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THE EFFECT OF NEUROLEPTICS ON IMIPRAMINE DEMETHYLATION IN RAT LIVER MICROSOMES AND IMIPRAMINE AND DESIPRAMINE LEVEL IN THE RAT BRAIN

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Abstract—A study of the cytochrome P-450 level and imipramine (IMI) demethylase activity in liver microsomes of rats treated concurrently with IMI and chlorpromazine (CPZ) or IMI and chlorprothixene (CPX) for two weeks were carried out. Concomitant administration of IMI and CPZ or IMI and CPX elevated the cytochrome P-450 level and accelerated IMI demethylation in *in vitro* study. Kinetic study of IMI demethylation carried out in the absence or in the presence of CPZ or CPX revealed that those neuroleptics inhibited IMI demethylation via competitive mechanism. Simultaneously with the enzymatic study the brain level of IMI and its demethylated metabolite desipramine (DMI) was assessed. It was found that 1 hr after withdrawal of IMI and CPZ or IMI and CPX the brain level of IMI was elevated in comparison with that of IMI treated animals, and the ratio between DMI/IMI brain concentration was decreased. When the assessment of IMI and DMI brain level was performed 24 hr after withdrawal of IMI and CPZ or IMI and CPX, there was no difference between the concentration of IMI and DMI in both, experimental and control animals.

As schizoaffective disorders and depression are often associated clinically, such agents as neuroleptics and antidepressants are frequently used in combination [1, 2]. For this reason a possible pharmacokinetic interaction between neuroleptics and tricyclic antidepressants (TAD) was investigated; it was found that such a combination of drugs induced an increase in the plasma and brain level of TAD in both man and rats [3–5], which might lead to potentiation of central and peripheral side effects of TAD [6].

A pharmacokinetic drug interaction was reported to be connected with the metabolism of drugs rather than their absorption, distribution or elimination. Actually, according to Gram *et al.* [4] the elevation in the TAD concentration in rats was due to the inhibition of hydroxylation and/or glucuronation of TAD by a concurrent administration of neuroleptics.

However, the main step in TAD biotransformation of the tertiary amine structure in man and rat is not hydroxylation but *N*-demethylation [7–9]; and this process may also be affected by a concurrent administration of neuroleptics.

There are no literature data concerning the effect of neuroleptics on the rate of *N*-demethylation; therefore we investigated the cytochrome P-450 level and imipramine (IMI) demethylase activity in liver microsomes of rats treated concurrently with IMI and chlorpromazine (CPZ) or IMI and chlorprothixene (CPX) for two weeks (the dosage schedule which simulated the clinical model of concurrent administration of those drugs [2, 5, 6]).

Simultaneously with the enzymatic study we assayed the brain level of IMI and its *N*-demethylated metabolite desipramine (DMI) in the same rats in order to find out whether concurrent administration of CPZ or CPX and IMI affected the level of IMI and DMI in the central nervous system (CNS) of rats.

The results of enzymatic and pharmacokinetic studies were compared with the corresponding results obtained earlier, when haloperidol (HAL) was given to rats in the same manner with IMI [10]. Such a comparison could lead to a more general conclusion concerning the effect of neuroleptics with a different chemical structure of the TAD biotransformation in rats.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats (200–250 g) kept under standard laboratory conditions at a fixed 12:12 hr light/dark cycle. The animals were fed on a standard granulated diet (Bacutil) until 18 hr before the experiment, and had free access to tap water. They received orally (with the drinking water) either IMI (hydrochloride, Polfa) in a dose of 20 mg/kg day, or CPZ (hydrochloride, Polfa) in a dose of 2.5 mg/kg/day, or CPX (hydrochloride, Hoffman-La Roche) in a dose of 2.0 mg/kg/day, or a combination of those drugs, i.e. IMI and CPZ or IMI and CPX in the doses mentioned above (it was found before that during 24 hr each rat drinks approx. 20 ml of water). The drugs were administered to rats for two weeks. Control animals received water.

Livers were excised 24 hr after the withdrawal of drugs. Microsomes were prepared according to the conventional methodology, by a differential centrifugation in Tris/KCl buffer (pH = 7.4). The cytochrome P-450 level was assayed according to Omura and Sato [11]. Protein was assessed according to Lowry *et al.* [12]. The *in vitro* studies of IMI

demethylation were carried out according to Daniel *et al.* [13]. The amount of IMI used as a substrate was 200 nmol/ml. The mixture was incubated for 25 min at 37°. Formaldehyde was measured according to Nash [14]. In kinetic experiments the following amount of IMI was used in *in vitro* demethylation: 40, 50, 60, 100, 200 and 300 nmol/ml. As the investigated neuroleptics were also demethylated, it was impossible to study the kinetics of IMI demethylation by the formaldehyde methodology [14]. Therefore each sample contained, in addition to the strictly determined amount of unlabelled IMI, a corresponding amount of ^{14}C IMI (48 mCi/mmol), i.e. 13–100,000 d.p.m. The incubations were performed either in the presence or in the absence of 300 nmol/ml of CPZ or CPX. After incubation the mixture was alkalized with ammonium hydroxide and extracted with 1 ml of 20% hexane solution in butanol. The organic phase was separated, evaporated to dryness and the residue was dissolved in 25 μl of methanol. Five μl of that solution was spotted on a chromatoplate. IMI and DMI were separated by a continuous TLC according to Rurak *et al.* [15], using a flat, horizontal tank of a sandwich type, equipped with an eluent distributor. The whole apparatus was constructed according to Soczewiński and Wawrzynowicz [16]. The spots were localized in the u.v. light, cut out and placed separately in the scintillation vial containing 0.5 ml of methanol; then 5 ml of the scintillation mixture (toluene solution of PPO and POPOP, 0.45% and 0.01% respectively) were added to the vial, and the radioactivity was measured with a 72% efficiency in a Packard TRIS-CARB 3255 scintillation counter. The results of these kinetic experiments were evaluated according to Lineweaver and Burk [17].

Pharmacokinetic studies were carried out at 1 or 24 hr after the withdrawal of drugs. The IMI and DMI level in the rat brain was assessed spectrofluorometrically according to Dingell *et al.* [9].

The results were elaborated statistically using Duncan's or Student's *t*-tests.

RESULTS

CPZ, CPX and HAL-when given to rats for two weeks did not affect the cytochrome P-450 level in liver microsomes of rats (Fig. 1); however, some tendencies were observed to increase the cytochrome P-450 level, particularly in the case of HAL [10].

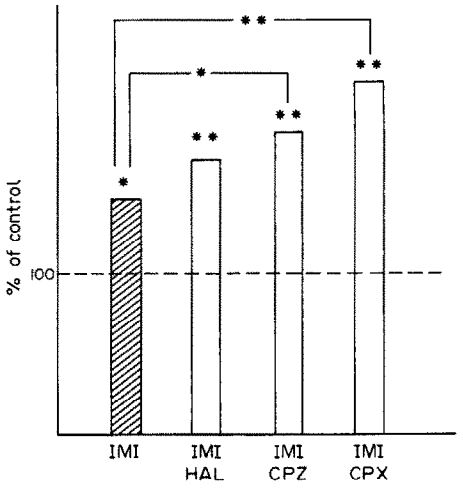

Fig. 2. Cytochrome P-450 level in liver microsomes of rats treated for two weeks with imipramine (IMI), 20 mg/kg/day, or a combination of IMI, 20 mg/kg/day, and haloperidol (HAL), 0.2 mg/kg/day, or IMI, 20 mg/kg/day, and chlorpromazine (CPZ), 2.5 mg/kg/day, or IMI, 20 mg/kg/day, and chlorprothixene (CPX), 2.0 mg/kg/day. Control = 0.47 ± 0.03 nmol/mg protein; N = 6; *P < 0.05; **P < 0.01 in comparison with control animals (Duncan's test).

A concurrent, prolonged administration of IMI and CPZ or IMI and CPX elevated the cytochrome P-450 level in liver microsomes of rats in a statistically significant manner in comparison with both control and IMI-treated animals (Fig. 2). A combination of IMI and HAL, investigated earlier in this respect [10] produced a similar change; however, the effect was not as potent as that induced by IMI and CPZ or IMI and CPX (Fig. 2).

A prolonged administration of CPX to animals did not change the rate of IMI demethylation in a statistically significant manner; however, HAL showed a tendency to accelerate IMI demethylation, and CPZ slightly inhibited that process (Fig. 3).

A two-week treatment with IMI and the investigated neuroleptics accelerated significantly the rate

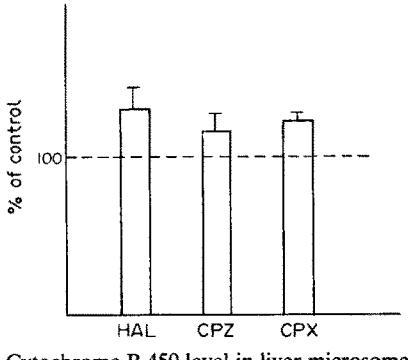

Fig. 1. Cytochrome P-450 level in liver microsomes of rats treated for two weeks with haloperidol (HAL), 0.2 mg/kg/day, chlorpromazine (CPZ), 2.5 mg/kg/day, or chlorprothixene (CPX), 2.0 mg/kg/day. The drugs were given orally. Control = 0.47 ± 0.03 nmol/mg protein; N = 6.

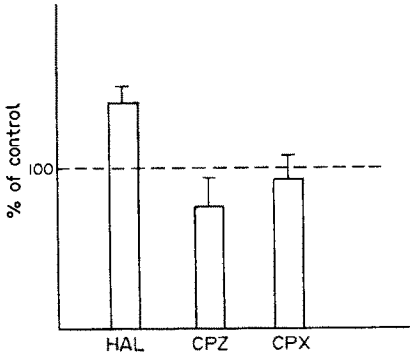

Fig. 3. The rate of IMI demethylation in liver microsomes of rats treated for two weeks with HAL, or CPZ, or CPX. For the key see Fig. 1. Control = 9.64 ± 0.84 nmol/25 min/mg protein; N = 6.

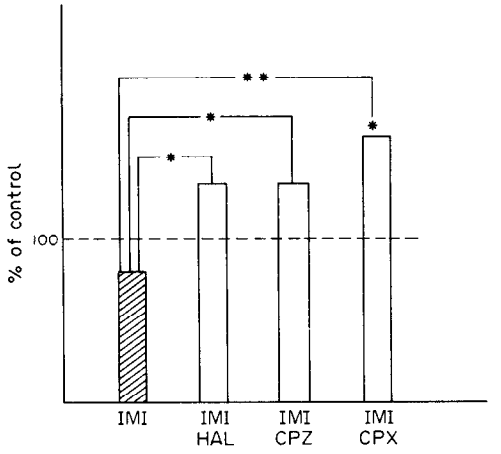

Fig. 4. The rate of IMI demethylation in liver microsomes of rats treated for two weeks with IMI, or IMI and HAL, or IMI and CPZ, or IMI and CPX. For the key see Fig. 2. Control = 9.64 ± 0.84 nmol/25 min/mg protein; N = 6; *P < 0.05; **P < 0.01 in comparison with control rats (Duncan's test).

of IMI demethylation in comparison with the rats treated with IMI alone. In rats treated with IMI and CPX, the change was statistically significant also when compared with that in control animals (Fig. 4).

Lineweaver–Burk plots revealed an enzymatic competition between IMI and the investigated neuroleptics (Fig. 5). The following values were obtained for the corresponding kinetic constants: $K_m = 0.54$ μmol, $V_{max} = 0.55 \times 10^{-2}$ μmol/mg protein/min, K_i(CPZ) = 0.208 μmol, K_i(CPX) = 0.108 μmol. Therefore CPX was twice as strong an inhibitor as CPZ, and three times more potent than HAL (K_i = 0.338 μmol acc. to Daniel and Melzacka [10]).

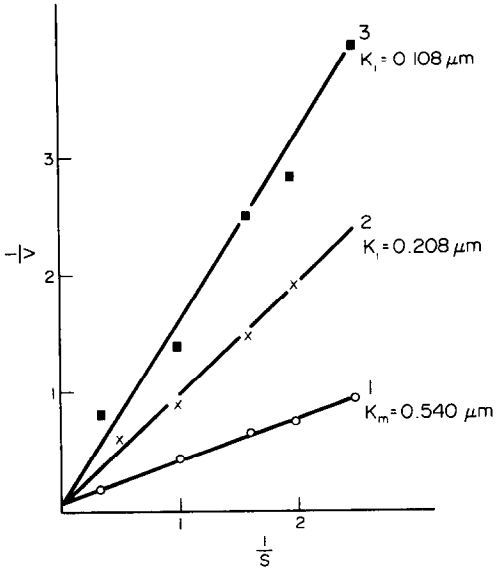

Fig. 5. Kinetics of the inhibition of IMI demethylation by CPZ or CPX *in vitro*. V = velocity of the reaction (mol × 10^{-8} of DMI/25 min/mg protein); s = concentration of IMI in the incubation mixture (mol × 10^{-7}/ml); 1—control (IMI), 2—IMI + CPZ, 3—IMI + CPX.

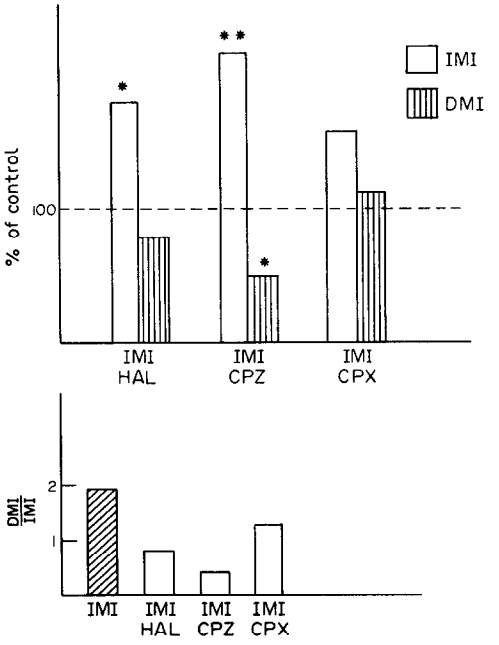

Fig. 6. The brain level of IMI and DMI and the ratio of DMI to IMI brain concentration 1 hr after the withdrawal of IMI and neuroleptics. Control (IMI-treated rats): IMI = 2.48 ± 0.20 μg/g, DMI = 4.52 ± 0.25 μg/g; N = 6; *P < 0.05; **P < 0.01 in comparison with IMI-treated animals (Student's *t*-test).

The brain level of IMI in rats treated concurrently with IMI and neuroleptics for two weeks, 1 hr after the withdrawal of drugs, was elevated in a statistically significant manner when compared with the IMI-treated animals (Fig. 6). It was followed by a decrease in the ratio of the brain concentration of IMI to that of DMI (Fig. 6).

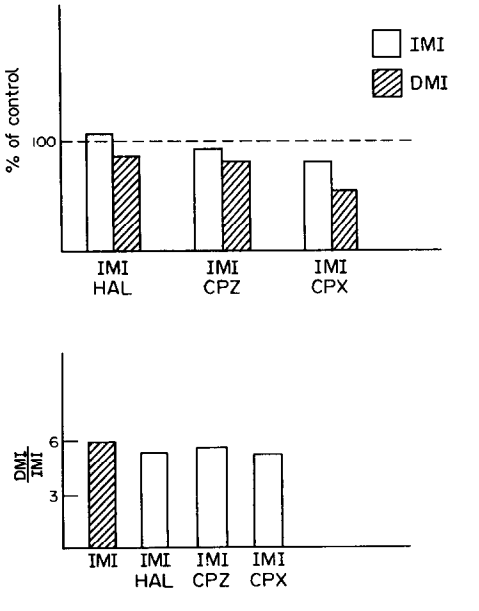

Fig. 7. The brain level of IMI and DMI and the ratio of DMI to IMI brain concentration 24 hr after the withdrawal of IMI and neuroleptics. Control (IMI-treated rats): IMI = 0.34 ± 0.05 μg/g; DMI = 1.12 ± 0.22 μg/g.

When assessment of IMI and DMI brain levels was carried out 24 hr after the withdrawal of drugs, neither the brain level of IMI nor the ratio of DMI to IMI brain concentration differed significantly from those of controls (Fig. 7).

DISCUSSION

A concurrent, prolonged administration of IMI and neuroleptics to rats elevated the brain level of IMI and decreased the ratio of DMI to IMI brain concentration. These pharmacokinetic results suggested inhibition of the IMI demethylase activity, evoked by a concurrent administration of IMI and neuroleptics. However, this inhibition did not proceed via hindrance of the IMI demethylase activity, as a prolonged administration of neuroleptics concurrently with IMI to rats accelerated the rate of IMI demethylation *in vitro*.

The discrepancy between the results of pharmacokinetic and enzymatic studies was probably due to the difference between experimental models used in these investigations. The method of microsome preparation used in our enzymatic study excluded the presence of previously administered IMI and the corresponding neuroleptics, and the pharmacokinetic studies were carried out 1 hr after the withdrawal of drugs, i.e. at the time when the concentrations of both IMI and neuroleptics in rats were very high [18, 19].

In order to simulate the *in vivo* situation and in *in vitro* experiments IMI and CPZ, or IMI and CPX were added concurrently to the incubation mixture (^{14}C-IMI as a substrate of the demethylation reaction), which resulted in inhibition of the IMI demethylation via a competitive mechanism.

When pharmacokinetic studies were performed 24 hr after the withdrawal of IMI and neuroleptics (at that time the concentration of IMI was still high [19], while the concentration of neuroleptic was low [18]), the brain level of IMI and DMI was almost the same as in the animals treated with IMI alone. This confirmed our assumption that inhibition of the IMI demethylation *in vivo* by CPZ, CPX or HAL required a respectively high concentration of neuroleptics in rats, the more so as neuroleptics accelerated the rate of IMI demethylation *in vitro*.

A comparison between the corresponding K_i values indicates that tricylic neuroleptics are stronger inhibitors of the IMI demethylation than the butyrophenone derivative HAL, and that the thioxantene derivative CPX is twice as strong as the derivative of phenothiazine CPZ.

The elevation of the cytochrome P-450 level in liver microsomes of rats and the acceleration of IMI demethylation *in vitro* by a concurrent administration of the investigated neuroleptics and IMI also depends on the chemical structure of neuroleptics. CPX was the most effective compound in both tests, whereas the weakest effect was exerted by HAL.

The final result of the discussed metabolic interaction between IMI and neuroleptics, i.e. the amount of DMI formed *in vivo*, depends on the potency of two opposite effects: the enhancement of the IMI demethylase activity by the investigated combination of drugs and the binding ability of the investigated drugs to the active centre of the enzyme. CPZ was as potent as HAL in acceleration of the IMI demethylation *in vitro*, when the drug was given to rats concurrently with IMI but it bound more readily to the active centre of the enzyme; therefore the ratio of DMI to IMI brain concentration 1 hr after the withdrawal of IMI and CPZ was more depressed than after a prolonged IMI and HAL treatment. When IMI was administered to rats jointly with CPX, the ratio of DMI to IMI brain concentration was higher than that observed in the IMI and HAL or the IMI and CPZ-treated animals. This was probably due to the most potent induction of the IMI *N*-demethylase and the strongest competitive inhibition showed by a combination of CPX and IMI. Apparently, these findings do not exclude participation of other factors in the final result, such as a different distribution of the investigated neuroleptics within rat tissues, a different rate of their elimination which might affect the actual concentration of drugs at enzymatic binding sites. In addition the investigated neuroleptics could compete at the plasma protein-binding sites with IMI or/and DMI and could also change the rate of penetration of these antidepressants through the biological membranes.

Another question concerns the mechanism of elevation of the cytochrome P-450 level and acceleration of IMI demethylation *in vitro*, induced by a concurrent administration of IMI and neuroleptics. Our results indicate that HAL and tricyclic neuroleptics change the pharmacokinetics of IMI via different mechanisms. When IMI and HAL were given jointly to rats, the final result in both the elevation of the cytochrome P-450 level and the acceleration of IMI demethylation *in vitro* was a summation while the elevation of the cytochrome P-450 concentration after a concurrent administration of IMI and CPZ, or IMI and CPX was a supra-addition. The combination of IMI and CPZ, or IMI and CPX accelerated the IMI demethylation *in vitro*; therefore its effect was opposite to that exerted by these drugs given individually to rats.

On the base of the presented results it is difficult to elucidate the mechanism of changes in the cytochrome P-450 level and IMI demethylase activity, produced by a prolonged administration of IMI and tricyclic neuroleptics. It could be suspected that they are due to some complex effect induced by the presence of IMI and CPZ, or IMI and CPX in rats, which affects the synthesis of enzymatic protein.

Our earlier study [20] found that the IMI demethylation proceeded via two different oxygenase systems, one dependent and the other independent of cytochrome P-450. On the basis of the results obtained in the present study it is difficult to determine which system is activated by the combination of IMI and investigated neuroleptics. The elevation of the cytochrome P-450 level induced by a concurrent administration of IMI and neuroleptics, followed by the acceleration of the IMI demethylation *in vitro*, suggests an activation of the cytochrome P-450-dependent system, however, an activation of another system, independent of cytochrome P-450, cannot be excluded.

The presented results lead us to a more general conclusion that the pharmacokinetic changes of IMI,

induced by a concurrent administration of IMI and neuroleptics are mainly due to the competitive inhibition of the IMI *N*-demethylation by investigated neuroleptics. However, the other enzymatic changes, such as the inhibition of hydroxylation and/or glucuronation [5, 21], as well as the induction of IMI demethylase that was discovered *in vitro* can also affect the final result of the pharmacokinetic interaction between IMI and the investigated neuroleptics.

REFERENCES

1. J. M. Davis, G. L. Klerman and J. J. Schildkraut, in *Psychopharmacology. A Review of Progress* (Ed. D. H. Efran), pp. 1957–1967. Washington Public. Health Service Publication.
2. M. Del Zampo, L. Ferini-Strambi, G. Martis and G. M. Corsini, *Proc. 4th CINP Congress, Florence*, S502 (1984).
3. R. W. Fuller, H. D. Snodoly and I. H. Slater, *Toxic. appl. Pharmac.* **29**, 259 (1974).
4. F. Gram, J. Christiansen and K. Fredricson-Overø, *Acta Pharmac. Toxic.* **35**, 223 (1974).
5. L. Bock, J. C. Nelson, S. Gray and P. I. Jatlow, *Clin. Pharmac. Ther.* **33**, 322 (1983).
6. J. C. Nelson and P. J. Jatlow, *Am. J. Psychiat.* **137**, 10 (1980).
7. M. H. Bickel, *Xenobiotica* **1**, 313 (1971).
8. P. L. Gigon and M. H. Bickel, *Biochem. Pharmac.* **20**, 1921 (1971).
9. J. V. Dingell, F. Sulser and J. R. Gillette, *J. Pharmac. exp. Ther.* **143**, 14 (1964).
10. W. Daniel and M. Melzacka, in *Cytochrome P-450 Biochemistry, Biophysics and Induction* (Eds. L. Vereczkey ann K. Magyar) p. 285. Akademiai Kiado, Budapest (1985).
11. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
13. W. Daniel, J. Friebertshäuser and C. Steffen, *Naunyn-Schmiedeberg's Archs Pharmac.* **328**, 83 (1984).
14. T. Nash, *Biochem. J.* **55**, 416 (1953).
15. A. Rurak, M. Melzacka and E. Soczewiński, *J. Chromatogr.* in press.
16. E. Soczewiński and T. Wawrzynowicz, *Chromatographia* **11**, 466 (1978).
17. H. Lineweaver and D. Burk, *J. Am. chem. Soc.* **56**, 658 (1934).
18. J. Lewi, J. J. P. Heykants, F. T. N. Allewijn, J. G. H. Dony and P. A. J. Janssen, *Arzneimittel-Forsch.* **20**, 943 (1970).
19. W. Daniel, A. Adamus, M. Melzacka and J. Syzmura, *J. Pharm. Pharmac.* **34**, 678 (1982).
20. W. Daniel and M. Melzacka, *Abstracts of 9th IUPHAR Congress London*, 1984, 564 pp.
21. C. von Bahr, E. Spina, C. Birgersson, Ö. Ericsson, M. Göransson and T. Henthorn, *Biochem. Pharmac.* **34**, 2501 (1985).