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CALCIUM-RESERPINE INTERACTIONS STUDIED BY ELECTRON SPIN RESONANCE SPECTROSCOPY IN SPIN LABELED ARTIFICIAL MEMBRANES

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Abstract

1. The interactions of reserpine with calcium on labeled phospholipid membranes are studied by electron spin resonance (ESR) spectroscopy.
2. Using TEMPO as spin label it is demonstrated that reserpine has a fluidizing effect on the lecithin-cholesterol system which corresponds to a membrane expansion and could represent the molecular basis of the tissue calcium losses reported after in vivo reserpine administration.
3. The reserpine-induced membrane expansion is partially reversed by the subsequent addition of calcium. By clustering the lipid molecules, the divalent cation would squeeze reserpine out of the lipid membrane. On the other hand, calcium administered before reserpine prevents the membrane expansion. Its binding to the phospholipid heads would inhibit reserpine's penetration into the artificial membrane.
4. These findings could provide a molecular basis for some previously reported in vivo calcium-reserpine interactions.

Key words: reserpine, calcium, artificial phospholipids membranes, ESR spectroscopy, spin labeled membranes

Introduction

Reserpine is well known for its ability to deplete tissue monoamines stores. Convergent data have recently shown that it also induces a significant decrease in the calcium content of vascular, cardiac and brain tissues (Carrier et al. 1969; Ross et al. 1974). It has been suggested that reserpine achieves this effect by interacting with one or more components of the plasma membrane.

The spin label technique provides a means to explore this hypothesis. The small amphiphilic spin label TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) which is known to be partitioned in both the aqueous and lipidic regions of phospholipid-water systems represents an adequate paramagnetic molecule for examining the interactions of various molecules and drugs with membrane model systems and biological membranes (Hsia and Boggs, 1972; Hubbell et al. 1970; McConnell et al. 1972).

In this paper, we report the results obtained using TEMPO as spin label and a phospholipid-water system as membrane model to study the reserpine membrane interactions.

Methods

The artificial membrane was prepared by two thirty second hand dispersions whereby 0.2 ml of TEMPO (12.8 mM) and 0.2 ml of Ringer solution were successively added to egg yolk lecithin (EYL) or to a mixture of EYL (31 mg) and cholesterol (5.1 mg). For single substance testing, the adduct was solubi-

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lized in the TEMPO solution. For interaction studies, one substance was introduced with the TEMPO solution (before the first dispersion) and the second in the Ringer solution (before the second dispersion).

The system was then sucked in 50 µl capillaries (Corning Co.) and again homogenized for 30 sec with a microdrill using a stretched coil spring (Rocky Mountain Orthodontics Canada Ltd., no F 98) for another 30 seconds. The capillary was then put in a standard quartz ESR tube. All spectra were recorded on a JEOL JES-ME-3X (X-band) ESR spectrometer using a cylindrical cavity.

TEMPO was synthesized according to the method of Rozantsev and Neiman (1964). Egg yolk lecithin was purchased from ICN Pharmaceutical. Cholesterol (Fisher) was recrystallized in methanol m.p. 148°C. Reserpine phosphate was a gift of Ciba Geigy. The chemicals for the Ringer solution, the organic solvents and anhydrous calcium chloride were reagent grade.

Results

The general feature of the ESR spectrum of TEMPO in an aqueous dispersion of a EYL-cholesterol system is presented in Fig. 1.

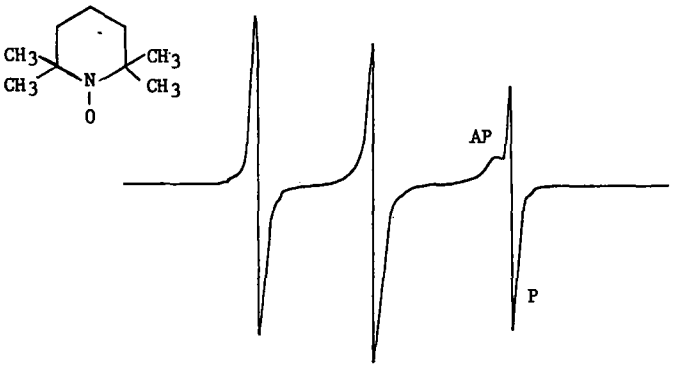

Fig. 1. Structure and ESR spectrum of TEMPO in a dispersion of lecithin-cholesterol in Ringer solution.

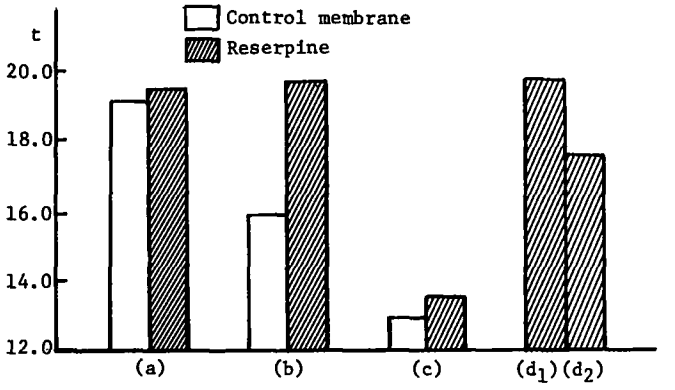

Fig. 2. Modifications of the TEMPO partitioning parameter (t) when reserpine is added to the aqueous egg yolk lecithin (EYL) system (a), to the EYL-cholesterol system (b) and to the EYL-cholesterol-calcium system (c). The EYL-cholesterol-reserpine system before (d_1) and after (d_2) administration of calcium.

The intensities of the two peaks of the $M_I = -1$ hyperfine line components give a quantitative evaluation of the partitioning of TEMPO in the lipid and aqueous phases. The proportion of TEMPO soluble in the lipid phase is given by the TEMPO partitioning parameter $t = (AP/AP + P) \times 100$, where AP and P represent the peak intensities of TEMPO soluble respectively in the apolar lipid and in the polar aqueous regions. The value of this parameter was taken as an index of the fluidity of the lipid phase.

The effect of reserpine on the conformation state of various lipid systems has been evaluated by the induced changes of the TEMPO partitioning parameter t. The results of the successive steps of our experimental approach are given in Fig. 2.

a. Effect of reserpine on the EYL system

The fluidity of this system is relatively high ($t = 19.2$). It was not significantly modified by reserpine ($t = 19.5$). Reserpine might have modified the pattern of the lipid arrangement. However, if present, this effect did not change the distribution of TEMPO significantly.

b. Effect of reserpine on the "EYL + cholesterol" system

When cholesterol is inserted into EYL, there is a decrease in the concentration of TEMPO in the lipid phase ($t = 15.9$), which suggests an increase in the molecular order and rigidity of the artificial membrane.

Reserpine, when added to this system, changed its ordered state into a disordered state ($t = 19.7$), similar to that of EYL alone ($t = 19.2$). The reversal by reserpine of the packing effect of cholesterol suggests that reserpine, which is like cholesterol a large rigid molecule, also enters the lipid bilayer. However, due to its suggested lipophilic-lipophobic characteristic, it fluidifies the lipid bilayer instead of packing it.

c. Effect of reserpine on the "EYL + cholesterol + calcium" system

When a high concentration of calcium (50 mM) was added to the "EYL + cholesterol" system, there was a further decrease in the concentration of TEMPO in the lipid phase ($t = 12.9$), which suggests a tightening of the packing of the phospholipid bilayer.

Reserpine when added to this system, was not able to modify significantly its packing ($t = 13.4$). This confirms that the above mentioned reserpine-induced expansion of the "EYL + cholesterol" system was due to reserpine entering into the phospholipid bilayer, a phenomenon which is no more possible in the highly packed "EYL + cholesterol + calcium" system.

d. Effect of calcium on the "EYL + cholesterol + reserpine" system

As mentioned before, this system is a rather disordered one ($t = 19.7$). The addition of a high calcium concentration strongly reduced its structural disorganization ($t = 17.3$). It is probable that calcium acts by clustering the expanded lipid molecules, squeezing thereby both reserpine and TEMPO out of the lipid bilayer.

Discussion

The results confirm that the addition of cholesterol and calcium increases the molecular order of a lecithin bilayer. As discussed more in details elsewhere (Wang and Radouco-Thomas, 1978), cholesterol would increase the rigidity of the system (Butler et al., 1970; Hsia et al., 1970, 1972; Kroes et al., 1972) by restricting the freedom of motion of the hydrocarbon chains (Hsia and Boggs, 1972; Hubbell and McConnell, 1971; Long et al., 1970). Calcium, on the other hand, would increase the tightening of the packing of the phospholipid bilayer by binding to the polar heads of lipid molecules from the same and from different lecithin vesicles (Hubbell and McConnell, 1968; White and Lakshminayanaiah, 1969).

Concerning reserpine, two main findings emerge from this ESR study.

First, the results indicate that reserpine increases the fluidity of the lipidic matrix of artificial membranes. Reserpine was expected to penetrate the lecithin film due to its oil-water partitioning coefficient (Seeman and Bialy, 1963). Its fluidizing effect corresponds to its suggested ability to expand the membrane (Seeman, 1966).

It is highly probable that the membrane expansion induced by reserpine results in the release of some of the calcium ions, previously bound to the packed head groups of the lipids. This mechanism could explain the calcium losses in brain (Chawla et al., 1978; Radouco-Thomas et al., 1971), vascular (Carrier and Shibata, 1967) and cardiac tissues (Clower et al., 1969), reported from in vivo and in vitro studies with reserpine. The increase in blood calcium level observed after reserpine administration (Radouco-Thomas et al., 1978) suggests that calcium losses may also occur in other body tissues, thus confirming the non-specificity of the reserpine-membrane interaction.

The membrane expansion induced by reserpine is also linked with an increase in the plasma membrane permeability. At the presynaptic level, the resulting sodium influx has been suggested to inhibit amine storage and promote amine leakage from the granules (Radouco-Thomas et al., 1971). The non-specific increase in sensitivity to calcium observed four hours after reserpine in isolated rabbit atria and aortic strips has also been suggested to be due to an increase in membrane permeability to calcium followed by an alteration in the intracellular homeostasis of calcium (Carrier and Hester, 1976; Carrier and Shibata, 1967; Jurevics and Carrier, 1973).

Second, the experimental results concerning the interactions between reserpine and exogenous calcium demonstrate that the conformational state of the membrane is highly dependent on the sequence of administration of calcium and reserpine.

It might be hypothesized that the ability of calcium to inhibit reserpine's penetration into the lipid membrane or to squeeze reserpine out of the membrane is due to the high affinity of this divalent cation for the negatively charged polar heads of the membrane lipids.

This physical model could explain the competitive-like pattern of the interaction between exogenous calcium and reserpine. In Hela cell culture, opposite effects on the cellular proliferation have been observed as a function of the amounts of calcium and reserpine added to the medium (Huot et al., 1978). In experimental animals, the behavioral and biochemical effects of reserpine have been shown to be inhibited in animals pretreated with calcium high doses (Boyaner and Radouco-Thomas, 1971a, b; Radouco-Thomas, 1971a; Radouco-Thomas et al., 1975). The protection afforded by calcium was dose-dependent (Radouco-Thomas, 1971b). The proposed mechanism of an interaction between calcium and reserpine at the pre-synaptic membrane, which was later controversed (Manara et al., 1974, 1976), is confirmed by the present results.

Finally, the finding that calcium, when added to a reserpine-treated membrane squeezes reserpine out and re-establishes the initial order of the membrane has also its biological counterpart. In vivo, the calcium ions, initially released by reserpine in the extracellular space, could thereafter by binding again to the outer surface of the cell membrane, facilitate the penetration of reserpine into the cell sap. As a matter of fact, some behavioral effects of reserpine have been shown to be enhanced when calcium was administered after reserpine (Radouco-Thomas et al., 1971).

The good correlation found between the reserpine-induced modifications of the spin label membrane system and the in vivo effects of reserpine, suggest that the mechanism of action of other drugs which are known to affect membrane properties could also be approached efficiently by the ESR-technique.

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