MECHANISM OF MEMBRANE STABILIZING AND
LYTIC EFFECTS OF TRICYCLIC ANTIDEPRESSANTS

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Accepted February 6, 1980

There have been reports of hepatic damage related to several tricyclic antidepressants (1, 2). The mechanism by which these drugs can produce hepatic damage is poorly understood. The author (3) reported that the differences in membrane damage produced by tricyclic antidepressants may be related to their surface activities which in turn may determine the extent of adsorption onto cell membrane. The present studies were undertaken to clarify the mechanism of membrane stabilizing and lytic effect of tricyclic antidepressants, using rat erythrocytes and hepatocytes.

Isolated rat hepatocytes were prepared by a modification of the method of Berry and Friend (4). Cell suspensions (2 x 10^5 cells/2 ml) were incubated with drugs in culture tubes for 1 hr at various temperatures. Cytotoxicity to isolated rat hepatocytes of the tricyclic antidepressants was determined by the leakage of glutamic oxalacetic transaminase (GOT) into the surrounding media. Inhibition of hypotonic hemolysis (70%) of rat erythrocytes was measured as hemoglobin concentration, according to the method of Elonen (5). A preliminary experiment for determination of hemoglobin concentration showed a good reproducibility. Therefore, the determination was repeated twice for each concentration of drugs used. Surface tension was measured with a Cahn electrobalance. The surface tension of Hanks’ solution in the absence of a drug was 71 dynes/cm.

Figure 1 shows the effect of tricyclic antidepressants on enzyme leakage from the cells at various temperatures. The enzyme leakage by chlorimipramine (CIM) was related to the incubation temperature and increased as the temperature was elevated. There were two breaks of increase in enzyme leakage. The rate of increase in enzyme leakage was mild when the incubation temperature was between 0° and 20°C and a rapid increase in enzyme leakage occurred at 20° to 25°C. There were no significant changes in enzyme leakage between 25° and 30°C. The second increase in enzyme leakage occurred at 30° to 35°C and a remarkable increase in the leakage was observed at 37°C. On the other hand, the effects of imipramine (IM) and desipramine (DMI) on enzyme leakage differed. Although a mild increase in enzyme leakage occurred at a temperature of 0° to 20°C by both drugs, decrease in enzyme leakage was observed at 20° to 35°C and a mild increase in enzyme leakage occurred at 37°C. Figure 2 shows the effect of tricyclic antidepressants on hypotonic hemolysis of erythrocytes. CIM protected hypotonic hemolysis at a concentration of
1 x 10^{-5} M to 1 x 10^{-4} M but facilitated the hemolysis at 2 x 10^{-4} M. IM and DMI also protected the hypotonic hemolysis at a concentration of 1 x 10^{-5} M to 4 x 10^{-4} M and facilitated the hemolysis above 4 x 10^{-4} M. CIM had the greatest surfactant effect since it lowered the surface tension by 24.2 dynes/cm at 1 x 10^{-4} M, while IM and DMI lowered the surface tension by 8.3 and 10.0 dynes/cm at 1 x 10^{-4} M, respectively.

CIM induced a marked incubation temperature dependent enzyme leakage from the hepatic cells, had a membrane stabilizing action on erythrocytes in lower concentrations (5 x 10^{-6}-1 x 10^{-4} M) and a hemolytic action in higher concentrations (over 2 x 10^{-4} M). The concentration (2 x 10^{-4} M) of CIM, in which hemolysis occurred, corresponded to the concentration which caused a marked enzyme leakage from hepatocytes. In contrast, CIM caused only a slight enzyme leakage at the concentration of 1 x 10^{-4} M (3). Thus a concentration of 2 x 10^{-4} M was discussed in detail. While IM and DMI also had a membrane stabilizing action on erythrocytes and hepatocytes depending on the incubation temperature, these effects were fewer than that of CIM. These effects of tricyclic antidepressants

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**Fig. 1.** Effects of temperatures on enzyme leakage. Cytotoxicity to isolated rat hepatocytes of tricyclic antidepressant (2 x 10^{-4} M) was determined by the leakage of glutamic oxalacetic transaminase (GOT) into surrounding media during 1 hour. Values are expressed as Karmen units per ml media. Means and S.D. of four experiments.
Salhab et al. (6) reported that the rank order of the surface activity of the drugs correlated with the rank order of their degree of uptake by the cells and suggested that surface active properties could play a role in differences in bioavailability and toxicity to liver cell membranes. The difference between membrane stabilizing and lytic effect of drugs was directly related to the extent of the drug concentration.

Biological membranes have been postulated to exist in a fluid mosaic (7) fashion. An essential feature of this model is that the lipids of functional cell membranes at physiological temperatures are in a mixture of fluid and solid states (8). Gordon et al. (9) reported that lipid phase separations occur in liver plasma membranes between 19˚C and 28˚C from examination of the temperature dependence of approximate and polarity-corrected orderparameters and suggested the following phase separation model, that is, the 19˚C break corresponds to solid lipid domains (S) → S + liquid lipid domains (L) transition and the 28˚C break reflects S + L → L transition. A wide variety of membrane associated functions may be influenced by these thermotropic phase separations. In our study, CIM damaged the liver plasma membrane when the tissue was exposed to temperatures between 20˚C and 25˚C. Within these temperatures, plasma membrane may be fragile due to the structural incompatibility between the solid and liquid domains, the junctional area being leaky. IM and DMI stabilized the plasma membrane at the same temperature range and decreased enzyme leakage from the cells. Terris and Steiner (10) reported that isolated hepatocytes
metabolized $[^{125}I]$-insulin negligibly at $0^\circ$ to $20^\circ$C, but that the rate increased steeply at temperatures between $20^\circ$ and $30^\circ$C. Inglot and Wolna (11) reported that erythrocyte stabilization-lysis by the drug is temperature dependent, and is reduced considerably at $4^\circ$C. These results suggest that drug effects on plasma membrane are influenced by the state of the lipid domain.

Another possible site of action of tricyclic antidepressants may be the integral proteins embedded in the lipid bilayer of the membrane (12). Samuels and Carey (13) showed that chlorpromazine inhibited the activities of Mg$^{++}$-, and Na$^+$, K$^+$-ATPase and that there was a linear dose-response relationship. Tricyclic antidepressants are similar in structure to chlorpromazine and thus may affect these enzymes regulating membrane permeability. Houslay et al. (14) reported that the Arrehenius plots of the enzymatic activities of Na$^+$, K$^+$- and Mg$^{++}$-ATPase exhibited breaks between $26^\circ$–$32^\circ$C. In our study, the second increase in enzyme leakage by CIM corresponded to these temperature ranges. It is possible that the disappearance of the phase separation at $28^\circ$C dramatically influences the function of these enzymes.

The concentrations of drugs investigated in the present experiments were much higher than the plasma concentration found in patients ingesting tricyclic antidepressants. However, Abernathy et al. (15) reported that the liver/plasma ratio of tricyclic antidepressants is 20–100, in other words, tricyclic antidepressants accumulated in the hepatocytes. Thus, the level of tricyclic antidepressants calculated from the above ratio is still one tenth that needed to induce damage in the hepatocytes. Nevertheless, isolated hepatocytes are a useful model for screening of hepatotoxicity.

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CHANGE IN CATECHOLAMINE-SENSITIVE Na⁺,K⁺-ATPase ACTIVITY IN THE RAT STRIATUM MICROSOMES FOLLOWING ELECTROLYTIC OR 6-HYDROXYDOPAMINE-INDUCED LESIONS OF DOPAMINERGIC NEURONS

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Accepted February 12, 1980

Ungerstedt (1) demonstrated that the degeneration of nigrostriatal dopamine (DA) system by microinjection of 6-hydroxydopamine (6-OHDA) produced a postsynaptic supersensitivity to inhibitory action of DA in the caudate neurons. Since then, there have been a number of reports concerned with the development of supersensitivity to DA in the caudate neurons following various procedures, e.g. surgical or chemical denervation of nigrostriatal DA neurons, sustained attenuation or blockade of dopaminergic transmission, etc. (2–6). DA stimulates the synthesis of cyclic AMP in the caudate nucleus (7) and, there is controversy about the role of adenylate cyclase in the development of supersensitivity (8–10).

On the other hand, current evidence suggests that the inhibitory action of DA on the caudate neurons is mediated through a stimulation of Na-pump via an activation of Na⁺, K⁺-ATPase, thereby presumably resulting in hyperpolarization of the caudate neurons (11–13). These views led to the assumption that the supersensitivity is attributed, at least in part, to an increase in the sensitivity of Na⁺, K⁺-ATPase to DA. We investigated the effects of electrolytic or 6-OHDA-induced lesions on the DA-induced in the Na⁺, K⁺-ATPase activity in the striatum in an attempt to elucidate the mechanism of supersensitivity.

The striatum was removed from male Wistar rats weighing approx. 300 g and homogenized in 10 vol. of 0.32 M sucrose containing 5 mM Tris (pH 7.4), using a ground glass homogenizer. The homogenate was centrifuged at 900×g for 10 min and the pellet was