# Intact human lymphocyte membranes respond to muscarinic receptor stimulation by oxotremorine with marked changes in microviscosity and an increase in cyclic GMP

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The muscarinic agonist oxotremorine produced a linear dose-dependent increase in membrane fluidity of intact and viable human lymphocytes in vitro. This effect proved to be receptor-mediated because preincubation with $10^{-5}$ M atropine shifted the dose-response curve one order of magnitude rightward. Pirenzepine preincubation did not affect membrane fluidity variation. A cGMP increase was also found after oxotremorine treatment. The results are discussed in terms of possible modulation of guanyl cyclase and adenyl cyclase through membrane fluidity variations.

*Oxotremorine* *Cyclic GMP* *Atropine* *Pirenzepine* *Membrane fluidity* *Lymphocyte*

## 1. INTRODUCTION

Membrane fluidity changes consequent to the association of a membrane receptor with agonists or antagonists have been reported for a variety of compounds in a number of tissues [1,2]. These membrane changes were observed concomitantly with the stimulation of certain biochemical reactions within the cell including protein phosphorylation, $Ca^{2+}$ mobilization, methyltransferase induction and extracellular ion influx [3–6]. Peripheral blood lymphocytes respond to muscarinic agonists with increased cGMP levels. Muscarinic agonists like carbamylcholine and acetylcholine alter lymphocyte function causing changes in motility and immunological activity. This effect was stopped by drugs eliciting cAMP synthesis [7]. This fact suggests an involvement of the muscarinic M2 receptor.

The muscarinic cholinergic receptor has been demonstrated to be present on the human lymphocyte surface [8]. The purpose of our work was to determine whether the muscarinic agonist oxotremorine modified membrane fluidity in intact and viable human lymphocytes. Such an alteration could permit modulation of adenyl cyclase and guanyl cyclase activities, since they are membrane-associated enzymes whose activity is strongly dependent on membrane physical parameters. Knowledge of such molecular mechanisms would lend some support to the use of human lymphocytes as a model for studies on the cholinergic muscarinic system.

## 2. MATERIALS AND METHODS

Human lymphocytes were obtained from blood serum of healthy male volunteers by the method of Boyum [9]. Their purity was always more than 90%. Membrane microviscosity was determined according to Shinitzky and Barenholz [10] using 1,3,5-diphenylhexatriene (DPH) as a probe.

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The fluorescence polarization value was determined on an Elscint MV-1 microviscosimeter (Haifa, Israel). Lymphocytes were incubated with $2 \times 10^{-6}$ M DPH in PBS (phosphate-buffered saline) at 37°C for 30 min.

The in vitro changes of lymphocyte microviscosity were determined by adding oxotremorine dissolved in PBS at the appropriate concentration to 3 ml DPH-labelled lymphocytes (0.1–0.25 mg protein/ml) to obtain a drug concentration of $10^{-5}$–$3 \times 10^{-4}$ M. DPH-labelled lymphocyte samples were preincubated with atropine ($10^{-5}$ M) or pirenzepine at the same concentration at 37°C before the addition of oxotremorine.

The membrane-fluidizing effect was measured as follows: the *P* value was determined before drug addition and every 2 min until no further microviscosity change was observed. The data for all the determinations refer to this peak time.

The possible spectral interaction of DPH with the drugs was verified by checking there was no interfering absorbance at the DPH emission wavelength.

Proteins were determined according to Lowry et al. [11]. Lymphocyte cGMP was determined according to Study et al. [12]. Atropine sulfate was obtained from Sigma, oxotremorine sesquifumarate from Aldrich, pirenzepine-HCl from Dr Karl Thomae, Biberach (FRG), carbamylcholine from Janssen Pharmaceuticals and nicotine from Sigma.

## 3. RESULTS

Fig.1 shows the time course of changes in membrane microviscosity after addition of $10^{-4}$ M oxotremorine. The maximum decrease was attained at 20 min, and remained stable for at least another 20 min. This figure also shows for comparison the effect of $10^{-5}$ M atropine alone and prior to incubation with oxotremorine. Neither treatment had any significant effect on membrane microviscosity.

Fig.2 reports the data after addition of oxotremorine at various doses ($10^{-5}$–$3 \times 10^{-4}$ M) to untreated lymphocytes or to cells preincubated with $10^{-5}$ M atropine. The data are expressed as the ratio of the membrane microviscosity 20 min after addition of oxotremorine (i.e. $\eta_D$) and the basal value (i.e. $\eta_0$) vs the logarithm of the drug concentration. The fluidizing effect of ox-

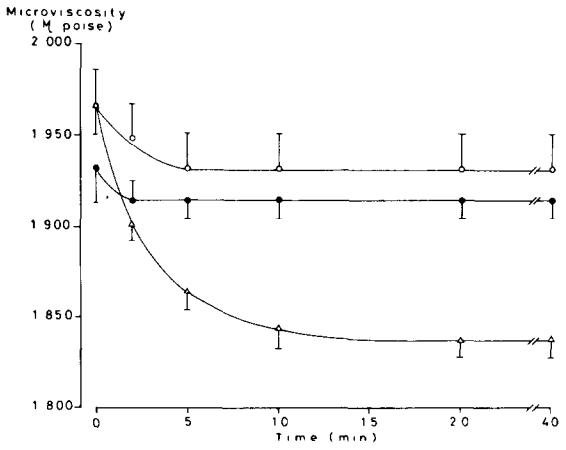


Fig.1. Effects of $10^{-4}$ M oxotremorine (△), $10^{-5}$ M atropine (○) and $10^{-4}$ M oxotremorine after preincubation with $10^{-5}$ M atropine (●). The abscissa shows the time (in min) after the addition of oxotremorine to the cuvette. Atropine was added 15 min before oxotremorine. The ordinate shows the microviscosity (in P).

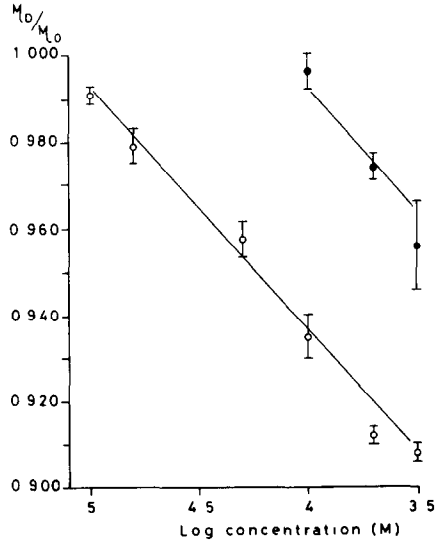


Fig.2. Dose-response curve of oxotremorine-induced fluidity change in intact and viable human lymphocytes (○) and the effect of atropine (●). The abscissa shows the concentration in logarithmic units. The ordinate shows the relative microviscosity measured 20 min (peak time) after addition of oxotremorine. The results are expressed as the mean ± SE of 4 separate experiments. In the experiments with atropine, $10^{-5}$ M atropine was preincubated with lymphocytes for 15 min at 37°C before oxotremorine addition.

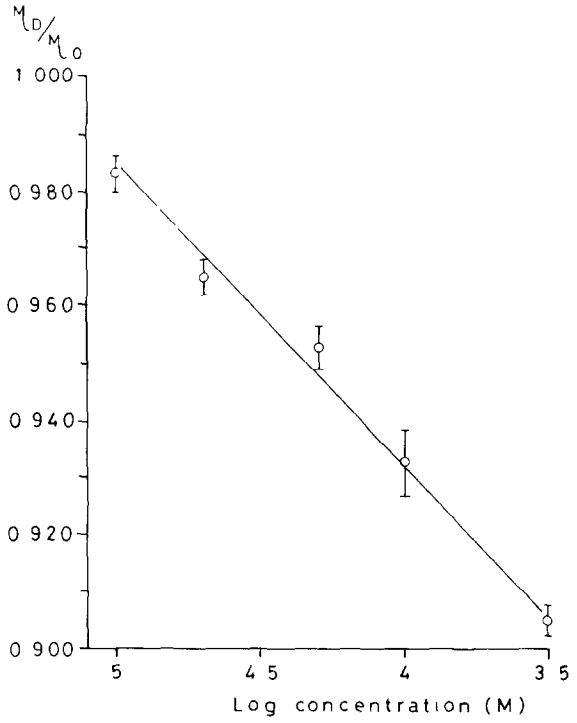


Fig.3. Dose-response curve of oxotremorine after preincubation with $10^{-5}$ M pirenzepine. The abscissa shows the concentration used in logarithmic units. The ordinate shows the relative microviscosity measured as in fig.2. Pirenzepine was preincubated with lymphocytes for 15 min at 37°C.

otremorine was linear with concentrations in the range $10^{-5}$–$3 \times 10^{-4}$ M. Preincubation with $10^{-5}$ M atropine caused a powerful rightward shift of the dose-response curve of about one order of magnitude. Atropine alone did not significantly alter lymphocyte membrane microviscosity.

Fig.3 shows membrane microviscosity variations following the treatment with oxotremorine in the same range of doses after the preincubation with $10^{-5}$ M pirenzepine. No variation vs oxotremorine-induced membrane microviscosity changes can be observed. Pirenzepine was absolutely inactive per se.

Table 1 shows cGMP levels in lymphocytes after 20 min incubation with $10^{-5}$ M atropine, $10^{-4}$ M oxotremorine and a combination of both. Oxotremorine caused a significant (2-fold) increase in cGMP. Atropine alone did not significantly alter cGMP content and completely reversed the action of oxotremorine. The table also reports the membrane fluidity values (expressed in P) obtained in parallel experiments. Table 2 shows for comparison the effects on membrane fluidity of carbamylcholine and nicotine. The effect of car-

Table 2

Effect of carbamylcholine and nicotine on membrane microviscosity of human lymphocytes

| Drug | Dose (M) | Microviscosity (P) |
|---|---|---|
| None | – | 1.957 ± 0.006 |
| Carbamylcholine | $10^{-4}$ | 1.794 ± 0.007[a] |
| Nicotine | $10^{-4}$ | 1.915 ± 0.012 |

[a] $p < 0.01$ vs controls

Results are the mean ± SE of 4 different determinations

Table 1

Effect of atropine and oxotremorine on cyclic GMP content and membrane fluidity of human lymphocytes

| Drug | Dose (M) | Cyclic GMP (pmol/g protein) | Microviscosity (P) |
|---|---|---|---|
| None | – | 0.121 ± 0.004 | 1.966 ± 0.020 |
| Atropine | $10^{-5}$ | 0.130 ± 0.029 | 1.932 ± 0.019 |
| Oxotremorine | $10^{-4}$ | 0.219 ± 0.020[a] | 1.838 ± 0.010[a] |
| Atropine + oxotremorine | $10^{-5}$ $10^{-4}$ | 0.166 ± 0.052 | 1.915 ± 0.010 |

[a] $p < 0.01$ vs controls

Each value is the mean ± SE of 4 different determinations

bamylcholine is somewhat stronger than that of oxotremorine, whereas nicotine is inactive.

## 4. DISCUSSION

This paper reports for the first time that membrane microviscosity can be modified by exposure to oxotremorine, a muscarinic agonist. To verify this point we made similar experiments using another muscarinic agonist, i.e. carbamylcholine. The microviscosity modification induced by this compound was comparable to that observed with oxotremorine in the same concentration range (table 2).

The effect was completely blocked by atropine, suggesting that the membrane-fluidizing effects are mediated by muscarinic receptors present on the lymphocyte surface. This presence was demonstrated for both human and murine lymphocytes [8,13].

Under our experimental conditions nicotine added to lymphocytes up to $10^{-4}$ M did not significantly alter the basal membrane microviscosity, as shown in table 2. This further confirms the muscarinic nature of the receptors under study.

The change in membrane fluidity caused by oxotremorine and carbamylcholine was firstly ascribed to the phosphoinositide turnover, that is a well-known biochemical event related to the occupancy of the muscarinic M1 receptor. But we were not able to find inositol phosphate accumulation after treatment with oxotremorine (not shown). Furthermore, it is reported that oxotremorine is a very weak phosphoinositide breakdown inducer in brain and in chick embryo heart cells even at concentrations an order of magnitude larger [14,15], whereas carbamylcholine is much stronger from this point of view.

So our findings could not be related to phosphoinositide breakdown. In addition pirenzepine, that is a selective M1 muscarinic receptor antagonist [16] and should therefore impair completely phosphoinositide breakdown, was absolutely ineffective in modifying membrane fluidity variations promoted by oxotremorine.

Oxotremorine is conversely reported to be very effective as an M2 muscarinic agonist, inhibiting adenylate cyclase activity in purified synaptic plasma membranes from rat striatum [17]. Some authors [18,19] report a concomitant variation in cAMP and cGMP levels in various tissues.

Since adenyl cyclase and guanyl cyclase activities are strongly dependent on membrane physical parameters [20,21] they could be at least partially regulated by membrane fluidity variations. Moreover, the long-lasting effect on membrane fluidity induced by oxotremorine could be explained in terms of long-range effects induced by occupancy of cholinergic sites by ligands [22].

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