J. Pharm. Pharmacol. 1991, 43: 107–110
Received July 5th 1990



# Effect of Some Cationic Amphiphilic Drugs on Phospholipid Methylation in the Central Nervous System of Rats

HALINA NOCON AND MIROSLAWA MELZACKA

*Institute of Pharmacology, Polish Academy of Sciences, 31-343 Krakow, Poland*

**Abstract**—Imipramine, desipramine, citalopram and chlorpromazine in concentrations which corresponded with their concentration in the central nervous system of rats after pharmacological doses, potentiated phospholipid methylation in the synaptic cortical membranes of naive rats in-vitro. Chronic administration of imipramine, desipramine or citalopram induced changes in the activity of phospholipid methyltransferases since none of these drugs stimulated phospholipid methylation in the synaptic cortical membranes of rats treated with these antidepressants for two weeks. In contrast, chronic treatment with chlorpromazine did not change the sensitivity of phospholipid methyltransferases to the stimulating effect of chlorpromazine, whereas addition of haloperidol to the synaptic cortical membranes of rats treated chronically with haloperidol led to a decrease of phospholipid methylation.

Phospholipid methylation and rapid vectorial rearrangement of the synthesized phospholipids within the membrane has been shown to affect the membrane fluidity (Hirata & Axelrod 1978). Since many membrane-bound enzymes and receptors have subunits which interact with the membrane, changes in the fluidity of membranes can affect the activity of enzymes or receptors by altering rotation, diffusion and association of various subunits. For example, it was found that conversion of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) in the rat reticulocyte ghosts regulates the activity of $\beta$-adrenoceptors (Strittmatter et al 1979) and adenyl cyclase (Hirata & Axelrod 1980). There is also evidence for the coupling between dopaminergic receptors and phospholipid methylation in the mouse B lymphocytes (Le Fur et al 1981), for the phospholipid methylation and [$^3$H]diazepam and [$^3$H]GABA binding in membrane preparations of the rat cerebellum (Di Perri et al 1983), and for the phospholipid methylation and $Ca^{2+}$ influx and histamine release in rat mast cells (Ishizaka et al 1980). Therefore phospholipid methylation is partially involved in the mechanism by which biochemical signals are transmitted through membranes (for review see Hirata & Axelrod 1980).

It has been shown that some amphiphilic drugs, such as imipramine or chlorpromazine, bind non-specifically to biological membranes (Bickel & Steele 1974), and that administration of tricyclic antidepressants in a high single dose, or prolonged exposure to these drugs leads to phospholipid accumulation in cells of an intact organism of various species (Lüllmann-Rausch 1979). Moreover, Honegger et al (1983) demonstrated a lysosomotropic action of desipramine in cultured human fibroblasts. It was also found that single doses of desipramine potentiate phospholipid methylation in the rat cerebral cortex (Racagni et al 1983). All these data suggest that tricyclic antidepressants induce phospholipidosis within membranes, this phenomenon being possibly connected—at least partially—with the well known adaptive changes of membrane receptors and membrane enzymes, produced by these drugs (for review see Brunello et al 1987).

The present work was aimed at assessing the effect of some antidepressant and neuroleptic agents on phospholipid methylation in in-vitro and ex-vivo experiments in the synaptic membranes of the rat cerebral cortex. The cerebral cortex was chosen for the experiments because most of the binding studies and the studies of the second messenger systems after administration of antidepressant drugs have been performed in this brain area.

Correspondence to: M. Melzacka, Institute of Pharmacology, Polish Academy of Sciences, 31-343 Krakow, Poland.

## Materials and Methods

Experiments were carried out on male Wistar rats, 190–200 g, which had free access to tap water and standard laboratory diet (Bacutil).

The drugs used were imipramine hydrochloride (IMI) (Polfa, Starogard), desipramine hydrochloride (DMI) (Serva, Heidelberg), citalopram hydrobromide (CIT) (Lundbeck, Copenhagen), chlorpromazine hydrochloride (CPZ) (Polfa, Starogard) and haloperidol hydrochloride (HAL) (Gedeon-Richter, Budapest).

*In-vitro experiment*

The animals were decapitated, the cortex was isolated and a synaptosomal fraction was prepared by the method of Hajos (1975). The total phospholipid (PL) methylation (phosphatidyl-*N*-methylethanolamine + phosphatidyl-*N*, *N*-dimethylethanolamine + phosphatidylcholine) in the cortical synaptosomal fraction was assayed by the method of Crews et al (1980). Briefly, 100 $\mu$L of synaptosomes suspended in a Tris-HCl buffer, pH 7·4 containing 0·2-0·4 mg protein $L^{-1}$ (Lowry et al 1951), was incubated with *S*-adenosyl-L-[$^3$H]methylmethionine (0·4 $\mu$Ci), Amersham UK spec. act. 15 Ci $mmol^{-1}$, 2 mM $MgCl_2$, 5 $\mu$M IMI or 5 $\mu$M DMI or 5 $\mu$M CIT or 10 $\mu$M CPZ or 10 $\mu$M HAL and Tris-HCl buffer, pH 7·4. The concentrations of antidepressants or neuroleptics added to the incubation mixtures corresponded to the mean cerebral concentrations of those drugs after their administra-

tion in pharmacological doses to rats. The reaction mixture was prepared at 4°C. The incubation was performed at 37°C for 30 min. The reaction was stopped by the addition of 20% aqueous solution of disodium trichloroacetate, and the samples were centrifuged at 27000 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 was added to the vial and the radioactivity was measured in a Beckman LS-3801 scintillation counter at 40% efficiency.

*Ex-vivo experiment*

The animals were treated with single or multiple doses of IMI, DMI or CIT (10 mg $kg^{-1}$ i.p. or 14 × 10 mg $kg^{-1}$ i.p., respectively), or with single or multiple doses of CPZ (2 mg $kg^{-1}$ i.p. or 14 × 2 mg $kg^{-1}$ i.p.) or HAL (0·1 mg $kg^{-1}$ i.p. or 14 × 0·1 mg $kg^{-1}$ i.p.). The rats were decapitated 24 h after administration of the respective drug (the chronically treated animals were killed 24 h after the last dose), the cerebral cortex was excised and the total PL methylation in the synaptosomes was assayed as described above.

The results were evaluated using an analysis of variance followed by Dunnet's test.

## Results

When added to the incubation mixture in a concentration of 5 μM, IMI DMI or CIT potentiated incorporation of [$^3$H]methyl into the cortical membrane of naive rats. A similar effect was produced by 10 μM CPZ, whereas HAL when added to the incubation mixture in a concentration of 10 μM did not change the basal activity of the system (Fig. 1).

Single doses of IMI, DMI, CIT, CPZ or HAL neither changed the basal activity of PL-methyltransferases in the synaptic cortical membranes of rats nor affected the sensitivity of those enzymes to the stimulating effect of IMI, DMI, CIT or CPZ, respectively (Figs 2, 3).

Repeated stress connected with chronic i.p. injection of water neither affected the activity of PL-methyltransferases in the synaptic cortical membranes of rats nor changed the response of the system to the stimulating effect of IMI, DMI, CIT or CPZ (Fig. 4).

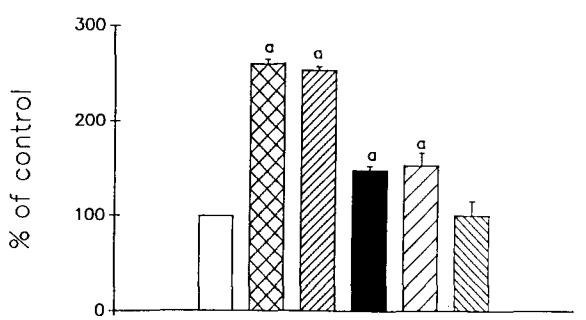


FIG. 1. The effect (left to right) of control (5442·63 + 247·66 dpm $mg^{-1}$ protein) imipramine (5 μM), desipramine (5 μM) citalopram (5 μM), chlorpromazine (10 μM) or haloperidol (10 μM) on phospholipid methylation in the synaptic cortical membranes of naive rats. n = 7-9. a—statistically significant when compared with the control.

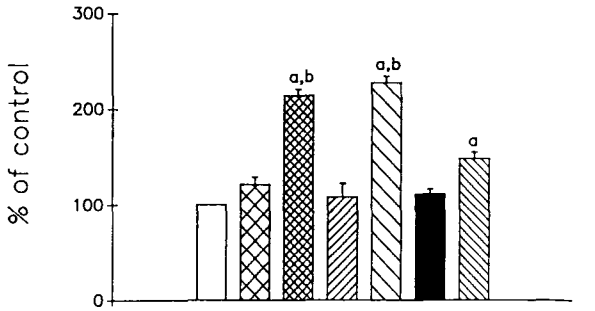


FIG. 2. The effect of a single dose (1 × 10 mg $kg^{-1}$ i.p.) of imipramine, desipramine or citalopram on phospholipid methylation in the synaptic cortical membranes of rats, in the presence of imipramine, desipramine or citalopram. Rats were killed 24 h after administration of antidepressant. Left to right: control (4989·05 + 321·98 dpm $mg^{-1}$ protein), imipramine (1 × 10 mg $kg^{-1}$ i.p.) imipramine (1 × 10 mg $kg^{-1}$ i.p.) + imipramine (5 μM), desipramine (1 × 10 mg $kg^{-1}$ i.p.) desipramine (1 × 10 mg $kg^{-1}$ i.p.) + desipramine (5 μM), citalopram (1 × 10 mg $kg^{-1}$ i.p.), citalopram (1 × 10 mg $kg^{-1}$ i.p.) + citalopram (5 μM). n = 7-9. a—statistically significant when compared with the control; b—statistically significant when compared with group treated with the corresponding antidepressant.

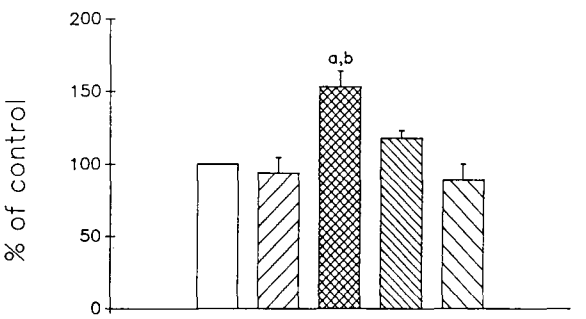


FIG. 3. The effect of a single dose of chlorpromazine (2 mg $kg^{-1}$ i.p.) or haloperidol (0·1 mg $kg^{-1}$ i.p.) on phospholipid methylation in the synaptic cortical membranes of rats, in the presence of chlorpromazine or haloperidol. The rats were killed 24 h after administration of chlorpromazine or haloperidol. Left to right: control (4398·90 + 354·12 dpm $mg^{-1}$ protein), chlorpromazine (1 × 2 mg $kg^{-1}$ i.p.) chlorpromazine (1 × 2 mg $kg^{-1}$ i.p.) + chlorpromazine (10 μM), haloperidol (1 × 0·1 mg $kg^{-1}$ i.p.), haloperidol (1 × 0·1 mg $kg^{-1}$ i.p.) + haloperidol (10 μM). n = 5-7. a—statistically significant when compared with the control; b—statistically significant when compared with the group treated with the corresponding neuroleptic.

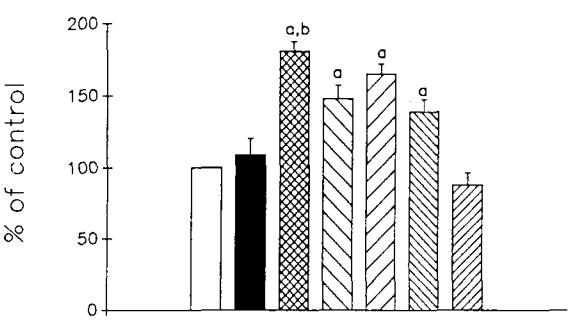


FIG. 4. The effect of repeated stress (two-week intraperitoneal injection of water) on phospholipid methylation in the synaptic cortical membranes of rats, in the presence of imipramine, desipramine, citalopram, chlorpromazine and haloperidol. The rats were killed 24 h after the last injection of water. Left to right: control, naive rats (5078·89 + 456·11 dpm $mg^{-1}$ protein), 14 × $H_2O$, 14 × $H_2O$ + imipramine (5 μM), 14 × $H_2O$ + desipramine (5 μM), 14 × $H_2O$ + citalopram (5 μM), 14 × $H_2O$ + chlorpromazine (10 μM), 14 × $H_2O$ + haloperidol (10 μM). n = 5-7. a—statistically significant when compared with the control; b— statistically significant when compared with the group treated with $H_2O$ for two weeks.

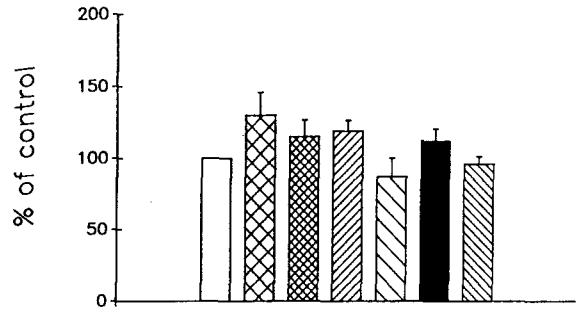


FIG. 5. The effect of prolonged administration of imipramine, desipramine or citalopram (14 × 10 mg $kg^{-1}$ i.p.) on phospholipid methylation in the synaptic cortical membranes of rats, in the presence of imipramine, desipramine or citalopram. The rats were killed 24 h after the last dose of antidepressant. Left to right: control (14 × $H_2O$) (5355·72 + 439·99 dpm $mg^{-1}$ protein), imipramine (14 × 10 mg $kg^{-1}$ i.p.), imipramine (14 × 10 mg $kg^{-1}$ i.p.) + imipramine (5 μM), desipramine (14 × 10 mg $kg^{-1}$ i.p.), desipramine (14 × 10 mg $kg^{-1}$ i.p.) + desipramine (5 μM), citalopram (14 × 10 mg $kg^{-1}$ i.p.), citalopram (14 × 10 mg $kg^{-1}$ i.p.) + citalopram (5 μM). n = 7-9.

Chronic administration of IMI, DMI or CIT (14 × 10 mg $kg^{-1}$ i.p.) to rats led to a slight increase in the basal PL-methyltransferase activity of animals treated chronically with IMI, when compared with animals receiving water for two weeks. However, addition of IMI, DMI or CIT in a concentration of 5 μM to incubation mixtures did not potentiate the incorporation of [$^3$H]methyl into the synaptic cortical membranes of rats treated with the respective antidepressants for two weeks (Fig. 5).

Chronic treatment of rats with CPZ (14 × 2·0 mg $kg^{-1}$ i.p.) or HAL (14 × 0·1 mg $kg^{-1}$ i.p.) did not induce any changes in the basal activity of PL-methyltransferases. Addition of HAL to the incubation mixture (10 μM) significantly inhibited incorporation of [$^3$H]methyl groups into the synaptic cortical membranes of rats treated chronically with HAL. CPZ (10 μM) potentiated the reaction in the synaptic cortical membranes of rats receiving CPZ for 14 days (Fig. 6).

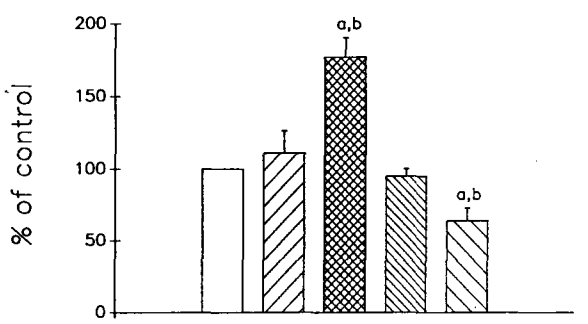


FIG. 6. The effect of prolonged administration of chlorpromazine (14 × 2 mg $kg^{-1}$ i.p.) or haloperidol (0·1 mg $kg^{-1}$ i.p.) on phospholipid methylation in the synaptic cortical membranes of rats, in the presence of chlorpromazine or haloperidol. The rats were killed 24 h after the last dose of chlorpromazine or haloperidol. Left to right: control (14 × $H_2O$) (5123·85 + 400·23 dpm $mg^{-1}$ protein), chlorpromazine (14 × 2 mg $kg^{-1}$ i.p.), chlorpromazine (14 × 2 mg $kg^{-1}$ i.p.) + chlorpromazine (10 μM), haloperidol (14 × 0·1 mg $kg^{-1}$ i.p.), haloperidol (14 × 0·1 mg $kg^{-1}$ i.p.) + haloperidol (10 μM). n = 5-7. a—statistically significant when compared with the control; b—statistically significant when compared with the group treated with the corresponding neuroleptic.

## Discussion

In concentrations corresponding to those found in the rat brain after pharmacological, single or multiple doses, all drugs, except HAL, potentiated the PL-methylation in cortical synaptic membranes in in-vitro experiments. This effect seems to be partly independent of the chemical structure of the agent used, as CIT a non-tricyclic drug, induced a similar, though weaker effect, to IMI or DMI, comparable in potency with the effect of CPZ.

Our results are consistent with those of Racagani et al (1983), who found an increase in PL-methylation in rat cortical membranes 1 h after administration of DMI at a dose of 10 or 20 mg $kg^{-1}$ i.p. According to the phamacokinetic data, the concentration of DMI in the rat brain 1 h after its administration at a dose of 10 mg $kg^{-1}$ i.p. was approximately 3–4 μM (Hrdina & Dubas 1981), i.e. similar to the DMI concentration used in our in-vitro experiments.

Potentiation of PL-methylation by drugs added externally to the incubation mixture may be explained in the following way. Molecules of amphiphilic drugs may interact with membranes (Römer & Bickel 1979; Zimmer & Schulze 1981), and may thus produce changes in the membrane organization. IMI given to rats in a single dose or added to the incubation mixture in a concentration equivalent to that found in the rat brain after pharmacological doses of IMI increased the fluidity of the cortical membranes of rats (Melzacka et al 1988; Wesemann et al 1988). Also CPZ induced changes in the fluidity of membranes isolated from ox brain white matter and erythrocytes (Neal et al 1976; Zimmer & Schulze 1981) and of artificial membranes (Römer & Bickel 1979). The increase in membrane fluidity potentiates the turnover of enzymatic processes (Gavish & Werber 1979), as well as the rate of lateral encounters (Hanski et al 1979) which, in turn, may facilitate the methylation of membrane PL.

The modulation of PL-methylation by altering the level of the substrate (phosphatidylethanolamine—PE) by externally added IMI, DMI, CIT or CPZ cannot be excluded; however the results of Honegger et al (1983), Lüllmann-Rausch (1979), Moor et al (1988) and Allan & Mitchell (1975) indicate that only prolonged exposure of the tissues to antidepressants and CPZ led to phospholipidosis in the membranes.

The stress connected with repeating intraperitoneal injections seems not to affect the rate of the PL methylation in the synaptic cortical membranes of rats. The responses of animals treated with water for two weeks to the stimulating effect of IMI, DMI, CIT and CPZ were similar to the respective responses of the control rats.

A two-week treatment of rats with IMI, DMI or CIT induced some changes in the sensitivity of PL-methyltransferases to the stimulating effect of the investigated antidepressants, since the 5 μM concentration of IMI, DMI or CIT added externally to the incubation mixture did not potentiate PL-methylation in the synaptic cortical membranes of rats treated chronically with antidepressants. We found previously (Melzacka et al 1988; Wesemann et al 1988) that chronic treatment of rats with IMI led to the resistance of the cortical membranes to the fluidizing effect of IMI. It was also found that, in contrast to short time exposure, long-

term treatment with IMI or DMI led to the accumulation of PL and induced marked differences in the PL contents in various tissue (Lüllmann-Rausch 1979; Moor et al 1988). Therefore, the chronic response of rats to the antidepressants might be a decrease in the level of the substrate (phosphatidylethanolamine—PE) and/or increase in the level of the product (phosphatidylcholine—PC) in the cerebral cortices. These changes might not affect the basal activity of PL-methyltransferases; however, they might inhibit the stimulating effect of externally added antidepressant. Therefore it is arguable that chronic treatment of rats with IMI, DMI or CIT produces changes in the physical and/or chemical properties of the membrane therefore different from those induced by short-term exposure of membranes to those agents. This membrane effect, produced by antidepressants given chronically, may be a primary one—at least partially—to the well-known adaptive changes in the membrane receptors and enzymes induced by long-term treatment with antidepressants (for review see Brunnelo et al 1987).

The effects of chronic CPZ and HAL differed from those of IMI, DMI or CIT. CPZ given to rats for two weeks did not change the sensitivity of PL-methyltransferases to the stimulating effect of CPZ, whereas chronic HAL altered to some extent PL-methyltransferases but differed from the effect induced by chronic antidepressants. The addition of HAL to synaptosomal membranes of rats treated chronically with HAL evoked the inhibition of the PL-methylation in-vitro.

The difference between the effect of chronic CPZ and chronic IMI or other antidepressants may be due, among others to a diverse mechanism of their interaction with the membrane. According to Römer & Bickel (1979) and Zimmer & Schulze (1981), IMI binds near the surface of liposomes only, whereas CPZ binds to the surface of the membrane and also penetrates into the inner hydrocarbon phase of the bilayer. Another explanation is that chronic CPZ did not affect the PL turnover in the same way as did prolonged administration of the antidepressant.

At the present it is difficult to explain the action of chronic HAL on phospholipid methylation. According to Zimmer & Schulze (1981), HAL, like CPZ, showed at low concentrations an increase of the order at the surface of the membrane (decrease of fluidity), while an increased fluidity of the hydrophobic interior of the membrane was induced only by CPZ and not by HAL. This could suggest some differences between membrane effects of CPZ and HAL. Since no data are available on the effect of HAL on PL turnover, some additional experiments are reqired to clarify the event.

As chronic CPZ and HAL exert contrary effects on the PL-methylation in the synaptic cortical membranes of rats, it seems that their action on the PL-methylation is not connected with the antipsychotic effect of either compound.

*Acknowledgements*

The authors wish to thank Mrs J. Drabik for her skilful technical assistance.

## References

Allan, D., Mitchell, R. H. (1975) Enhanced synthesis de novo of phosphatidylinositol in lymphocytes treated with cationic amphiphilic drugs. Biochem. J. 148: 471–478

Bickel, M. H., Steele, J. W. (1974) Binding of basic and acidic drugs to rat tissue subcellular fractions. Chem. Biol. Interact. 8: 151–162

Brunello, L., Cicerano, U., Rovescalli, A.C., Riva, M., Galimberti, R., Franzetti, C., Racagni, G. (1987) Receptor interactions in antidepressant neurochemical mechanisms: modulation by membrane components. In: Fuxe, K., Agnati, L. F. (eds) Receptor-Receptor Interaction. McMillan Press Ltd, London, pp 398–406

Crews, F. T., Hirata, F., Axelrod, J. (1980) Identification and properties of methyltransferases that synthesize phosphatidylcholine in rat brain synaptosomes. J. Neurochem. 34: 1491–1498

Di Perri, B., Calderini, G., Battistella, A., Raciti, R., Toffano, G. (1983) Phospholipid methylation increases $^3$H-diazepam and $^3$H-GABA binding in membrane preparations of rat cerebellum. Ibid. 41: 302–308

Gavish, B., Werber, M. (1979) Viscosity-dependent structural fluctuations in enzyme catalysis. Biochemistry 18: 1269–1275

Hajos, F. (1975) An improved method for the preparation of synaptosomal fractions in high purity. Brain Res. 93: 485–489

Hanski, E., Rimon, G., Levitzki, A. (1979) Adenylate cyclase activation by the $\beta$-adrenergic receptors as a diffusion-controlled process. Biochemistry 18: 846–853

Hirata, F., Axelrod, J. (1978) Enzymatic methylation of phosphatidylethanolamine increases erythrocyte membrane fluidity. Nature 275: 219–220

Hirata, F., Axelrod, J. (1980) Phospholipid methylation and biological signal transmission. Science 209: 1082–1090

Hrdina, P. D., Dubas, T. C. (1981) Brain distribution and kinetics of desipramine in the rat. Can. J. Physiol. Pharmacol. 59: 163–167

Honegger, U. E., Roscher, A. A., Wiesmann, U. N. (1983) Evidence for lysosomotropic action of desipramine in cultured human fibroblasts. J. Pharmacol. Exp. Ther. 225: 436–441

Ishizaka, T., Hirata, F., Ishizaka, K., Axelrod, J. (1980) Stimulation of phospholipid methylation, $Ca^{2+}$ influx and histamine release by bridging of IgE receptors on rat mast cells. Proc. Natl. Acad. Sci. 77: 1903–1906

Le Fur, G., Phan, T., Canton, T., Tur, C., Uzan, A. (1981) Evidence for a coupling between dopaminergic receptors and phospholipid methylation in mouse B lymphocytes. Life Sci. 29: 2737–2749

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Lüllmann-Rausch, R. (1979) Drug-induced lysosomal storage disorders. In: Dingle, J. T., Jacques, P. J., Shaw, I. H. (eds) Lysosomes in Applied Biology and Therapeutics vol 6, North-Holland Publishing Co., Amsterdam, pp 49–130

Melzacka, M., Wesemann, W., Sontag, K. H., Nocon, H., Danek, L. (1988) Membrane effects of imipramine within the central nervous system of rats. Abstracts of 12th International Symposium on Clinical Pharmacology. Poland, Wroclaw, p. 23

Moor, M., Honegger, U.E., Wiesmann, U.N. (1988) Organspecific, qualitative changes in the phospholipid composition of rats after chronic administration of the antidepressant drug desipramine. Biochem. Pharmacol. 37: 2035–2039

Neal, M. J., Butler, K. W., Polnaszek, C. F., Smith, I. C. P. (1976) The influence of anesthetics and cholesterol on the degree of molecular organization and mobility of ox brain white matter. Mol. Pharmacol. 12: 144–155

Racagni, G., Mocchetti, I., Calderini, G., Battistella, A., Brunello, N. (1983) Temporal sequences in central noradrenergic system of rat after prolonged antidepressant treatment: receptor desensitization and neurotransmitter interactions. Neuropharmacology 22: 415–424

Römer, J., Bickel, M. H. (1979) Interactions of chlorpromazine and imipramine with artificial membranes investigated by equilibrium dialysis, dual-wavelength photometry and fluorimetry. Biochem. Pharmacol. 28: 799–805

Strittmatter, W. J., Hirata, F., Axelrod, J. (1979) Phospholipid methylation unmasks cryptic $\beta$-adrenergic receptors in rat reticulocytes. Science 204: 1205–1207

Wesemann, W., Melzacka, M., Lausch, R. O., Weiner, N., Mennel, H. D., Sontag, K. H. (1988) Age- and drug-dependent changes of serotonin binding sites and membrane architecture. Abstracts of XVIth C.I.N.P. Congress. Psychopharmacology 96(Suppl.): p 165

Zimmer, G, Schulze, P. (1981) Membrane action of tricyclic drugs. Arzneim. Forsch. 31: 1389–1392