Biochemical Pharmacology, Vol. 23, pp. 1503–1507. Pergamon Press, 1974. Printed in Great Britain.

IMIPRAMINE-MEDIATED EFFECTS ON LEVODOPA METABOLISM IN MAN

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(*Received* 25 *June* 1973; *accepted* 19 *October* 1973)

Abstract—The effect of imipramine (IMP) on levodopa (L-dopa) metabolism was studied in four normal subjects. IMP pretreatment for 3 consecutive days prior to the administration of 500 mg L-dopa moderately decreased the urinary excretion of dopa. Similarly, IMP pretreatment decreased the urinary excretion of dopamine, norepinephrine and their major acid metabolites. In contrast, IMP pretreatment decreased the urinary excretion of serotonin but increased the excretion of its major acid metabolite, 5-hydroxyindoleacetic acid. The findings suggest that IMP-mediated decreased excretion of dopa and its metabolites studied is partially related to retarded gastrointestinal absorption of L-dopa and that IMP may alter the metabolic pathway of serotonin during L-dopa administration in man.

THE CLINICAL efficacy of imipramine (IMP) in the treatment of depressive states[1,2] and of levodopa (L-dopa) in the management of Parkinson's disease[3] is well established. IMP and L-dopa are administered simultaneously to patients with Parkinson's disease who develop depressive symptoms. IMP exerts an inhibitory effect on the reuptake of the catecholamines into the nerve endings,[4–6] while L-dopa increases the amounts of neuronally stored catecholamines. Therefore, co-administration of IMP with L-dopa may interfere in L-dopa metabolism. To test this possibility we studied the effect of IMP on the urinary excretion of exogeneously administered L-dopa and some major metabolites in normal subjects.

METHODS

Four normal healthy males, aged 22–25, volunteered for the study. They received a 500 mg dose of L-dopa by mouth, and urine samples were collected serially over 6·0 N HCl at hourly intervals during the initial 6 hr and at 24 hr. After a 2-week drug-free interval, the subjects received a 100 mg dose of IMP divided into four equal portions on each of 3 consecutive days. Then, L-dopa was given 1 hr after the last dose of IMP, and urine samples were collected as before. All urine specimens were aliquoted and kept frozen at -20° until chemical analyses were performed.

A portion of each urine specimen was fractionated for separation of urinary dopa from its amine metabolites as earlier described.[7] A second urine aliquot of the urine specimens was subjected to chromatography on Amberlite CG-50 resin column in H^+ cycle to retain L-dopa and the catecholamines. The effluent, containing the neutral and acid metabolites, was rechromatographed on basic aluminum hydroxide for the

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separation of homovanillic acid (HVA) and subsequent differential elution of dihydroxyphenylacetic acid (DOPAC), dihydroxymandelic acid (DOMA) and vanillylmandelic acid (VMA) from basic aluminum hydroxide column as previously described.[8] A third urine aliquot served for the determination of 5-hydroxyindoleacetic acid (5-HIAA) after the elimination of dopa from the sample by chromatography on Amberlite CG-50 in H^+ cycle and the extraction of 5-HIAA from the acidified effluent with butyl acetate. Quantitative determinations of dopa and dopamine (DA) followed a modification [9,10] of the hydroxyindole procedure of Carlsson and Waldeck.[11] VMA and HVA were determined according to the procedures of Pisano *et al.*[12] and Sato[13] respectively. The ethylenediamine condensation procedure was used for the quantitative estimation of DOMA and DOPAC in the separate functions as outlined by Werdinius.[14] Norepinephrine (NE) and epinephrine (E) were assayed by the method of Häggendal.[15] Also, serotonin (5-HT) and its major acid metabolite, 5-HIAA, were determined.[16,17]

The results for the quantitative measures are corrected for the per cent recovery of authentic internal standard compounds and reflect both free and conjugates, liberated by acid hydrolysis, of dopa, DA, NE, E and 5-HT and the free form of their acid metabolites studied. Their recoveries ranged from 65 to 75 per cent for dopa, the monoamines and their major acid metabolites and from 52 to 60 per cent for DOPAC and DOMA. The data are analyzed by *t*-test for correlated means.

RESULTS

Figure 1 presents urinary excretion of dopa, DA and their major acid metabolites, DOPAC and HVA, that was obtained after the oral administration of 500 mg L-dopa and also after the administration of 100 mg/day of IMP for 3 consecutive days prior to the administration of an identical dose of L-dopa. A decrease in the urinary excretion of dopa, DA, DOPAC and HVA was observed during the first 2 hr of the test that followed pretreatment with IMP. This initial decrease amounted to 18·5 per cent for dopa, 39·8 per cent for DA, 71·3 per cent for DOPAC ($P < 0{\cdot}01$) and 55·5 per

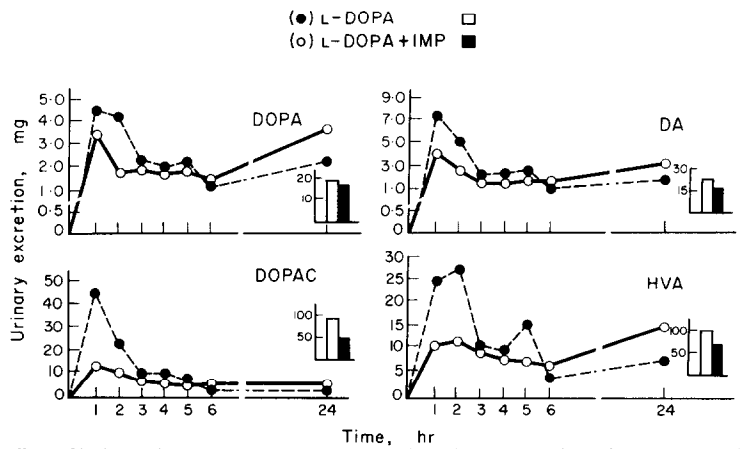

FIG. 1. Effect of imipramine (IMP) pretreatment on the urinary excretion of dopa, dopamine (DA) and their major acid metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) derived from orally administered L-dopa. Bar graphs display total cumulative 24-hr urinary output of metabolites studied. Results are given as means of four subjects at time intervals studied.

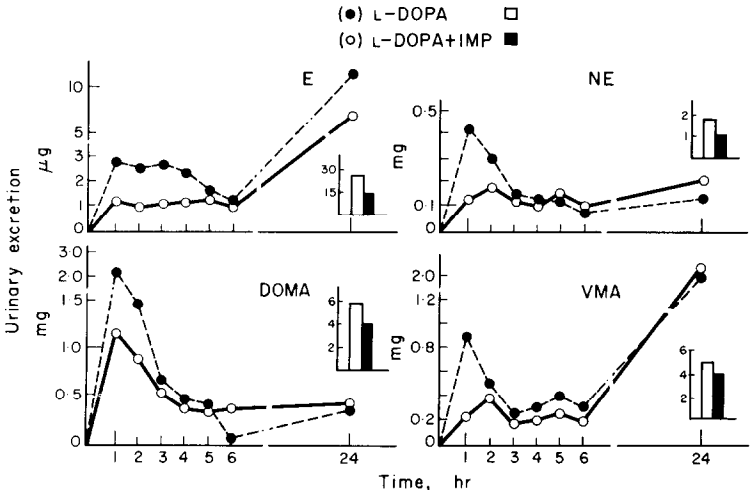

FIG. 2. Effect of imipramine (IMP) pretreatment on the urinary excretion of norepinephrine (NE), epinephrine (E) and their acid metabolites, dihydroxymandelic acid (DOMA) and vanillylmandelic acid (VMA). Total cumulative 24-hr output is given in the bar graphs.

cent for HVA ($0{\cdot}1 < P < 0{\cdot}05$). After pretreatment with IMP, the total 24-hr urinary excretion of DOPAC and HVA remained decreased by approximately 50 per cent ($P < 0{\cdot}05$) and 30 per cent ($P < 0{\cdot}01$) respectively. In contrast, the decrease in the excretion of dopa and DA failed to persist throughout the 24-hr period studied.

Figure 2 illustrates the graphic analysis of the urinary excretion of E, NE and their major acid metabolites, DOMA and VMA, that were obtained during the two test periods. A prolonged but non-significant decrease in the urinary excretion of E was observed after the administration of IMP prior to L-dopa. IMP pretreatment decreased the urinary excretion of NE and DOMA by 3·3- and 2·0-fold at 2 hr respectively. Peak urinary excretion of VMA was reduced from $0{\cdot}88 \pm 0{\cdot}05$ mg/hr to $0{\cdot}22 \pm 0{\cdot}07$ mg/hr ($0{\cdot}1 < P\ 0{\cdot}05$) as a consequence of IMP pretreatment with little apparent differences in the total 24-hr output. Conversely, IMP pretreatment reduced the urinary excretion of NE and DOMA by 28·8 and 44·2 per cent at 24 hr respectively.

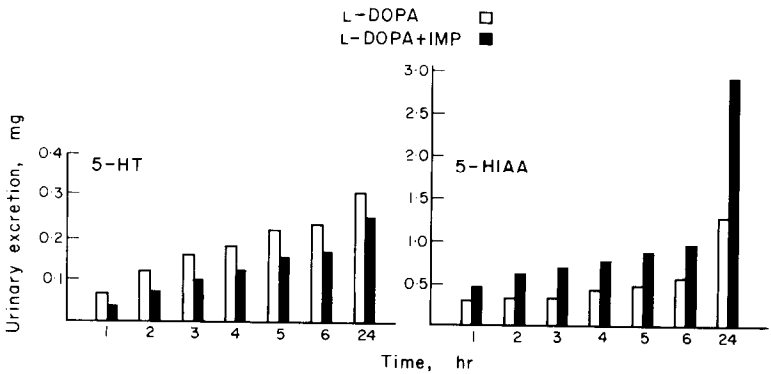

FIG. 3. Effect of imipramine (IMP) pretreatment on L-dopa-mediated changes in endogenous serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). The results are plotted cumulatively as a function of time.

Figure 3 shows the cumulative urinary excretion of 5-HT and its major acid metabolite, 5-HIAA, for the two phases of the study. Preadministration of IMP for 3 consecutive days prior to L-dopa administration leads to a moderate reduction in the amount of endogenous 5-HT excreted in the urine. In contrast, a marked increase in the urinary excretion of 5-HIAA from 1·3 mg/24 hr to 2·9 mg/24 hr was evident for the 24-hr period after IMP pretreatment.

DISCUSSION

The mode of action of IMP is ascribed to its blockade of the reuptake inactivation mechanism of neuronally released NE[4-6] and possibly of 5-HT and DA.[18,19] As a result, the pharmacologic effect of the monoamines is potentiated at the post-synaptic receptor. Further, IMP decreases deaminated metabolites derived from NE.[20] In the present study, pretreatment with IMP decreased urinary excretion of endogenous VMA, the major acid metabolite of NE. This decrease in VMA excretion is consistent with the findings obtained for endogenous VMA excretion during administration of IMP alone to depressed patients[21] and to normal subjects.[22]

In this study, administration of L-dopa to IMP-pretreated subjects moderately decreased urinary excretion of dopa from base line value obtained with L-dopa alone at the initial 2 hr. It is conceivable that administration of IMP delayed gastric emptying time due to its anticholinergic effect[23] causing a decreased absorption of L-dopa. Consequently, reduced amounts of L-dopa are recovered in urine. It has been reported[24] that administration of IMP after a single 200 mg dose of DL-dopa decreased peak plasma catecholamine concentrations and conversly increased their 24-hr urinary output. However, dopa was not separated from DA prior to quantitative determinations. This is of importance because the metabolism of D-dopa in man differs from L-dopa, in that a greater proportion of ingested D-dopa is excreted in urine unmetabolized[25] as compared with less than 1 per cent of the L-dopa dose.[26-31] Therefore, IMP-produced increase in catecholamine excretion[24] may be due to the racemic mixture administered.

It appears that IMP does not inhibit aromatic L-amino acids decarboxylase,[32] the enzyme which converts dopa to DA, but may inhibit monoamine oxidase (MAO) *in vitro*.[33] However, IMP-mediated decreases in the excretion of DOPAC and DOMA relative to DA and NE are unlikely due to inhibition of MAO, since less dopa was absorbed. Therefore, IMP-mediated decreased excretion of DA and NE may be indicative of a delayed absorption, release and subsequent metabolism of L-dopa by IMP. Approximately 3·8 per cent of the L-dopa dose was recovered in urine over a 24-hr period when L-dopa was given alone. This is greater than the 1 per cent reported[26-31] and may reflect quantitative differences in the metabolism between an acute small dose and a greater dose regimen of L-dopa.

In the present study, treatment with IMP prior to L-dopa moderately decreased the excretion of endogenous 5-HT but markedly increased its major acid metabolite, 5-HIAA, for the 24-hr period studied. However, IMP administration for 3 weeks to depressed patients decreased the urinary excretion of 5-HIAA,[34] while no similar changes occurred in normal subjects given IMP for 2 consecutive weeks.[22] Further, administration of L-dopa to Parkinsonian patients is associated with decreased excretion of endogenous 5-HIAA[28,31,35,36] caused by a displacement of 5-HT from its storage sites by dopa and/or DA[37] or as a result of their inhibitory effect of trypto-

phan hydroxylase, the rate-limiting step in 5-HT biosynthesis. It should be noted that L-dopa[35] and IMP[38] may increase tryptophan pyrolase activity, and administration of L-dopa to IMP-pretreated experimental animals negated an L-dopa-elicited decrease of tissue content of 5-HT.[39] Therefore, administration of L-dopa with IMP may interfere in the metabolism of L-dopa and negate an L-dopa-mediated effect on endogenous 5-HT metabolism, which may account for some of the antidepressant effects produced by IMP administration during L-dopa therapy in Parkinson's disease.

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