J. Pharm. Pharmacol. 1991, 43: 564–568
Received October 17, 1990

Effect of Imipramine on the Membrane Anisotropy and on the Phospholipid Methylation in the Central Nervous System of the Rat*

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Abstract—An ex-vivo and in-vitro study of the effects of imipramine on the membrane anisotropy and phospholipid methylation in the rat cortical membranes was carried out. A comparative study of the membrane fluidity in various brain regions indicated different basal anisotropy of the areas and different reaction of these membranes to imipramine. It was found that imipramine when given to rats chronically (14×10 mg kg^{-1}, i.p.) or added externally to the cortical membranes of naive rats or rats treated with a single dose of imipramine (10 mg kg^{-1}, i.p.) decreased the anisotropy of cortical membranes. Chronic imipramine produced some changes of the membrane architecture in the cortex, whereas imipramine in different concentrations did not fluidize these membranes in-vitro. Imipramine in concentrations corresponding to its mean concentration in the rat brain after administration at a dose of 10 mg kg^{-1} i.p., potentiated phospholipid methylation in the cortical membranes of naive rats and rats receiving imipramine in a single dose of 10 mg kg^{-1} i.p. in an in-vitro study, whereas the prolonged administration of imipramine decreased the sensitivity of phospholipid methyltransferases to the stimulating effect of the drug in-vitro.

There is evidence indicating that some tricyclic antidepressants bind non-specifically to the biological membrane, changing its organization (Römer & Bickel 1979; Zimmer & Schulze 1981) and increase in-vivo the incorporation of methyl groups into membrane phospholipids in the rat brain (Racagni et al 1983). Since Hirata & Axelrod (1978, 1980) demonstrated that phospholipid methylation regulated the fluidity characteristics of erythrocyte membranes, and Zimmer & Schulze (1981) have found by the spin-label method that amitriptyline and some other tricyclics disturbed the order of the outer part of the red cell membrane, it could be argued that these drugs, when given to rats, might affect the membrane anisotropy in the central nervous system (CNS) of the animals via alteration of the phospholipid methylation in the lipid bilayer or via other mechanisms.

As the membrane fluidity plays an important role in many membrane-mediated events (Hirata & Axelrod 1980; Brunello et al 1987) changes of the membrane anisotropy produced by drugs might be associated with hypo- or hyperfunction of membrane receptors and enzymes, ion channels, or ion pumps, produced by prolonged administration of the agents (for review see Sugrue 1983).

It was therefore worth investigating whether the exogenous, chronic administration of imipramine affects the membrane anisotropy and the phospholipid methylation in the cerebral cortex of rats. The cerebral cortex was chosen for our study since many binding studies and studies on the second messenger transmission after prolonged administration of tricyclic antidepressants have been carried out in this brain area.

* A part of the results concerning the effect of imipramine on the membrane anisotropy in-vitro was presented at the XVIth C.I.N.P. Congress, Munich, 1988 (Wesemann et al 1988).

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Materials and Methods

Drug

Imipramine hydrochloride was obtained from Sigma.

Assay of membrane anisotropy

Ex-vivo study. Male Wistar rats, 250 ± 10 g, were used for the preparation of the crude cortical membrane fraction. Imipramine was administered in a single dose (10 mg kg^{-1}, i.p.) or chronically, for 14 days at the same dose. Thirty min after administration (in chronic experiments 30 min after the last dose) the animals were killed by decapitation. The brain was removed and dissected into cortex, hippocampi, striatum, cerebellum and pons + medulla. The tissue was homogenized in 10 vol ice-cold 0·32 M sucrose solution with an Ultraturrax homogenizer (setting 6 for 1 min). The homogenate was centrifuged at 700 *g* for 10 min, and the supernatant was centrifuged at 40 000 *g* for 20 min. The resulting pellet was resuspended in 20 vol Tris-HCl buffer (pH = 7·4) and centrifuged at 40 000 *g* for 20 min. The final pellet was stored at −20°C until membrane fluidity assay. Control membranes were prepared using rats treated with saline.

Membrane anisotropy was assessed with 1,6-diphenyl-1,3,5-hexatriene (DPH) as the fluorescence probe (Heron et al 1980). The pellet prepared as described above was suspended in an appropriate volume (2–6 mL) of Tris-HCl buffer (pH = 7·4) and the protein in each sample was measured (Lowry et al 1951). Each sample was diluted with Tris-HCl buffer to give approximately 25 μg protein in 100 μL. Samples (100 μL) were incubated with 900 μL of Tris-HCl buffer (pH = 7·4) and 1000 μL of DPH (5 μM) for 30 min at 25°C. After incubation the fluorescence intensity was measured with a Perkin Elmer LS-5 spectrofluorometer equipped with two glass prism polarizers (Ex. 346 nm, em. 450 nm). The fluorescence polarization (P) was calculated (Azumi & McGlynn 1962) assuming that 1/P is approxima-

tely proportional to the anisotropy of the membrane region in which the fluorescence probe is incorporated (Shinitzky & Barenholz 1978). Anisotropy (r) was calculated from the equation $r = 2P/3 - P$.

In-vitro study. Samples (100 μL) of the cortical membrane preparation (see above) obtained from naive rats or rats receiving single (10 mg kg^{-1}, i.p.) or multiple (14 × 10 mg kg^{-1}, i.p.) doses of imipramine were incubated for 30 min at 25°C with 250 μL of DPH and 1650 μL of imipramine buffer (Tris-HCl, pH = 7·4) solutions of different concentrations. The final concentration of imipramine in the incubation mixture was $10^{-10} - 10^{-3}$ M. The membrane anisotropy was measured as described above, against the control, i.e. the sample containing 1650 μL of Tris-HCl buffer, without imipramine.

Assay of phospholipid methylation

Ex-vivo study. Animals were treated either with a single (10 mg kg^{-1}, i.p.) or multiple (14 × 10 mg kg^{-1}, i.p.) dose of imipramine. Thirty min after administration (chronic animals 30 min after the last dose) the rats were killed by decapitation, the cortex was isolated and the synaptosomal fraction was prepared (Hajos 1975). The total phospholipid methylation (the amount of phosphatidyl-*N*-methylethanolamine + phosphatidyl-*N*, *N*-dimethylethanolamine + phosphatidylcholine) in the cortical synaptosomal fraction was assayed according to Crews et al (1980). Briefly, 100 μL of synaptosomal membrane suspended in a Tris-HCl buffer (pH 7·4) containing 0·2–0·4 mg protein mL^{-1} (Lowry et al 1951) was incubated with *S*-adenosyl-L-[^{3}H]methylmethionine (0·4 μCi, Amersham UK, sp. ac. 15 Ci $mmol^{-1}$), 2 mM $MgCl_2$ and 5 μM of imipramine. The reaction mixture was prepared at 4°C. The incubation was carried out at 37°C for 30 min. The reaction was stopped by the addition of 20% aqueous disodium trichloroacetate and the samples were centrifuged at 24000 *g*. The methylated phospholipids were extracted with 3 mL of chloroform-methanol (2:1, v/v), the chloroform phase was washed twice with 0·1 M KCl in 50% methanol and the chloroform was evaporated under a stream of nitrogen. Three mL of a scintillation fluid (POPOP, PPO, naphthalene, ethylene glycol, methanol, dioxane) was added to the vial and the radioactivity was measured in a Beckman LS-3801 scintillation counter at 40% efficiency. Control synaptic membrane was prepared using cerebral cortices of naive rats or rats treated with 0·9% NaCl (saline).

In-vitro study. Animals receiving imipramine either in a single (10 mg kg^{-1}, i.p.) or multiple (14 × 10 mg kg^{-1}, i.p.) dose were killed by decapitation 30 min after administration. Control animals received saline. The cerebral cortex was excised and the total phospholipid methylation was assayed as described above in the presence of 5×10^{-6} M imipramine added externally to the incubation mixture (5×10^{-6} M imipramine corresponded to the mean cerebral level of the drug when given to rats in a single or multiple dose of 10 mg kg^{-1} i.p. (Daniel et al 1981)).

The results were evaluated statistically using analysis of variance followed by Student's *t*-test or Dunnett's test.

Results

Effect of imipramine on the membrane anisotropy in various regions of rat brain—ex-vivo experiments

The basal membrane anisotropy in investigated regions of the rat brain was different being the highest in the pons + medulla (Table 1).

Imipramine when given to rats in a single dose of 10 mg kg^{-1} i.p. increased the membrane anisotropy of hippocampi and cerebellum. The membrane anisotropy of the remaining areas investigated was similar to that of the corresponding control (Table 1).

Chronic treatment of rats with imipramine (14 × 10 mg kg^{-1}, i.p.) led to a significant decrease of the membrane anisotropy in the cortex compared with the control, and in the hippocampi and cerebellum when compared with the membrane anisotropy of these regions after a single dose of imipramine (Table 1).

Effect of imipramine on cortical membrane anisotropy—in-vitro experiments

When the crude membrane preparation of cerebral cortex of naive rats or of animals treated with a single dose of imipramine (10 mg kg^{-1}, i.p.) was incubated with different concentrations of drug ($10^{-10} - 10^{-3}$ M), a significant decrease of membrane anisotropy was produced by $10^{-4} - 10^{-3}$ M imipramine. Lower concentrations (10^{-5} and 10^{-6} M) showed a tendency to increase the fluidity of the membranes; however, this effect was not statistically significant (Fig. 1).

The external addition of $10^{-10} - 10^{-3}$ M imipramine did not change anisotropy of the cortical membrane of rats treated chronically with the drug (Fig. 1).

Effect of imipramine on phospholipid methylation in rat cerebral cortex

The stress connected with a single or repeated intraperitoneal injection of saline did not affect the rate of phospholipid methylation in the cortical synaptic membranes of rats (Fig. 2).

Imipramine, when given to rats in a single dose of 10 mg kg^{-1} i.p., slightly potentiated phospholipid methylation in rat cerebral cortex; however, the effect did not reach the statistically significant level (Fig. 3). Chronic treatment of rats (14 × 10 mg kg^{-1}, i.p.) did not affect the activity of phospholipid methyltransferases in the rat cerebral cortex although a tendency to an inhibition of the reaction was found (Fig. 3).

Imipramine when added externally (5×10^{-6} M) to the cortical synaptic membranes of rats treated with saline (1 × saline) or rats treated acutely with drug (10 mg kg^{-1}, i.p.), significantly potentiated phospholipid methylation, whereas external addition of 5×10^{-6} M imipramine to the cortical synaptic membranes of chronically treated rats (14 × 10 mg kg^{-1}, i.p.) did not induce any change in the phospholipid methyltransferase activity (Fig. 4).

Discussion

Our results indicate that the regions of the rat brain investigated were characterized by various basal anisotropy of the membranes. As lipid content and composition regulate

Table 1. Effect of imipramine given acutely or chronically to rats on the membrane anisotropy (r) in various regions of rat brain in ex-vivo experiments. n = 3–4; [a] $P < 0.05$ as compared with the control; [b] $P < 0.05$ as compared with corresponding anisotropy of the area after a single dose of imipramine; [c] $P < 0.01$ as compared with the anisotropy of the cortex (Dunnett's test).

Region	Control	Imipramine	
		1 × 10 mg kg^{-1} i.p.	14 × 10 mg kg^{-1} i.p.
Cortex	0·2372 ± 0·0012	0·2347 ± 0·0031	0·2235 ± 0·0030[a]
Hippocampus	0·2376 ± 0·0022	0·2466 ± 0·0018[a]	0·2336 ± 0·0047[b]
Cerebellum	0·2400 ± 0·0009	0·2482 ± 0·0018[a]	0·2332 ± 0·0024[b]
Striatum	0·2391 ± 0·0033	0·2440 ± 0·0123	0·2328 ± 0·0010
Pons + medulla	0·2536 ± 0·0033[c]	0·2547 ± 0·0020[c]	0·2532 ± 0·0022[c]

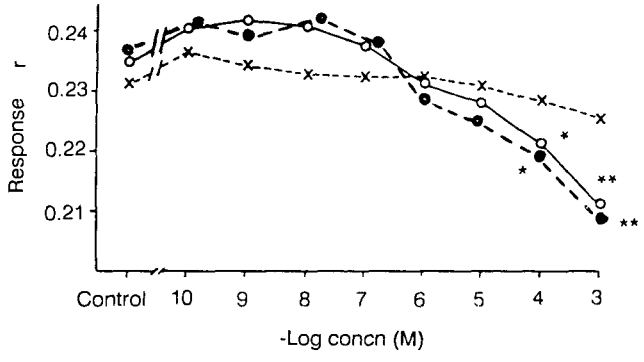

FIG. 1. Effect of different concentrations of imipramine on cortical membrane anisotropy in in-vitro experiments. Membranes were isolated from cortex of naive rats ●– –●, rats treated with single (10 mg kg^{-1} i.p.) o—o or multiple (14 × 10 mg kg^{-1} i.p.) x– – –x dose of imipramine. Each point is a mean of 3 assessments. * $P < 0.05$; ** $P < 0.01$ as compared with basal anisotropy (Student's t-test). r—anisotropy.

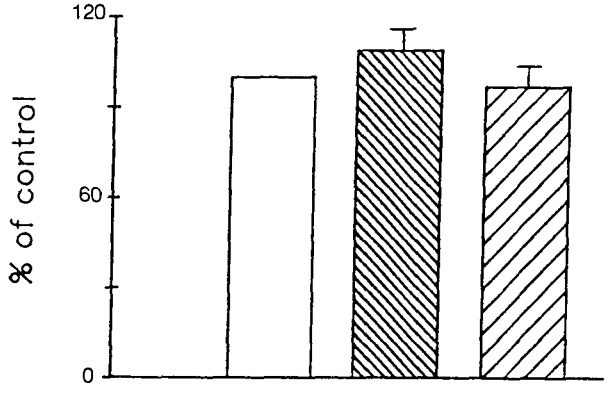

FIG. 2. Effect of acute and chronic intraperitoneal administration of saline on phospholipid methylation in the cortical synaptic membrane of rats. The results are expressed as a percentage of the control (naive rats). Control (4487·80 + 412·09 d min^{-1} (μg prot)$^{-1}$), open column; 1 × saline, right-hatched column; 14 × saline, left-hatched column. n = 5.

the membrane anisotropy (Papahadjopoulos et al 1973) it can be assumed that the different basal anisotropies of the membranes isolated from various parts of rat brain were due to the regional variation of lipid content and composition within rat CNS (Tayyaba & Hasan 1985).

Imipramine when given to rats in single or multiple doses

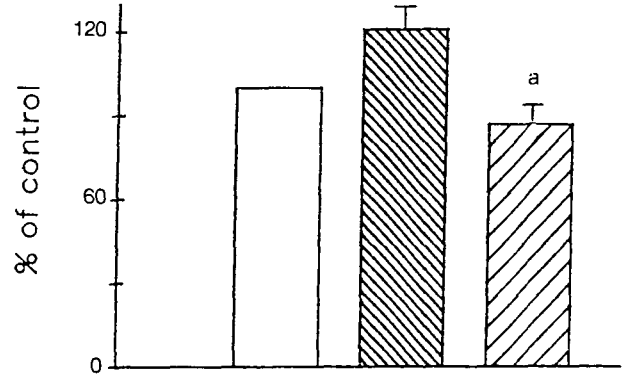

FIG. 3. Effect of a single (10 mg kg^{-1} i.p.) or multiple (14 × 10 mg kg^{-1} i.p.) dose of imipramine on phospholipid methylation in the cortical synaptic membrane of rats. The results are expressed as a percentage of the control (saline). Rats were killed 30 min after administration of imipramine. Control (4989·05 ± 321·98 d min^{-1} (mg prot)$^{-1}$), open column; imipramine, 10 mg kg^{-1} i.p., right-hatched column; imipramine, 14 × 10 mg kg^{-1} i.p. n = 5–7, left-hatched column. a—statistically significant as compared with the cortical synaptic membranes of rats treated acutely with imipramine (Dunnett's test).

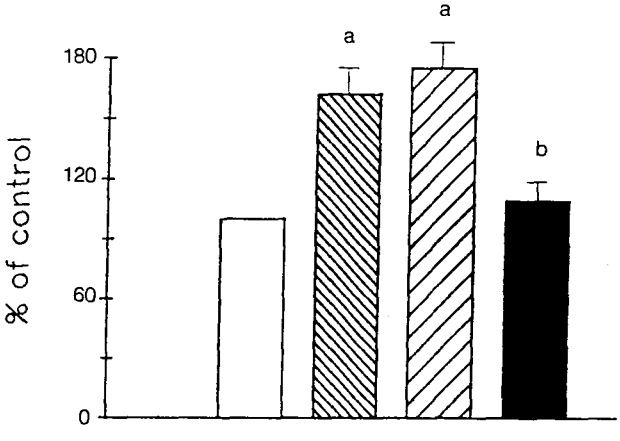

FIG. 4. Effect of 5 × 10^{-6} M imipramine added externally to cortical synaptic membranes on phospholipid methylation in-vitro. Control (saline, 5005·68 + 435·23 d min^{-1} (mg prot)$^{-1}$), open column; saline + 5 × 10^{-6} M imipramine, right-hatched column; imipramine 10 mg kg^{-1} i.p. + 5 × 10^{-6} M imipramine, left-hatched column; 14 × imipramine 10 mg kg^{-1} i.p. + 5 × 10^{-6} M imipramine, solid column. The results are expressed as a percentage of the control. n = 5–7. a—statistically significant as compared with the control; b—statistically significant as compared with saline + 5 × 10^{-6} M imipramine and imipramine 10 mg kg^{-1} i.p. + 5 × 10^{-6} M imipramine (Dunnett's test).

induced changes of membrane anisotropy in some of the investigated regions of rat brain as assessed in the ex-vivo study, and the direction of the changes depended on the brain area and the dosage schedule. Single doses of imipramine produced a significant increase of membrane anisotropy in hippocampi and cerebellum, while chronic treatment with the drug led to a decrease of membrane anisotropy in cortex, hippocampi and cerebellum. The latter effect was significant as compared with the membrane anisotropy of these areas after a single dose of imipramine. The result might be due to the interaction of imipramine molecules with the outer part of the lipid bilayer (Römer & Bickel 1979; Zimmer & Schulze 1981) which could produce either a decrease or an increase of the order of the membrane, the direction of the changes depending on the lipid content and composition of the area.

The reverse changes of membrane anisotropy within the CNS of rats after single and prolonged administration of imipramine suggest that prolonged exposure of the membranes to imipramine led to changes of membrane architecture different from those induced by a single dose of the drug. This is consistent with the results of our in-vitro study which indicated that the membranes isolated from the cortex of animals treated chronically with imipramine were resistant to the "fluidizing" effect of the drug, whereas the same concentrations of the agent (10^{-4}–10^{-3} M) produced a significant decrease of anisotropy of cortical membranes isolated from the brain of rat treated acutely with imipramine.

Imipramine when given to rats in a single dose showed a tendency to increase phospholipid methylation in the cerebral cortex of rats. Moreover imipramine, when added externally to the incubation mixture in the concentration corresponding to that found in the rat brain after pharmacological doses significantly potentiated phospholipid methylation in the cortical synaptic membranes of saline-treated rats and rats treated acutely with imipramine. These results are in agreement with those of Racagni et al (1983) who found an increase of phospholipid methylation in the rat cortical membrane 1 h after administration of desipramine in a dose of 10 or 20 mg kg^{-1} i.p. Potentiation of phospholipid methylation by impramine may be connected with its effect on cortical membrane anisotropy. The increase in membrane fluidity potentiates the turnover of enzymatic processes (Gavish & Werber 1989) as well as the rate of lateral encounters (Hanski et al 1979) which, in turn, might facilitate the methylation of the membrane phospholipid. Although the modulation of phospholipid methylation by altering the level of the substrate (phosphatidylethanolamine) by imipramine cannot be excluded, the results of others (Lüllmann-Rauch 1979; Honegger et al 1983; Moor et al 1988) indicate that only prolonged exposure of the tissue to imipramine or to other amphiphilic cationic agents led to phospholipidosis in the membrane.

Treatment of rats with imipramine for fourteen days produced changes in the sensitivity of phospholipid methyltransferases to the stimulating effect of imipramine, since 5×10^{-6} M imipramine added externally to the incubation mixture did not potentiate the phospholipid methylation in the synaptic cortical membranes of rats treated chronically with imipramine. Lüllmann-Rauch (1979) and Moor et al (1988) have shown that—in contrast to short time exposure—long-term treatment with imipramine or desipramine led to the accumulation of phospholipid and induced marked differences in the phospholipid content of various tissues. Therefore, we cannot exclude the possibility that chronic exposure of rats to imipramine might decrease the level of substrate (phosphatidylethanolamine) or increase the level of the product (phosphatidylcholine) in the cortical membranes of rats and this could inhibit the stimulating effect of externally added imipramine. A tendency to a decrease of phospholipid methylation in the cerebral cortex of rats treated chronically with imipramine found in ex-vivo experiments might confirm this possibility. This result is in agreement with the result indicating that chronic treatment of rats with imipramine led to the resistance of the cortical membranes to the fluidizing effect of externally added drug.

Hirata & Axelrod (1978) proposed that phospholipid methylation and vectorial rearrangement of the synthesized phospholipid within the membrane increases the membrane fluidity. However, many workers in the field are sceptical of this, as phospholipid methylation involves only a small proportion of the membrane lipids. Our results of the in-vitro study confirm to some extent the link between membrane anisotropy and phospholipid methylation, i.e. fluidization of the membrane and potentiation of phospholipid methylation by imipramine added externally to the cortical membrane of naive rats and rats treated acutely with imipramine, resistance of the membrane to the fluidizing effect of imipramine and decreased sensitivity of phospholipid methyltransferases to the stimulating effect of imipramine in cortical membranes of rats treated with imipramine for two weeks. On the other hand the results of the corresponding ex-vivo study are against the hypothesis of Hirata & Axelrod (1978), as chronic treatment decreased cortical membrane anisotropy and did not potentiate but rather inhibited phospholipid methylation in the cerebral cortex of rats. The resolution of the problem needs a more detailed physical and chemical study of membrane organization.

Acknowledgements

Dr M. Melzacka thanks Max-Planck-Gesellschaft for financial support and Professor Wesemann, Head of Department of Neurochemistry, Philipps University, Marburg for suggesting the experiments and for fruitful discussions. This work was supported by grant 06.02.I.

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