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REVERSIBLE CONVERSION BETWEEN AFFINITY STATES FOR AGONISTS OF THE MUSCARINIC ACETYLCHOLINE RECEPTOR FROM RAT BRAIN

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Studies of the binding of radiolabeled ligands to muscarinic acetylcholine receptors of mammalian brain and smooth muscle suggest the presence of multiple receptor populations which differ in their affinities for receptor agonists, but not antagonists (1,2). An increase in the affinity of muscarinic receptors for agonists was demonstrated subsequent to alkylation of, or heavy metal interaction with, sulfhydryl groups in rat neural membranes (3,4). It led to the suggestion that this change may reflect a conversion of receptors from a state of low affinity for agonists to one of high affinity. However, disulfide reducing reagents and membrane oxidizing ones did not significantly alter receptor binding properties (4). In this report, we are able to demonstrate reversible conversion of agonist binding affinities by disulfide reducing reagents as well as oxidizing ones. Reduction results in a decrease in overall agonist affinity, while oxidation increases agonist affinity.

MATERIALS AND METHODS

A neural membrane preparation was obtained from the brains of 150-250 g Wistar rats as previously described (5), except the buffer used was 20 mM HEPES, pH 8.4. Membranes (1 mg protein/ml) were exposed to oxidizing and reducing reagents for 30 min at 37°; then the reagents were removed by centrifugation at 30,000 g for 20 min. The pellets were rinsed with distilled water and rehomogenized in 50 mM sodium phosphate buffer, pH 7.4, prior to the measurement of binding. Muscarinic receptor binding was measured with [^{3}H]3-quinuclidinyl benzilate (QNB) (29.4 Ci/mmole, New England Nuclear) by filtration on Whatman GF/B filters as detailed elsewhere (4,6). Binding of QNB that was inhibited by 10^{-6} M scopolamine was considered to be specific binding. The association of carbamylcholine with the muscarinic receptor was inferred from its ability to inhibit the specific binding of radiolabeled QNB (2,4).

RESULTS

There is no change in QNB binding subsequent to treatment with 5 mM dithiothreitol (DTT), 5 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), 1 mM N-ethylmaleimide (NEM), 5 mM 2-mercaptoethanol (ME), 5 mM potassium ferricyanide, or 5 mM potassium ferricyanide followed by 5 mM DTT treatment. The dissociation constants obtained (x 10^{-10} M) are 1.6 ± 0.1; 1.5 ± 0.2; 1.3 ± 0.2; 1.5 ± 0.2; 1.4 ± 0.1; 1.3 ± 0.2, respectively, while that of the control is 1.5 ± 0.1.

The effects of disulfide bond reduction and sulfhydryl alkylation on carbamylcholine binding to the muscarinic acetylcholine receptor are depicted in Fig. 1. DTT (5 mM) causes a shift of the binding curve to lower affinity, while 5 mM NEM has the opposite effect, shifting the binding curve to higher affinity. Treatment with NEM subsequent to DTT reduction results in a binding curve coincident with that observed with NEM treatment alone (not shown). A similar reversal of the DTT-induced decrease in agonist affinity is effected by subsequent treatment with 5 mM DTNB. Scatchard plots of the carbamylcholine binding data, derived from its inhibition of QNB binding (Fig. 2), indicate changes in the proportion of receptors demonstrating the highest affinity agonist binding.

Dose-response curves demonstrating the effects of organic sulfhydryls on muscarinic binding are shown in Fig. 3. DTT inhibits 10^{-10} M QNB binding at concentrations above 3 mM, while inhibition by ME is seen only at concentrations above 10 mM. Both reagents significantly decrease the ability of carbamylcholine to inhibit 10^{-10} M QNB binding. Both DTT and ME decrease agonist binding at concentrations greatly below those necessary to inhibit QNB binding, causing half of their maximal effect on carbamylcholine binding at 0.2 mM and 0.5 mM respectively.

The effects of consecutive oxidation and reduction of neural membranes on carbamylcholine

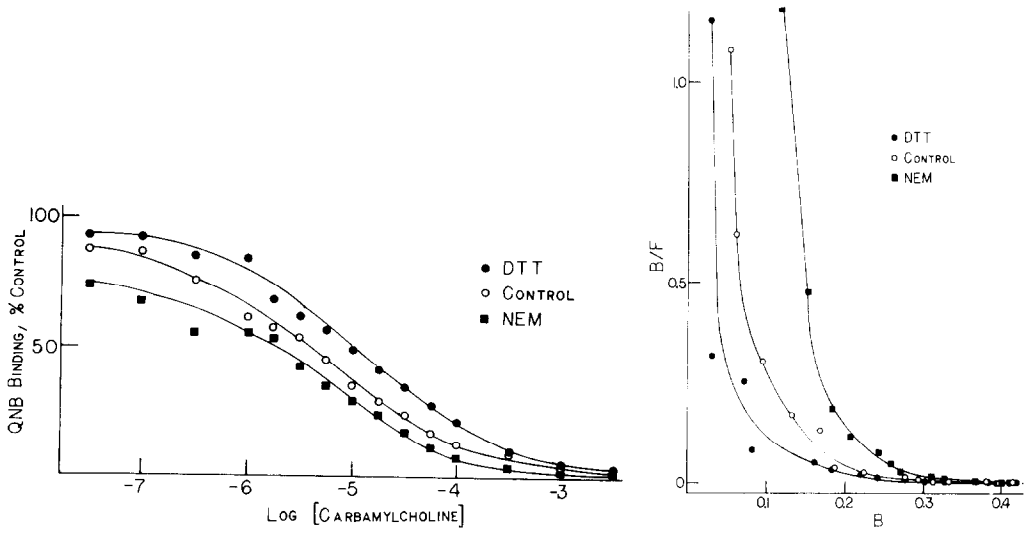

Fig. 1 (left). Inhibition of 10^{-10} M QNB by carbamylcholine in untreated (o), DTT-treated (•) and NEM-treated (■) membranes from rat brain.
Fig. 2 (right). Scatchard plots of carbamylcholine binding to control (o), DTT-treated (•) and NEM-treated (■) membranes. B, amount of QNB bound in pmoles/mg protein; F, concentration of QNB in nM.

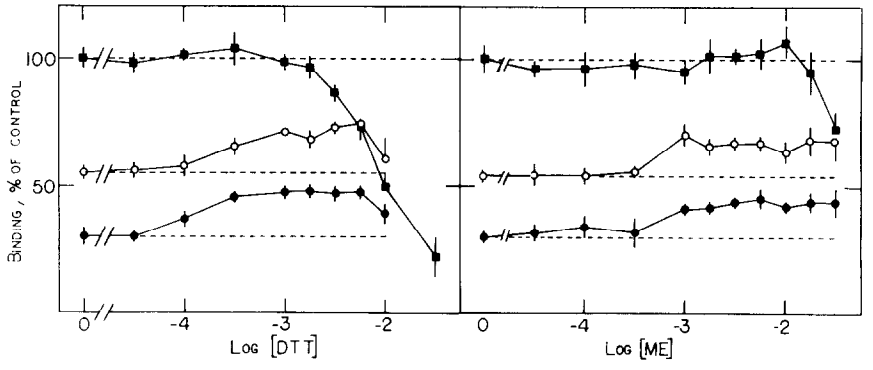

Fig. 3. Influence of DTT (left) and ME (right) on specific QNB (10^{-10} M) binding in the absence (■) and presence of 10^{-5} M (o) and 10^{-4} M (•) carbamylcholine.

binding are depicted in Fig. 4. Treatment with 5 mM of the oxidizing reagents, potassium ferricyanide and DTNB, for 30 min at 37° increases the ability of carbamylcholine to inhibit QNB binding. The magnitude of this effect is approximately the same as that seen with heavy metals such as 10^{-4} M copper, although alkylation of membrane sulfhydryls by NEM has an even greater effect (data not shown). Treatment with DTT (or ME, data not shown) subsequent to membrane oxidation with ferricyanide or DTNB decreases the ability of carbamylcholine to inhibit QNB binding. This decrease in carbamylcholine affinity is the same in control membranes as in membranes that have been previously oxidized with DTNB or ferricyanide.

DISCUSSION

Oxidation and reduction of sulfhydryl-disulfide groups on the receptor protein decreases and increases, respectively, the affinity of neural muscarinic receptors for an agonist (carbamylcholine) without affecting the binding of the receptor antagonist QNB. These changes are reversible, and may represent a mechanism for control of receptor function. The oxidation state of a disulfide group in the vicinity of the binding site of acetylcholine receptors from fish electric organs has been reported to influence nicotinic electrophysiological responses

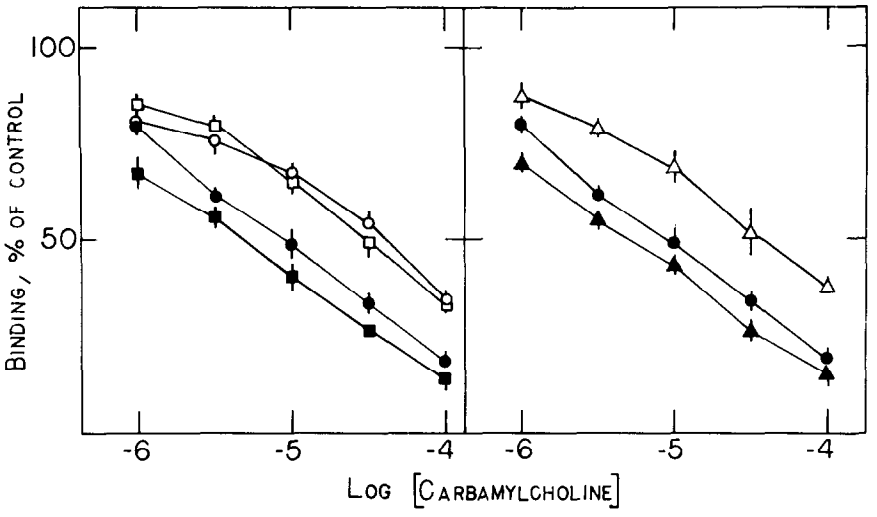

Fig. 4. Specific QNB (10^{-10} M) binding to membranes treated with oxidizing and reducing reagents in the presence of various concentrations of carbamylcholine. Untreated (●); 5 mM DTT (o); 5 mM potassium ferricyanide (■); 5 mM potassium ferricyanide followed by 5 mM DTT (□); 5 mM DTNB (▲); 5 mM DTNB followed by 5 mM DTT (Δ).

(7) and binding properties (8). Thus, a similar mechanism may control receptor function at both muscarinic and nicotinic cholinergic synapses.

Although increases in the affinity of muscarinic agonists after sulfhydryl alkylation or interaction with heavy metals were previously observed, no changes in agonist binding were detected after disulfide bond reduction by organic mercurials or treatment of membranes with oxidizing reagents (3,4). The reason for our current success probably arises from the more favorable reaction conditions used in the present experiments. Specifically, the pH was raised from 7.4 to 8.4, a condition which favors disulfide reduction by DTT and ME, the concentration of tissue being treated was reduced from 2 mg membrane protein/ml to 1 mg/ml, higher concentrations of reducing reagents were used and the incubation time was increased from 20 min at 35° to 30 min at 37°.

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REFERENCES

1. N.J.M. Birdsall and E.C. Hulme, J. Neurochem. 27, 7 (1976).
2. N.J.M. Birdsall, A.S.V. Burgen and E.C. Hulme, Mol. Pharmacol. 14, 723 (1978).
3. R.S. Aronstam, W. Hoss and L.G. Abood, Eur. J. Pharmacol. 46, 279 (1977).
4. R.S. Aronstam, L.G. Abood and W. Hoss, Mol. Pharmacol. 14, 575 (1978).
5. R.S. Aronstam, L.G. Abood and J. Baumgold, Biochem. Pharmacol. 26, 1689 (1977).
6. H.I. Yamamura and S.H. Snyder, Proc. Nat. Acad. Sci. USA 71, 1725 (1974).
7. A. Karlin, J. Gen. Physiol. 54, 245s (1969).
8. M.E. Eldefrawi and A.T. Eldefrawi, Proc. Nat. Acad. Sci. USA 69, 1776 (1972).