}H]-4NMPB binding.

displacement of [^{3}H]-4NMPB by agonists was measured in the presence and absence of the inhibitors (Fig. 3). If the inhibitors were to bind non-selectively to sites with low or high affinity towards agonists, as does [^{3}H]-4NMPB, they would be expected to yield only a rightward shift in the agonist displacement curves. However, interaction with a specific class of sites (e.g. high-affinity sites) or allosteric interactions are expected to lead to a change in the ratio between the dissociation constants of agonists to the low and high affinity sites (K_L/K_H) (16). The fraction of high affinity agonist binding sites (α) may also change; however, changes in this parameter are considerably less sensitive experimentally. The results with the brain preparation (Fig. 3, Table I) clearly support the notion of a preferential effect (either directly or through an allosteric site) on the high affinity agonist binding sites. Similar results were observed in the atria and ventricular preparations. Thus, the K_L/K_H ratio changes by a factor of 4-6 in the case of lidocaine ($2x10^{-4}$ M), and by a factor of $\sim$ 1.5 in the case of quinidine (6 μM) using the agonists oxotremorine and carbamylcholine (Table I). Our findings are in line with a recent report (17) on the action of quinidine on rat

TABLE I: The effect of lidocaine and quinidine on agonist binding parameters as determined in brain stem membranes

	α (%)	K_H (μM)	K_L (μM)	K_L/K_H
Carbamylcholine				
No drug	56 ± 3.5	0.2 ± 0.03	42.5 ± 0.9	213
+ 6 μM quinidine	54 ± 2.7	0.4 ± 0.02	48.5 ± 0.9	120
+ 200 μM lidocaine	52 ± 4.2	2.0 ± 0.04	78.0 ± 1.2	39
Oxotremorine				
No drug	55 ± 5	0.017±0.001	3.8 ± 0.25	223
+ 6 μM quinidine	59 ± 8	0.1 ±0.01	9.0 ± 0.1	90
+ 200 μM lidocaine	56 ± 4	0.049±0.08	2.86± 0.4	58.4

Binding parameters were derived from competition binding experiments as shown in Fig. 3, according to a two site model for agonis binding (see Methods). Mean values ± S.D. for the proportion of high affinity agonist binding sites (α) and the dissociation constants (K_H and K_L) of the agonists from the high and the low affinity binding sites respectively are given. Data are of 3 separate experiments.

heart muscarinic receptors, which presented kinetic evidence for allosteric interactions and with a report on the action of several local anesthetics on β-adrenergic receptors in frog erythrocyte membranes (18). The fact that the alterations in the K_L/K_H ratios are in the direction of minimizing the differences between the high and low affinity agonist binding sites again supports the suggestion of a preferential interaction with the high affinity agonist binding site.

As discussed above, the data can be explained either by direct or allosteric interactions of quinidine and lidocaine with the muscarinic receptors. These drugs are known as blockers of the voltage-sensitive sodium channels (6, 11, 12). They could therefore affect the muscarinic receptors by direct interaction with the sodium channels, or with a peripheral site on the muscarinic receptor itself. Allosteric interactions between the voltage-sensitive sodium channels and the muscarinic receptors are an attractive hypothesis, especially in view of the fact that BTX, a neurotoxin which induces opening of the voltage-sensitive sodium channels (10), has an effect opposite to that of lidocaine or quinidine on the muscarinic receptor (Fig. 3). Moreover, the effect of BTX is reversed by lidocaine (Fig. 3). Of course, the existence of a site on the muscarinic receptors similar to that which exists in the sodium channel cannot be ruled out. It should be noted that the effect of BTX on the muscarinic receptors is clearly allosteric, since the neurotoxin itself did not affect [^{3}H]-4NMPB binding.

In conclusion, the findings reported in the present communication support the existence of selective interactions of lidocaine and quinidine with muscarinic receptors which display high affinity towards agonists. Direct interaction of these drugs with preference to high-affinity agonist binding sites is a possible mechanism for this effect. However, several lines of evidence suggest the possibility of an allosteric interaction between these drugs and the muscarinic sites, with a site analogous to the sodium channel being an attractive candidate.

REFERENCES

1. Mirro, M.J., Malan, A.S., Bailey, J.C., and Watanabe, A.M. (1980) Circ. Res. 47, 855-865.
2. Fields, J.Z., Toeske, W.R., Morkin, E., and Yamamura, H.I. (1978) J. Biol. Chem. 253, 3251-3258.
3. Aronstam, R.S., Elderfrawi, A.T., and Elderfrawi, M.E. (1980) Biochem. Pharmacol. 29, 1311-1314.
4. Cohen-Armon, M., Schreiber, G., and Sokolovsky, M. (1984) J. Cardiovas. Pharmacol. in press.
5. Schreiber, G., Friedman, M., and Sokolovsky, M. (1984) Circ. Res. 55, 0000.
6. Hondeghem, L.M., and Katzung, B.G. (1977) Biochim. Biophys. Acta 472, 373-398.
7. Mason, J.W., Hodeghem, L.M., and Katzung, B.G. (1983) Pflugers Arch. 396, 79-81.
8. Feinstein, M.B., and Paimre, M. (1967) Nature 214, 151-153.
9. Polster, P., and Broekhuysen, J. (1976) Biochem. Pharmacol. 25, 131-134.
10. Catterall, W.A. (1977) J. Biol. Chem. 252, 8669-8676.
11. Catterall, W.A. (1981) Mol. Pharmacol. 20, 356-362.
12. Postma, S.W., and Catterall, W.A. (1983) Mol. Pharmacol. 25, 219-227.
13. Kloog, Y., Egozi, Y., and Sokolovsky, M. (179) Mol. Pharmacol. 15, 545-558.
14. Avissar, S., Egozi, Y., and Sokolovsky, M. (1981) Neuroendocrinology 32, 303-308.
15. Sokolovsky, M., Gurwitz, D., and Kloog, Y. (1983) in Advances in enzymology and related areas of molecular biology. A. Meister, ed. 55, 137-196, John Wiley and Sons Inc.
16. Henis, Y.I., and Sokolovsky, M. (1983) Mol. Pharmacol. 24, 357-365.
17. Waelbroeck, M., De Neef, P., Robberecht, P., and Christophe, J. (1984) Life Sci. 35, 1069-1076.
18. Voeikov, V.L., and Lefkowitz, R.J. (1980) Biochim. Biophys. Acta 629, 266-281.