some drugs difference in serum protein binding and free drug concentration may account for variation within and among species.

Acknowledgement—This work was supported in part by the United States Public Health Service Grant HE-06664, by the intramural funding program of the Addiction Research Foundation, Canada, and by a Burroughs Wellcome Fund Clinical Pharmacology Award (Dr. Koch-Weser).

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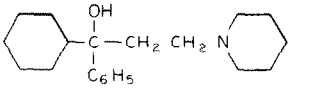
Biochemical Pharmacology, Vol. 26, pp. 2447–2448. Pergamon Press, 1977. Printed in Great Britain.

Interactions between phosphatidylcholines and trihexyphenidyl and benztropine

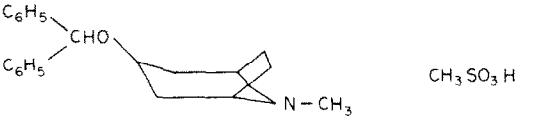
(*Received* 13 *December* 1976; *accepted* 6 *May* 1977)

In principle, drugs can block the action potential in nerve by affecting any of the conductance parameters that control the nerve excitability. In practice, many local anaesthetics block the action potential by blocking the sodium current. The major problem in accounting for this effect is that it is surprisingly unselective, with many neutral, negatively charged and positively charged molecules acting as local anaesthetics [1]. This can be explained if the site of action of the anaesthetics is the lipid component of the nerve membrane, but it is then necessary to explain the connection between action on lipids and the blockage of sodium current. A general increase in fluidity will not do, since any changes in fluidity for the bulk lipids of the membrane will be insignificant at the relevant drug concentrations [2, 3]. A more specific effect has to be postulated, as in the annular transition model [4]. In this model, the sodium channels in nerve membranes are postulated to be surrounded by lipid molecules in a rigid or gel-like state. Addition of local anaesthetics triggers a change in the surrounding lipids to a fluid or liquid crystalline state, allowing the sodium channel to relax to an inactive configuration, in which the sodium current is reduced or blocked.

In previous publications it has been shown that for alcohols [5], amines [6], barbiturates [7], chlorpromazine [3] and β-blockers [3] the concentrations required to produce blocking of the sodium current in nerve also cause a decrease of ca 3° in the temperatures of the gel to liquid crystalline phase transition in phospholipids. In order to extend the correlation to further classes of compounds, the effects of the anti-Parkinsonian drugs, trihexyphenidyl (I) and benztropine (II), on the phase transition temperatures of lipids have now been studied. In a recent paper Wu and Narahashi [8] have shown that these compounds act as local anaesthetics, blocking the sodium current.



Trihexyphenidyl hydrochloride (I)



Benztropine mesylate (II)

The temperatures of the gel to liquid crystalline phase transitions in dipalmitoyl phosphatidylcholine were measured using chlorophyll *a* as a fluorescence probe, as described elsewhere [5]. Liposomes were prepared from lipid (6×10^{-7} moles) in 4 ml 0.01 M Tris–HCl containing 0.1 M NaCl at a final pH 7.2, the anaesthetic being first dissolved at the required concentration in the buffer. The anaesthetics were obtained as trihexyphenidylhydrochloride and benztropine mesylate.

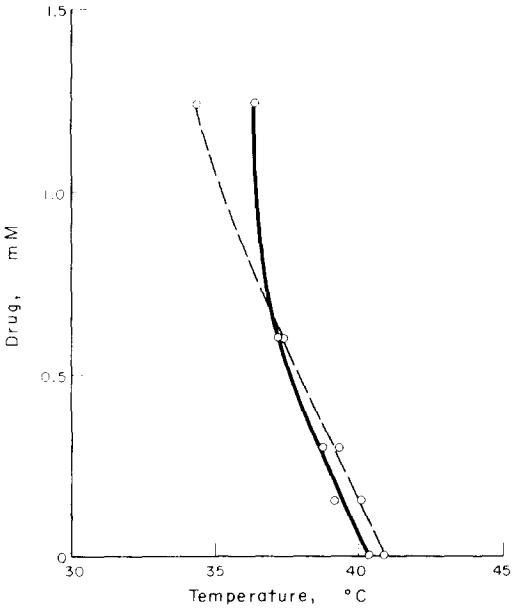


Fig. 1. The effect of benztropine on the phase transition temperature of dipalmitoyl phosphatidylcholine (solid line) and dipalmitoyl phosphatidylcholine plus 11 mole % myristic acid (broken line).

Figure 1 shows the effect of addition of benztropine on the mid-point transition temperature of the main gel to liquid crystalline phase transition of dipalmitoyl phosphatidylcholine. The effects of increasing concentration of benztropine were non-linear, presumably because of a build-up of positive charge on the liposomes. Similar effects have been observed with other positively charged drugs [3, 6]. Incorporation of negatively charged lipid into the bilayers should neutralize some of this charge, and thus increase the effect of the benztropine. Figure 1 shows that incorporation of 11 mole % of myristic acid into liposomes of dipalmitoyl phosphatidylcholine caused a slight increase in the temperature of the transition to 41°, but also caused an appreciable increase in the effect of benztropine on the phase transition temperature. Very similar results were obtained with trihexyphenidyl, and these are presented in Fig. 2.

These experiments have therefore shown that both trihexyphenidyl and benztropine interact with phosphatidylcholine bilayers causing a decrease in the temperatures of the lipid phase transition. Further, benztropine is the more potent of the two: the concentration of benzotropine required to produce a 3° drop in transition temperature is ca 0.5 mM and the concentration of trihexyphenidyl required for the same effect is ca 1.1 mM. These figures compare very favourably with those found by Wu and Narahashi to give the maximum observable blocking (80 per cent) of the sodium current in squid axon: ca 0.3 mM for benztropine and 1 mM for trihexyphenidyl [8].

In view of the very different structures of these two compounds, it seems very unlikely that they could be affecting sodium currents by direct binding to the sodium channel. The conclusion seems inevitable that their effects are mediated through the lipid component of the membrane. The smaller effect of trihexyphenidyl could then be due either to a less favourable partitioning into the membrane,

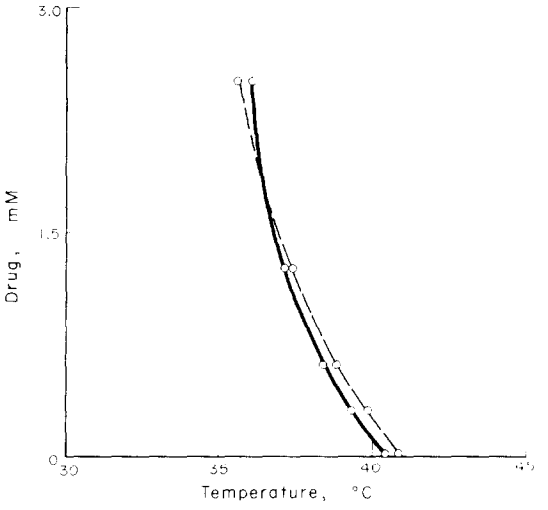


Fig. 2. The effect of trihexyphenidyl on the phase transition of dipalmitoyl phosphatidylcholine (solid line) and dipalmitoyl phosphatidylcholine plus 11 mole % myristic acid (broken line).

or to a slightly different effect on the lipids when in the membrane. Jain *et al.* [9] have shown for a series of adamantane derivatives that there is no simple correlation between effects on lipid transition temperatures and calculated partition coefficients.

Whether the postulated transition in the lipid annulus around the sodium channel should be likened to a normal gel to liquid crystalline phase transition, or whether it is a more gradual transition of the second-order type is not clear: this point is discussed at length elsewhere [10]. However, both benztropine and trihexyphenidyl have been observed to trigger a change in lipid state from rigid or gel-like to fluid at concentrations causing local anaesthesia, so that they fit into the model for local anaesthetic action described above.

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Acknowledgements—I thank Merck, Sharp and Dohme for a sample of benztropine and Cyanamid for a sample of trihexyphenidyl.

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