not produce any significant amounts of collagenase or other neutral proteases in culture. Nevertheless, when activated with LPS, they release a factor in the medium which induces the chondrocytes to synthesize proteases. The chondrocytes respond to this factor for 48–72 hr, after each addition of MCM [10]. The synovial cells obtained from normal or inflamed rabbit joints synthesize proteases in culture, after activation with latex particles or *Mycobacterium butyricum*. Nevertheless, these cells do not release any activators that will stimulate chondrocytic protease synthesis (K. Phadke *et al.*, unpublished data).

The osteoarthritic condition is associated frequently with a low grade inflammation and influx of some mononuclear cells into the joint cavity. The infiltrated macrophages, although few in number, may be activated by degradative products of the damaged tissue, local immune complexes, activators produced by the lymphocytes, etc., and may, in turn, stimulate the chondrocytes to produce proteolytic enzymes. As mentioned earlier, the synovium plays a minimum role in the process of cartilage destruction. The intrinsic enzymes, therefore, may be primarily responsible for the slow, but progressive cartilage degradation. The interference with the release of the majority of the enzymes may be an important factor in the treatment of osteoarthritis.

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Effects of deanol, choline and its metabolites on binding of [^{3}H]quinuclidinyl benzilate to rat brain membranes

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Choline and its analog, dimethylaminoethanol (deanol), elicit a variety of pharmacologic effects in both humans and animals which can be attributed to the activation of cholinergic neurons in the brain [1, 2]. However, neither compound is as active as acetylcholine (ACh) at cholinergic receptors in the peripheral nervous system, their potencies being 1000–100,000 times less than that of ACh [3–6]. Nevertheless, the possibility exists that these compounds, or one of their metabolites, stimulate cholinergic receptors in the brain. To test this hypothesis, we have compared deanol, choline and some of the metabolites of choline with cholinergic drugs for their ability to displace radiolabeled quinuclidinyl benzilate (QNB) from muscarinic receptors in rat brain membrane preparations.

Male Sprague–Dawley rats, weighing 120–150 g, were used. Binding of [^{3}H]–QNB to rat brain S_1 (whole brain minus debris and nuclei) fractions was measured by the method of Yamamura and Snyder [7]. Dimethylaminoethanol acetamidobenzoic acid (Deaner) was obtained from Riker Laboratories, Northridge, CA. All other compounds were purchased from the Sigma Chemical Co., St. Louis, MO.

Choline displaced [^{3}H]–QNB from its muscarinic binding site with an IC_{50} of approximately 1800 μM (Table 1). Choline was less potent than the other muscarinic receptor agonists by a factor that ranged from 20 for carbachol to 4000 for oxotremorine. The IC_{50} value for scopolamine, a muscarinic antagonist, was similar to that reported by Yamamura and Snyder [7], and more than 100,000 times as potent as choline.

Choline was more than three times as potent as its analog, dimethylaminoethanol, and was two to more than five times as potent as any of its metabolites, including phosphorylcholine, betaine aldehyde, CDP–choline or glycerylphosphorylcholine.

The finding that choline and deanol displace [^{3}H]–QNB from its muscarinic binding site in brain is consistent with results of previous studies showing that these compounds stimulate cholinergic receptors at high concentrations [3–5]. However, the fact that choline and its metabolites are considerably less potent than cholinomimetic drugs in displacing [^{3}H]–QNB from the mammalian brain muscarinic cholinergic receptors would indicate that the central cholinergic actions of choline cannot be attributed entirely to direct activation of these receptors.

In humans treated with therapeutically active doses of choline, the concentration of the compound in the cerebrospinal fluid is only 3 μM [8]. In studies in which choline has been administered to elicit an increase in the concentration of ACh in brain, the level of choline did not exceed 59 μmoles/kg (65 μM, assuming that 80 per cent of the tissue was aqueous) [9]. The concentration of choline needed to displace half the [^{3}H]–QNB from muscarinic receptors is more than 25 times this amount (Table 1.)

Table 1. Effects of choline analogs and cholinergic drugs on [^{3}H]-QNB binding in rat brain*

Compound	IC_{50} (μM)	
Anticholinergic		
Scopolamine	0.014 ± 0.008	(4)
Cholinergic		
Oxotremorine	0.540, 0.600	(2)
Arecoline	8.60 ± 2.5	(4)
Pilocarpine	4.8 ± 1.7	(3)
Carbachol	89.7 ± 3.2	(3)
Choline analogs		
Choline	1,800 ± 600	(4)
Betaine aldehyde	2,800 ± 800	(3)
Glycerylphosphorylcholine	6,200 − > 10,000	(4)†
Dimethylaminoethanol‡	7,900 ± 3,300	(3)
Cytidinediphosphocholine	>10,000	
Phosphorylcholine	>10,000	

* Results are given as the means ± S.D. with the number of observations in parentheses. Each compound was tested at two to four concentrations in triplicate for each separate IC_{50} determination. [^{3}H]-QNB (sp. act. 29.4 Ci/mmole, New England Nuclear, Boston, MA.) was present at a final concentration of 60 pM. Nonspecific binding was determined using 100 μM oxotremorine.

† Glycerylphosphorylcholine was insoluble at the concentrations used and gave varying IC_{50} values.

‡ The acetamidobenzoic acid salt (Deaner) was used.

Because of the efficiency of the cellular uptake processes for choline in brain, the majority of this choline is likely to be intracellular, and therefore the concentration of choline at the cholinergic receptor would be expected to be far lower than the level determined in whole tissue.

Furthermore, it seems unlikely that a metabolite of choline, formed after the compound is administered, could be responsible for a direct cholinomimetic action, as none of the metabolites tested was as potent as choline in displacing QNB. In addition, the amounts of these metabolites found in the brain when [^{3}H]-choline is administered to animals is quite small [10].

The central cholinergic action of choline would appear, therefore, to be elicited by a mechanism other than that of direct stimulation of muscarinic cholinergic receptors. One such possibility in this regard is that choline might stimulate nicotinic cholinergic receptors, but this seems unlikely since its central effects can be blocked by atropine [11, 12]. Another possibility is that choline acts pre-synaptically to stimulate the synthesis and, presumably, the release of ACh from cholinergic neurons. This latter possibility is consistent with the observations that (1) choline administration causes an increase in the concentration of brain acetylcholine [9], (2) both the choline-induced increase in brain levels of ACh and the central cholinergic effects [11, 13] are blocked by inhibitors of acetylcholine synthesis, and (3) choline stimulates *in vitro* the rate of release of ACh in the heart [14].

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Clofibrate-induced increase in carnitine and acylcarnitine content in rats

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It has been shown that clofibrate administration markedly increases fatty acid oxidation in rat liver [1–5]. A corresponding increase of several enzyme activities connected to the fatty acid oxidation sequence is found [6, 7]. Previously, it has been briefly reported that carnitine content in rat liver is increased after clofibrate administration [3, 8]. Carnitine plays a major role in the transport of activated fatty acyl groups from cytosol to sites of β oxidation in the mitochondria. In general, the changes in liver carnitine appear to correlate with the rate of fatty acid oxidation [9, 10]. As a consequence, a regulatory role for carnitine in fatty acid oxidation has been postulated [9, 11]. In this study the effect of clofibrate on carnitine and its derivatives in different tissues and total body of the rat was investigated. This report provides also a clarification regarding the role of hepatic carnitine in the hypotriglyceridaemic effect of the drug.

Male Wistar rats weighing 200–300 g were used in the experiments. The animals were allowed free access to standard laboratory chow and water. The treatment consisted of daily subcutaneous injections of clofibrate (200 mg/kg body wt) for 14 days. Control rats received saline. Rats were killed by decapitation and blood collected in heparin. The heart, a piece of liver and of skeletal muscle (hind leg)