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SHORT COMMUNICATION

Choline: Binding Studies Provide Some Evidence for a Weak, Direct Agonist Action in Brain

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SUMMARY

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The potential ability of choline to act as a muscarinic agonist in brain was examined. Binding curves for muscarinic agonists are flattened and their Hill coefficients are lower than unity, while antagonists exhibit mass action curves with Hill coefficients equal to unity. Taking into account these findings, we have studied the behavior of choline on brain muscarinic receptors as measured by competition studies with ^{3}H-quinuclidynyl benzilate. Choline displaces the ^{3}H-muscarinic antagonist in cortical and hippocampal homogenates with an IC_{50} value 100 times greater than that of acetylcholine. In addition, its displacement curve is flattened with a Hill coefficient of 0.77. We conclude that choline might act as a direct agonist on brain muscarinic receptors but probably only at high concentrations. The agonistic action of choline suggests an "inactivation" role for high affinity choline uptake as well as a role in supplying precursor.

INTRODUCTION

Several recent studies suggest that choline has a cholinomimetic action in the brain. Choline administration has a beneficial therapeutic effect on memory and in some illnesses involving a central cholinergic deficiency (1-4). Possible mechanisms for this cholinomimetic action include increasing releasable acetylcholine levels and acting directly as an agonist. In this study we test whether choline would act as a cholinergic muscarinic agonist.

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The ability of choline to act as a cholinomimetic is well known in studies of peripheral tissues. Bovet and Bovet-Nitti (5) have reviewed the cholinomimetic activity of several acetylcholine-like compounds in a variety of physiological preparations. Choline was reported to be quite variable in its ability to function as a cholinomimetic, that is, it was 1/200 to 1/100,000 times less potent than acetylcholine (5), depending on the preparation studied. However, no data are available on the cholinomimetic activity of choline in the central nervous system. Recent studies on cholinergic muscarinic receptors suggest that binding studies can be used as a potential indicator for agonistic activity (6-14).

Extensive studies (6-14) on the brain muscarinic receptor have revealed the complexity of the binding of agonists as com-

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pared to the relatively simple binding of antagonists. The complexity of the agonist binding seems to be due to a heterogeneity or multiplicity of agonist binding sites. This is reflected in the flattening of saturation curves with ^{3}H-agonist. Because of this flattening, one observes Hill coefficients of values lower than one for agonists, while antagonists feature a Hill coefficient of about 1.0 (6–14). While the reason for the heterogeneity of agonists versus the homogeneity of antagonist binding sites is unknown at this time, one can use this empirical finding to test whether a given compound has putative agonist or antagonist properties. An advantage of this approach is that one can measure direct potency at the receptor apart from other pre- or postsynaptic effects.

Thus, we have investigated the possibility that choline is a directly acting agonist at muscarinic receptors by testing whether choline displaces ^{3}H-QNB (quinuclidynyl benzilate) in a manner similar to that of other cholinergic muscarinic agonists, that is, over a wide concentration range with a resultant Hill coefficient of less than unity.

Male Sprague-Dawley rats were sacrificed by decapitation and the brains rapidly removed from the skull. Cerebral cortex or hippocampi were dissected and homogenized in Tris buffer (pH 7.7, 50 mM) at a dilution of 1:100. ^{3}H-QNB and displacing drugs were added to a further 10-fold dilution of the homogenate (2 ml) and incubated for one hour at 25°. The concentration of ^{3}H-QNB (29 Ci/mM, New England Nuclear Corp., Boston, Mass.) was 0.2 nM and the concentration on cholinergic muscarinic receptors was 75 pM. Blank values were obtained by adding atropine or scopolamine (10^{-6} M) to the sample. The incubations were terminated by rapid filtration over Whatman GF/B filters (15). In each of several experiments, complete displacement curves were constructed for at least one agonist, one antagonist, and choline.

The competition curves (Fig. 1A) for scopolamine and atropine showed typically steep curves over about two orders of magnitude. Similar steep displacement curves have been reported previously (6–15). On the other hand, the agonists, acetylcholine and carbachol, displaced over a much wider range of concentrations yielding flattened displacement curves. Again, this is similar to what has been reported (6–14). The differences between the agonist and antagonist displacement curves is reflected in the values of the Hill coefficients. The Hill coefficients for agonists were 0.5 and 0.62 (Fig. 1B). Choline behaved more like acetylcholine and carbachol rather than like scopolamine or atropine in that it displaced over a wide range of concentrations and had a Hill coefficient less than one (Fig. 1B). These data suggest that choline is a directly acting muscarinic agonist in brain, but with a potency of about 100 fold less than that of acetylcholine (Table 1). Similar results were found using hippocampal homogenates. It is unlikely that the displacing action of choline is due to the formation of small quantities of acetylcholine, since choline had the same weak potency whether an acetylcholinesterase inhibitor was present or not (data not shown). In preliminary experiments, 2-dimethylaminoethanol (Deanol), a drug used clinically for cholinergic deficiencies (16, 17), was an even weaker displacer of ^{3}H-QNB (5–10 fold less than choline, data not shown), and its Hill coefficient was not determined.

These binding data suggesting that choline is a directly acting agonist, although indirect and not conclusive, are consistent with recent electrophysiologic studies (18). Choline was found to have an excitatory action after iontophoretic application on cat cerebral cortical cells shown to be excited by acetylcholine. The excitatory action of choline was blocked by atropine suggesting a muscarinic rather than nicotinic effect. Simultaneous administration of hemicholinium-3 did not block the choline excitation suggesting that it is not acting by conversion to acetylcholine. In support of this, physostigmine potentiates the action of acetylcholine but not choline. While the ratio of potency between acetylcholine and choline was not rigorously determined in the electrophysiologic studies, it was judged to be greater than 8 which is compatible with our measured ratio of 100.

While one cannot rule it out completely,

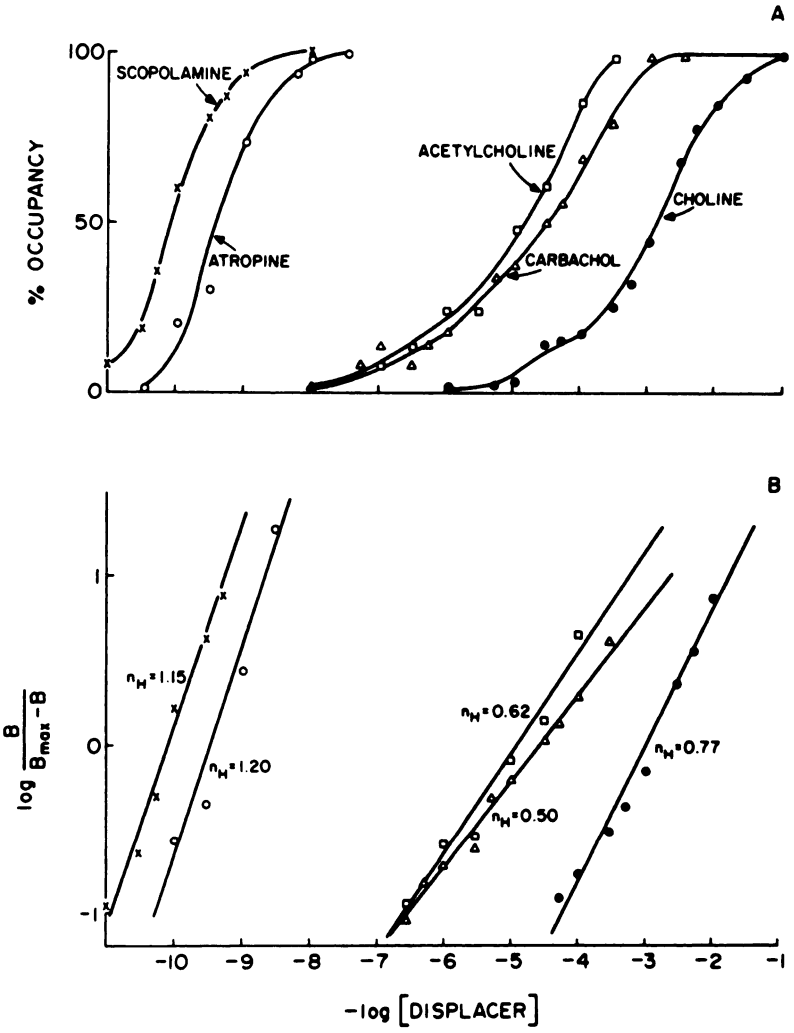

FIG. 1. *A. Inhibition of the binding of ^{3}H-QNB by scopolamine, atropine, acetylcholine, carbachol, and choline*

Except for acetylcholine (hippocampus), displacement curves were measured utilizing cerebral cortical homogenates. Experiments with acetylcholine required the additional presence of 5 μm eserine. See text for additional details. The data points represent the mean of 2–4 different experiments with triplicate determinations in each experiment. The standard errors were less than 10% of the mean.

B. Hill plots of the inhibition curves

it seems to us that the weak affinity of choline for receptors suggests that the cholinomimetic action of choline in dietary studies is not due to a direct muscarinic action since choline levels in brain do not reach mM concentrations. But, one must consider that the agonist binding data indicate the presence of both high and low affinity agonist sites (6–10, 12), and IC_{50} values for the whole curve will not accurately reflect agonist affinities at high affinity sites. Thus, choline would have substantial occupancy of high affinity sites at much lower concentrations than the mM IC_{50} value measured on the whole curve. On the other hand, it has been argued that the low affinity site rather than the high affinity one is the pharmacologically active one (9, 14). Thus, further studies will be needed to resolve the mechanism of action of choline

TABLE 1

IC_{50} values of the cholinergic drugs

Experiments were performed in triplicate and repeated 2–4 times. Results are the mean which differed from individual values by less than 15%. See text and Fig. 1 for further details. K_i values are calculated by multiplying IC_{50} values by one-third.

Drug	IC_{50}[a]
	(M)
Scopolamine	7×10^{-11}
Atropine	5×10^{-10}
Acetylcholine[b]	2×10^{-5}
Carbachol	3×10^{-5}
Choline	2×10^{-3}

[a] Determined graphically by log-probit analysis.
[b] Determined in the presence of 5 μM eserine.

in dietary studies. It has been proposed that its mechanism of action involves elevating acetylcholine levels (19–21) via a choline carrier (22) but not all workers find increased acetylcholine levels after choline administration (23, 24).

The observation that choline may be a directly acting agonist raises another interesting issue. Since acetylcholine concentrations in the synaptic cleft could reach as high as 1 mM after a single impulse (25–27), the choline concentrations could also reach very high levels following hydrolysis by acetylcholinesterase. If choline in such high concentrations were not removed from the cleft, then it could continually activate the receptor by a direct agonist action suggested here and elsewhere (5, 18). Thus, another important role for high affinity choline transport would be not only to recycle and gather precursor as has been previously assumed (22), but also to remove a potentially receptor-active metabolite from the synaptic cleft.

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