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THE EFFECTS OF IMIPRAMINE ON THE METHYLATION OF PHOSPHATIDYLETHANOLAMINE (PE) IN THE CORTICAL MEMBRANES OF WISTAR RATS

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Abstract—This study investigates the effect of imipramine (IMI) on the methylation of phosphatidylethanolamine (PE) in crude cortical membranes of rat brain *in vitro* and *ex vivo*. It was found that IMI enhanced the formation of phosphatidyl-*N*-monomethylethanolamine (PME) and phosphatidyl-*N,N*-dimethylethanolamine (PDE) and inhibited the formation of phosphatidylcholine (PC) in the cortical membranes of rats *in vitro*. The same effect i.e. increased incorporation of methyl groups in PE and PME and decreased formation of PC was found in the cortical membrane of rats killed 1 hr after intraperitoneal administration of IMI at a single dose of 10 mg/kg. Chronic treatment of rats with IMI for 14 days with a daily dose of 10 mg/kg i.p. led to further inhibition of PC formation but did not affect the formation of PME and PDE and abolished the stimulating effect of IMI on the formation of PME and PDE *in vitro*.

In spite of the fact that tricyclic antidepressants (TCAs†) have been used clinically for a long time, their mechanism of action remains unknown. All attempts to clarify the nature of TCA's therapeutic effect have been unsuccessful probably because of the heterogeneity of their pharmacological effects within the central nervous system (CNS) and the complexity of the brain.

It is well documented that after prolonged administration TCAs produce changes in the density and affinity of several membrane receptors [1, 2], affect the neurotransmitter turnover [3, 4] and alter the activity of some membrane enzymes [5]. The results of *in vitro* [6, 7] and *ex vivo* [6, 8] studies indicate that some TCAs exert effects on the membranes isolated from various brain areas and affect phospholipid (PL) metabolism. As the physicochemical state of the membranes can play an important role as a regulatory factor in many membrane-mediated events [9], a link may exist between functional alterations of membrane receptors and/or enzymes in the CNS and changes in biomembrane architecture induced by prolonged exposure of the tissue to TCAs.

We have found before [6, 8] that imipramine (IMI), desipramine (DMI) and citalopram (CIT) increased PL methylation in the cortical membranes of rats *in vitro*, and that chronic treatment of rats with these drugs decreased the sensitivity of phospholipidmethyltransferases (PLMT) to the stimulating effects of IMI, DMI or CIT *in vitro*. According to Hirata and Axelrod [10] and Mato and Alemany [11] PL methylation regulates the fluidity characteristics of membranes and plays an important role in many membrane-mediated events; therefore the effect of IMI, DMI or CIT on PL methylation in the cortical membranes of rats may be of importance for their pharmacological action in the CNS.

Earlier findings [6, 8] showed the stimulating effect of IMI, DMI and CIT on the total PL methylation in cortical membranes of rats. However, the process consists of at least two steps [12], the first resulting in formation of phosphatidyl-*N*-monomethyl-

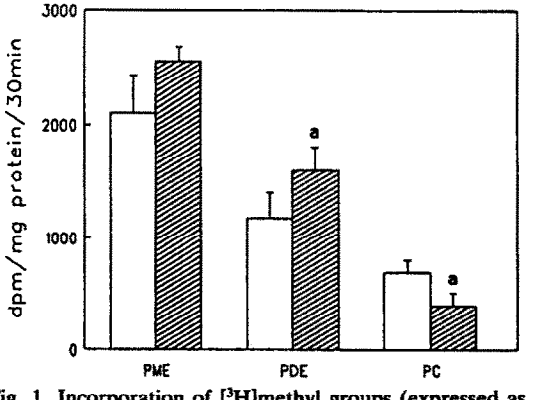

Fig. 1. Incorporation of [^{3}H]methyl groups (expressed as dpm/mg protein/30 min) into PE in the cortical membranes of rats killed 1 hr after administration of IMI at a single dose of 10 mg/kg i.p. (□) Control (rats treated acutely with saline); (▨) rats treated with a single dose of IMI. Results are the means of six to eight rats ± SD. a, Statistically significant as compared with the control.

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† Abbreviations: PE, phosphatidylethanolamine; PME, phosphatidyl-*N*-monomethylethanolamine; PDE, phosphatidyl-*N,N*-dimethylethanolamine; PC, phosphatidylcholine; TCAs, tricyclic antidepressants; IMI, imipramine; PL, phospholipid; DMI, desipramine; CIT, citalopram; PLMT, phospholipidmethyltransferases.

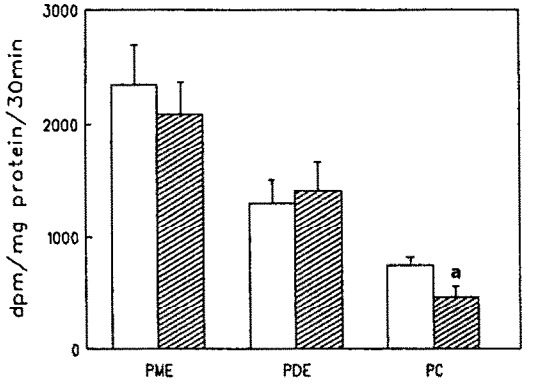

Fig. 2. Incorporation of [^{3}H]methyl groups into PE in the cortical membranes of rats killed 1 hr after administration of the last dose of IMI given to rats for 14 days in a daily dose of 10 mg/kg i.p. (□) Control (rats treated for 14 days with saline); (▨) rats treated chronically with IMI. Results are the means ± SD of five to seven animals. a, Statistically significant as compared with the control.

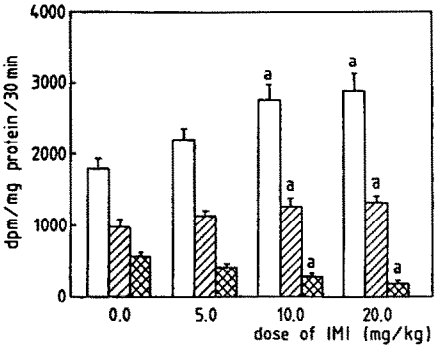

Fig. 3. The effect of different doses of IMI (5–20 mg/kg i.p.) on the formation of PME, PDE and PC in the cortical membranes of rats killed 1 hr after administration of IMI. (□) PME; (▨) PDE; (▩) PC. Results are the means ± SD of five rats. a, Statistically significant as compared with the control (IMI dose = 0).

ethanolamine (PME) from phosphatidylethanolamine (PE) and the second in an intermediate phosphatidyl-*N,N*-dimethylethanolamine (PDE) and the final product phosphatidylcholine (PC). As these three compounds are localized differently in biomembranes [12] and functionally play various roles in the interaction between exogenous amphiphilic compounds and PL [13], it seemed of importance to study how IMI given either acutely or chronically affects the first and the second step of PE methylation in the cortical membranes of rats *in vitro* and *ex vivo*.

MATERIALS AND METHODS

Animals. Experiments were carried out on male, Wistar rats, 180–200 g, which had free access to tap water and standard laboratory diet (Bacutil). They received IMI (hydrochloride, the Sigma Chemical Co, Poole, U.K.) either in a single dose of 10 mg/kg i.p. or chronically for 2 weeks in a daily dose of 10 mg/kg i.p. Some animals received IMI in a single dose of 5 or 20 mg/kg i.p. Control animals received saline in a corresponding dosage schedule.

Isolation of crude membranes. One or twenty-four hours after administration of IMI (in the case of chronically treated animals 1 or 24 hr after the last dose of IMI) the rats were decapitated, the brains were removed, the cortex was dissected and crude membranes (pellet P_2) isolated according to the following procedure: the tissue was homogenized in 10 vol. (10 mL per gram of the wet tissue) of ice-cold sucrose (0.32 M) and centrifuged at 770 *g* for 10 min. The supernatant was centrifuged at 40,000 *g* for 20 min and the resulting pellet was suspended in 20 vol. of Tris–HCl buffer (pH = 7.4). Then the suspension was incubated at 37° for 10 min and centrifuged at 40,000 *g* for 20 min. The resulting pellet was used for assay of PL methylation.

Assessment of PL methylation. PL methylation was measured according to Crews *et al.* [12]. Briefly, 100 μL of synaptosomes suspended in Tris–HCl buffer (pH = 7.4) containing 0.2–0.4 mg protein/mL [14] were incubated with *S*-adenosyl-L-[^{3}H]-methylmethionine (0.4 μCi; Amersham, U.K., sp. act. 15 Ci/mmol). To some samples 0.1–10 μM of IMI was added, i.e. the concentrations which corresponded to the mean cerebral levels of IMI at different time-intervals after its administration to rats at a dose of 10 mg/kg i.p. [15]. The incubation was carried out at 37° for 30 min. The reaction was stopped by the addition of 20% of trichloroacetic acid and methylated PLs were extracted with chloroform. Chloroform extracts after clarification were applied on a silica gel G plate (Merck, Darmstadt, F.R.G.) and the separation of methylated PL i.e. PME, PDE and PC was performed in chloroform + propionic acid + *n*-propylalcohol + water (2:2:3:1, by vol.) solvent system against the standards of PME, PDE and PC (all obtained from Sigma). After development the corresponding parts of the plate were placed in the vials together with the scintillation fluid and the radioactivity of the samples were measured in a Beckman LS-3801 scintillation counter at 38% efficiency.

The results were evaluated statistically using analysis of variance followed by Dunnett's test.

RESULTS

Ex vivo *study*

The cortical membranes of animals killed 1 hr after administration of a single, 10 mg/kg dose of IMI showed increased formation of PME and PDE and decreased formation of PC in comparison with the control (Fig. 1). These effects were not found when animals were killed 24 hr after administering a single dose of 10 mg/kg of IMI i.p.

Chronic treatment of rats with IMI (14 × 10 mg/kg i.p.) did not affect the formation of PME and PDE in the cortical membranes of rats killed 1 or 24 hr after the last dose of IMI. However prolonged

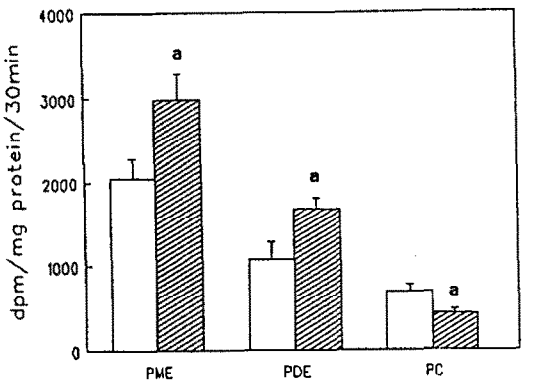

Fig. 4. The effect of 5 μM IMI on the incorporation of [^{3}H]-methyl groups into PE in the cortical membranes *in vitro*. (□) Control (naive rats); (□) 5 μM IMI added to cortical membranes of naive rats. Results are the means of five to eight animals ± SD. a, Statistically significant as compared with the control.

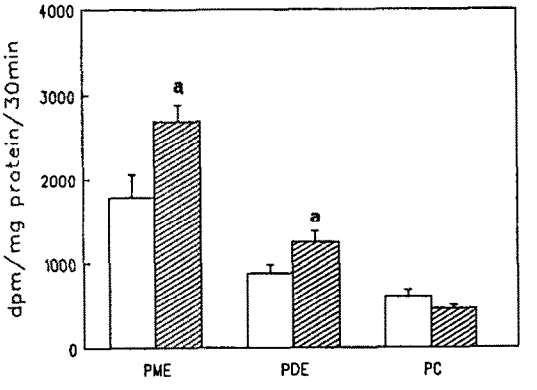

Fig. 5. Effect of 5 μM IMI *in vitro* on the incorporation of [^{3}H]methyl groups into PE in the cortical membranes of rats killed 24 hr after administration of IMI in a single dose of 10 mg/kg i.p. (□) Control (rats killed 24 hr after administration of IMI); (▨) 5 μM IMI added to cortical membranes of rats killed 24 hr after IMI administration. Results are the means ± SD of six to eight rats. a, Statistically significant as compared with control.

administration of IMI inhibited significantly the formation of PC in the cortical membranes of animals decapitated 1 or 24 hr after the last dose of IMI (Fig. 2).

Given to rats in different single doses (5, 10 or 20 mg/kg i.p.) IMI enhanced PME and PDE formation and decreased PC formation in the cortical membranes of animals killed 1 hr after administration of IMI. The effect was dose-dependent only for PC formation (Fig. 3).

In vitro *study*

IMI (5 μM) added to a mixture of cortical membranes of saline treated rats (control) enhanced significantly the formation of PME and PDE and decreased the formation of PC as compared to control (Fig. 4).

IMI (5 μM) when added to the cortical membranes of rats decapitated 24 hr after administration of IMI (1 × 10 mg/kg i.p.) enhanced the formation of PME and PDE and slightly decreased the formation of PC (Fig. 5). It must be emphasized that the addition of IMI (5 μM) to the cortical membranes of animals killed 1 hr after administration of IMI (1 × 10 mg/kg i.p.) neither potentiated nor inhibited the effects (shown in Fig. 1) produced by a single dose of IMI given to intact rats.

IMI (5 μM) added to the cortical membranes of animals treated chronically with IMI (14 × 10 mg/kg i.p.) and killed 24 hr after the last dose of the drug neither enhanced nor inhibited the formation of PDE or PME but potentiated (not significantly) the inhibitory effect of chronic IMI on PC formation found *ex vivo* (Fig. 6).

There was no linear relationship between IMI concentration (0.1–10 μM) and enhancement of PME or PDE formation *in vitro* (Fig. 7).

IMI (0.1–10 μM) inhibited PC formation *in vitro* in a concentration-dependent fashion (Fig. 7).

DISCUSSION

Our results indicate that IMI when added to the cortical membranes prepared from saline treated rats or to the cortical membranes of rats killed 24 hr after administration of IMI at a single dose of 10 mg/kg i.p. enhanced the formation of PME and PDE and inhibited the formation of PC. A similar effect i.e. an elevation of PME and PDE formation and decreased production of PC was found in the cortical membranes of rats killed 1 hr after administration of IMI at a single dose (1 × 10 mg/kg i.p.) while 24 hr after administration of IMI (1 × 10 mg/kg i.p.) the incorporation of methyl groups was at control levels. Our previous pharmacokinetic data indicate that 1 hr after intraperitoneal administration of IMI at a single dose of 10 mg/kg, its concentration in the rat brain was approximately 1 μM; however 24 hr after administration of the same dose the level in the rat brain was very low (less than 0.1 μM) [15]. This and the present results of *in vitro* experiments with different concentrations of IMI (0.1–10 μM) indicate that the enhancement of PME and PDE formation by IMI *in vivo* and *in vitro* needs a relatively high concentration of IMI in the medium.

The question arises what is the mechanism of the effect i.e. the increase of PME and PDE formation by relatively high concentrations of IMI. It is well documented that in contrast to inhibition, the induction of the enzymatic systems by exogenous agents needs prolonged exposure to the inducers [16]. Therefore the short exposure to IMI in our experimental models (30 min *in vitro* or 1 hr *in vivo*) could hardly activate corresponding methyltransferases (PLMT) in the cortical membranes of rats. We believe that the elevated incorporation of the first and second methyl group in PE is due to the interaction between the IMI molecule and membrane lipids [17] resulting in some alterations of the lipid bilayer which in turn facilitates the interaction between PLMT and PE. It was found before that IMI fluidized cortical membranes of rats

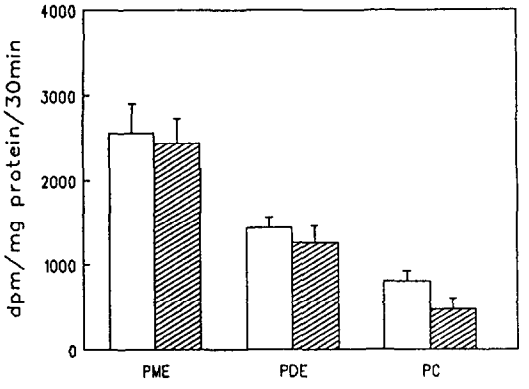

Fig. 6. Effect of 5 μM IMI *in vitro* on the incorporation of [^{3}H]methyl groups into PE in the cortical membranes of rats killed 24 hr after administration of the last dose of IMI given to rats for 14 days at a daily dose of 10 mg/kg i.p. (□) Control (rats treated for 14 days with IMI); (▨) 5 μM IMI added to the cortical membranes of rats treated for 14 days with IMI. The results are the means ± SD of six to seven rats.

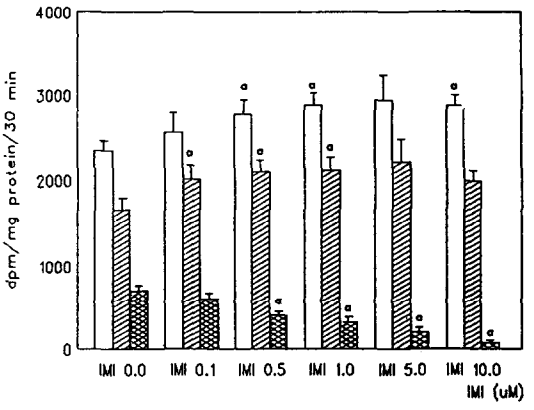

Fig. 7. Effect of 0.1–10 μM IMI on the formation of PME, PDE and PC in the cortical membranes of naive rats *in vitro*. (□) PME; (▨) PDE; (■) PC. Results are the means of seven to eight rats ± SD. a, Statistically significant as compared with the control.

in vitro [6] and this may potentiate the turnover of enzymatic processes and increase the rate of lateral encounters within the membrane [18, 19]. The lack of a linear relation between IMI at a higher concentration and the intensity of its stimulating effect on PME or PDE formation found *in vitro* and *ex vivo* (i.e. no significant differences between effects of 0.5–10.0 μM IMI *in vitro* and doses of IMI 10 and 20 mg/kg i.p. *ex vivo*) support this hypothesis.

The inhibition of PC formation by IMI found *in vitro* and *ex vivo* after single and prolonged administration of IMI may have a competitive character. Both IMI and PDE have tertiary amino groups with two methyl substituents and the third methyl group is introduced into PDE or IMI molecules through the same mechanism, i.e. formation of covalent bonds between the methyl group and the nitrogen atom of the tertiary amine to form the corresponding zwitterionic compounds. It cannot be excluded that PDE and IMI molecules compete for the same methyltransferase and the presence of IMI in the brain tissue inhibits the further methylation of PDE to PC. In fact we have found in this study a linear relationship between IMI concentration (0.1–5 μM) or the dose of IMI (5–20 mg/kg i.p.) and inhibition of PC production *in vitro* and *ex vivo*. This supports our hypothesis.

In the chronic experiment 1 and 24 hr after administration of the last dose of IMI the formation of PME and PDE was only slightly elevated despite the high brain concentration of IMI *in vivo* [15] and the addition of exogenous IMI (5 μM) into the mixture of cortical membranes of chronically treated animals. It was found before that in contrast to short exposure time, prolonged exposure of the tissue to IMI or DMI led to the accumulation of PL and change in the quantitative relations among particular PLs [17, 20 21]. Moreover chronic treatment of rats with IMI led to resistance of cortical membranes to the fluidizing effect of IMI *in vitro* [6] and decreased cholesterol level in the cerbral cortex of rats [22]. These data suggest that prolonged administration of IMI to rats produces changes in the membrane architecture in the rat brain which may hinder the interaction between PE and PLMT. It is also possible that in this experimental model (i.e. chronic treatment with IMI) the main metabolite of IMI in rats DMI can exert a direct action on the formation of PME and PDE in the cortical membranes of rat brain. After prolonged administration of IMI its metabolite DMI accumulates to a high extent in the rat CNS [15] as the capacity of the tissue to bind nonspecifically the parent drug and DMI increases during chronic treatment with IMI [23]. On the basis of the binding study [24] it cannot be excluded that after chronic treatment with IMI trace amounts of DMI remain in the crude membrane preparation (pellet P_2). DMI, as a secondary amine in the medium containing high concentration of methyl donor (SAM), can be methylated to IMI competing with PE and PME for the corresponding methyltransferases and only a small amount of the enzymes may remain to catalyse the formation of PME and PDE.

Apart from the reactions involved in the disturbances of PL methylation induced by IMI, the results presented indicate that: (1) IMI exerts some effects on the PL methylation in the cerebral cortex of rats, (2) the effects of IMI on PL methylation are not the same after single and prolonged administration of the drug, (3) when considering the link between PL methylation and the transduction of biological signals through membrane, the effect of IMI on PL methylation should be taken into consideration to explain the pharmacological effects of IMI on the CNS of rats.

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