*Biochemical Pharmacology*, Vol. 37, No. 10, pp. 2059–2067, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00


# ON THE MECHANISM OF IMIPRAMINE'S INFLUENCE IN LOWERING *p*-HYDROXYPHENYLGLYCOL CONCENTRATIONS IN THE BRAIN

## THE ROLE OF TYROSINE

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(*Received* 27 *February* 1987; *accepted* 6 *October* 1987)

**Abstract**—Administration of imipramine (IMI) to rats was shown to lower after 4.5 hr the brain concentration of the octopamine metabolite *p*-hydroxyphenylglycol (pHPG) in a dose-dependent manner over the range of 10–40 mg/kg of IMI. Assay of plasma and brain levels of tyrosine revealed that IMI produced a reduction in both but with a shorter time-course than for the depletion in pHPG, with the maximal decreases occurring at 1.5 hr, before there was any loss of pHPG. The reductions in tyrosine and pHPG levels could not be explained by an effect of IMI on food intake, since the levels were diminished even in 24-hr fasted animals. When rats were injected with IMI 4.5 hr before 200 mg/kg of tyrosine and 5.5 hr before being killed, the elevation in brain pHPG levels were attenuated by about 50%, as compared to the animals that received tyrosine alone. These data suggest that the ability of IMI to lower brain pHPG probably involves two distinct mechanisms: (1) a lowering of brain and plasma tyrosine concentrations, and (2) an inhibition of the conversion of tyrosine to pHPG. It is unclear whether these effects are due to IMI itself or to one of its metabolites, such as desmethylimipramine or didesmethylimipramine, which were found in the plasma in amounts equal to or greater than IMI.

Previous studies from our laboratory have shown that imipramine (IMI), an antidepressant drug, lowers brain and urine concentrations of *p*-hydroxyphenylglycol (pHPG), a neutral metabolite of octopamine [1, 2] which is structurally related to octopamine as 3-methoxy-4-hydroxyphenethylene glycol (MHPG) is related to norepinephrine (NE) [3]. If pHPG levels reflect octopamine turnover as MHPG levels reflect NE turnover [4, 5], the reduction in pHPG levels caused by IMI would suggest that the drug decreases octopamine turnover.

Unfortunately, at present little is known concerning the regulation of octopamine turnover. Whereas NE turnover is tightly regulated by adrenoceptors, it is likely that non-receptor mediated factors play a major role in controlling octopamine turnover. The concentration of the precursor amino acid, tyrosine, would be expected to have a particularly important influence in determining the rate of octopamine synthesis, since none of the required enzymes is near substrate saturation. In contrast, in the production of catecholamines, tyrosine hydroxylase is close to saturation by tyrosine and, therefore, catecholamine turnover ordinarily is not affected by the tyrosine concentration, except under certain conditions of increased cell firing that result in an activation of tyrosine hydroxylase [6]. Our finding that an injection of 200 mg/kg of tyrosine causes an 82% elevation in brain pHPG but only a 12% rise in MHPG [7] supports the idea that tyrosine availability plays a key role in octopamine turnover. Therefore, a report by Tagliamonte *et al.* [8] that desmethylimipramine (DMI) lowers the concentration of tyrosine in both brain and plasma raises the possibility that IMI, which is metabolized to DMI [9], causes a decrease in octopamine turnover as a result of a diminished tyrosine level. An alternate possibility is that IMI or one of its metabolites inhibits an enzymatic or transport step involved in the conversion of tyrosine to octopamine.

In this paper, we have attempted to elucidate further the mechanism by which IMI decreases pHPG levels in the brain. We first studied in more detail the effects of IMI on brain and plasma tyrosine in order to find out whether decreases in tyrosine levels could account for the depletion of pHPG. A second part of this paper deals with the question of whether IMI interferes with the conversion of tyrosine to octopamine. To answer this question, we examined the effects of IMI on the increases in brain pHPG induced by exogenous tyrosine.

### MATERIALS AND METHODS

*Animals and sample preparation.* Male Sprague–Dawley rats (200–250 g) were supplied by Zivic–Miller Laboratories (Allison Park, PA) and were singly housed in our animal facility with free access to food and water for 1 week prior to experimentation. Lights were on between 6:00 a.m. and 6:00 p.m. All

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animals were killed by decapitation between 2:00 p.m. and 4:30 p.m. Trunk blood from each rat was collected with constant swirling in a 100-ml beaker containing 200 μl of heparin. The blood samples were kept on ice until they were centrifuged (500 *g* for 15 min). The plasmas were stored at 4°. The brains were removed within 20 sec of decapitation, dissected longitudinally, and the halves frozen on dry ice. The tissue samples were stored at −50° until assayed.

*Assay of the neutral metabolites of octopamine and catecholamines in brain samples.* Brain concentrations of the glycol metabolites of octopamine (pHPG) and of NE (MHPG and 3,4-dihydroxyphenethylene glycol, DHPG), as well as the neutral metabolite of dopamine (DA), 3,4-dihydroxyphenethanol (DHPE), were measured by gas chromatography–mass spectrometry (GC–MS) operated in the chemical-ionization mode, using procedures developed in our laboratory [10, 11] but which now have been modified in several important respects: homogenizing the tissue samples in perchloric acid solutions so that catecholamine analyses can be performed on the same tissue samples; using 15-ml extraction tubes and smaller solution volumes; and evaporating samples simultaneously in a Speed-Vac concentrator (Savant Instruments, Farmingdale, NY) instead of individually with a rotary evaporator. Brain halves were homogenized in 3 ml of cold 0.1 N perchloric acid containing 0.05% EDTA with a Polytron (Brinkman Instruments, Westbury, NY) for 15 sec at a setting of 6. After centrifugation for 15 min at 17,000 *g* in a Sorvall RC-2B refrigerated centrifuge, the supernatant fractions were transferred with Pasteur pipettes to glass tubes and stored overnight at 4°. Then, 10 μg of the internal standard, 3-methoxy-4-hydroxyphenylpropanol (MHPP, synthesized as previously described [7]) in 100 μl was added to a 2-ml aliquot of the supernatant fraction of each sample, standards and a blank. Next, 250 μl of a solution containing 2% EDTA and 0.4% ascorbic acid and 500 μl of 1 M sodium acetate buffer, pH 6.0, containing 200 mg of type H-1 sulfatase (Sigma Chemical Co., St Louis, MO) was added to each tube, except that the sulfatase was omitted from the standards. The samples were adjusted to pH 6.0 by adding approximately 80 μl of 1 N NaOH, flushed with nitrogen, and incubated overnight at 37° with gentle shaking. Each tube was then extracted twice with 5-ml portions of freshly distilled ethyl acetate, and the combined ethyl acetate layers were transferred to a 15-ml silanized glass tube to which 0.5 ml of a 1 M potassium bicarbonate solution had been added. After shaking and centrifuging the tubes, the aqueous layers were removed by Pasteur pipette and discarded. Sodium sulfate (18 mg) was added to each tube and vortexed, and the organic solution was then transferred to a clean silanized tube. The solvent was evaporated from each tube in a Speed-Vac concentrator. Each residue was then redissolved in 1 ml of ethyl acetate, transferred to a 1-ml silanized Reacti-Vial (Pierce Chemical Co., Rockford, IL), and re-evaporated in the Speed-Vac concentrator. The extracts were derivatized by adding through a Tuf-Bond disc (Pierce) 15 μl of ethyl acetate and 5 μl of pentafluoropropionic anhydride (PFPA, Pierce).

Analyses were performed by selected-ion monitoring using a model 3200 Finnigan quadrupole GC/MS operated by chemical-ionization and with the following conditions: column, 6-ft × 2-mm 3% SP-2401 on 80/100 mesh Supelcoport (Supelco, Bellefonte, PA), 160° isothermal; injector, 200°; carrier and reagent gas, methane; ion-source pressure, 0.9 torr; transfer line, 250°; analyzer, 100°; electron energy, 130 eV; electron multiplier, 1900 V. The PROMIM channels were set at $m/z$ 429 for pHPG and DHPE, $m/z$ 459 for MHPG, $m/z$ 591 for DHPG, and $m/z$ 311 for MHPP [12].

*Assay of catecholamines, tyrosine and phenylalanine.* Brain concentrations of the catecholamines, NE and DA, and the acidic DA metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), were measured by high-performance liquid chromatography (HPLC) with electrochemical detection by the procedures of Reinhard and Roth [13] as modified in our laboratory [11]. Plasma and brain tyrosine levels were assayed by HPLC with electrochemical detection, using *m*-tyrosine as the internal standard [14]. Plasma phenylalanine was determined by a fluorometric assay [15].

*Determination of plasma levels of IMI and its metabolites.* Plasma concentrations of IMI and two of its major metabolites, DMI and didesmethylimipramine (DDMI), were measured by HPLC with electrochemical detection. A 0.5-ml aliquot of each plasma sample was added to an 8-ml silanized glass tube containing 300 ng of trimipramine as an internal standard. After adding 0.5 ml of 0.1 M Tris buffer, pH 3.0, the samples were applied to Bond-Elut end-capped CN columns (No. 624101, Analytichem International, Harbor City, CA), which had been prepared by successive washes of two column volumes each of methanol, distilled water and 0.1 M Tris, pH 3.0. The columns were then washed with one column volume each of Tris buffer and distilled water, and IMI and its metabolites were eluted with 1 ml of 0.5% $NH_4OH$ in methanol. The eluates were evaporated to dryness in the Speed-Vac concentrator, and the residues were redissolved in 100 μl of mobile phase buffer, which consisted of 0.12 M perchloric acid, 3 mM sodium citrate, 0.2 mM EDTA, 0.03% tridecylamine, 2.5 mM sodium octyl sulfate, and 40% acetonitrile, pH 5.5. An aliquot (20–50 μl) was injected into a model LC-153 HPLC (Bioanalytical Systems, West Lafayette, IN), equipped with a 25 cm, 5 μm Biophase ODS column and pumped with a flow rate of 2 ml/min (approximately 2500 psi). The peaks were detected by a LC-4B electrochemical detector equipped with a TL-5 glassy carbon electrode, maintained at 1.0 V versus an Ag/AgCl reference electrode.

*Drugs.* IMI hydrochloride (CIBA Geigy, Summit, NJ) was dissolved in saline at a concentration of 4 mg/ml of the free base (2–8 mg/ml for the dose–response study). Tyrosine ethyl ester (Sigma) was dissolved in saline at a concentration of 40 mg/ml of the free amino acid. Both drugs were injected i.p. in a volume of 5 ml/kg. DMI hydrochloride was provided by the USV Pharmaceutical Corp., Tuckahoe, NY. DDMI was a gift from Dr. J. Stephen Kennedy, Neurosciences Research Branch, NIMH, Rockville, MD.

## RESULTS

*Dose–response curve for IMI.* Figure 1a shows the dose–response curves for IMI between 10 and 40 mg/kg injected 4.5 hr before sacrifice. Brain pHPG was lowered significantly by all doses of IMI, declining maximally to 26% at the 40 mg/kg dose. Plasma tyrosine levels decreased in a dose-dependent manner, and the decreases reached statistical significance for all except the lowest dose. After the 40 mg/kg dose, plasma tyrosine was reduced to 76% of control. The brain tyrosine concentration was unchanged at all doses.

In contrast to the substantial decrease in brain pHPG concentrations, IMI caused much smaller, if any, changes in the levels of catecholamines and their metabolites (Fig. 1b). However, significant decreases were observed for both NE metabolites. MHPG levels were reduced significantly by all doses of IMI, except 30 mg/kg, to 86–89% of control. At all doses DHPG levels were lowered to a somewhat greater extent, decreasing to 61–79% of control. These results are consistent with seven other individual studies in which brain MHPG levels ranged from 86 to 91% of control (achieving statistical significance in four of the studies) and DHPG ranged from 48 to 83% of control (being lower than MHPG in each of these experiments) 4.5–8 hr after a dose of 20 mg/kg of IMI (see Table 3 and data not shown).

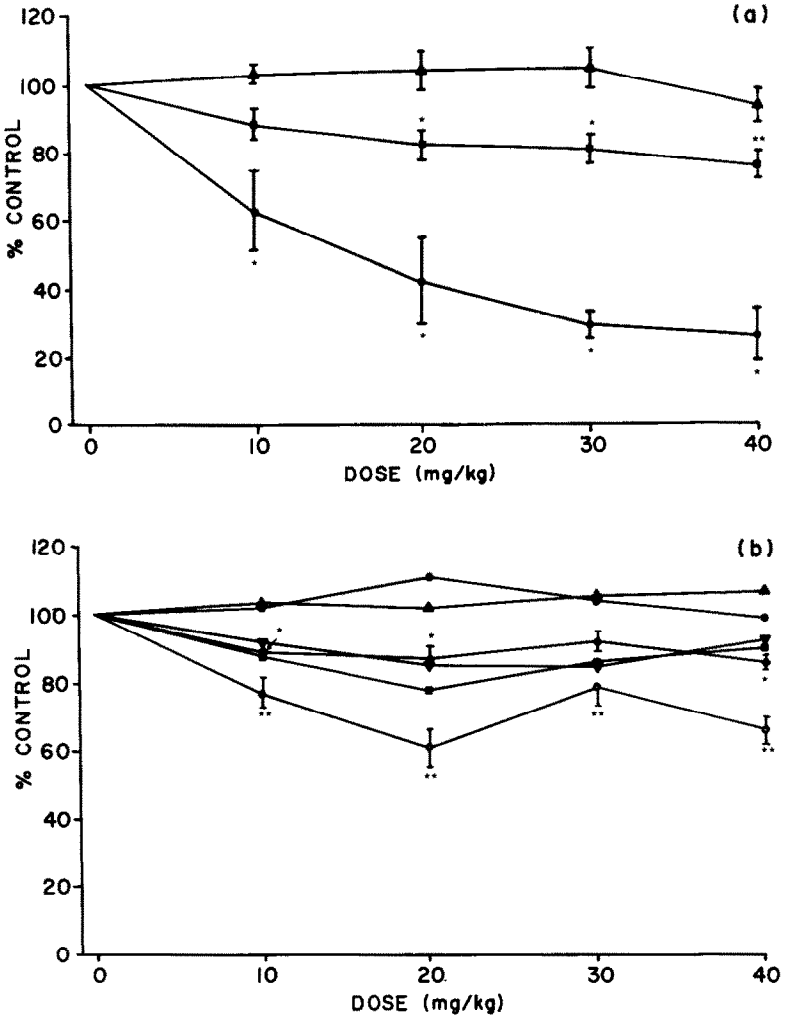


Fig. 1. Dose–response curve for IMI. Rats were injected i.p. with a volume of 5 ml/kg of solution of IMI hydrochloride in saline and decapitated 4.5 hr later. Doses refer to the free base. The data represent the mean ± SE of six animals in each group, except for tyrosine levels, which were combined from two separate experiments (N = 12). Statistical comparisons were performed with one-way ANOVA, followed by a Newman–Keuls test: (*) $P < 0.05$; and (**) $P < 0.01$. (a) Brain and plasma tyrosine and brain pHPG. Control values were the following: brain tyrosine (▲), 16.3 ± 1.4 and 11.1 ± 0.9 μg/g; plasma tyrosine (■), 16.3 ± 0.4 and 16.4 ± 1.3 μg/ml; and brain pHPG (●), 2.72 ± 0.64 ng/g. (b) Catecholamines and their metabolites in brain. Control values were the following: NE (●), 239 ± 7 ng/g; DA (▲), 559 ± 23 ng/g; MHPG (◆), 55 ± 0.8 ng/g; DHPG (○), 80 ± 2.4 ng/g; DHPE (▼), 13 ± 2 ng/g; and DOPAC (■), 125 ± 11 ng/g. Error bars are omitted for clarity, except for MHPG and DHPG.

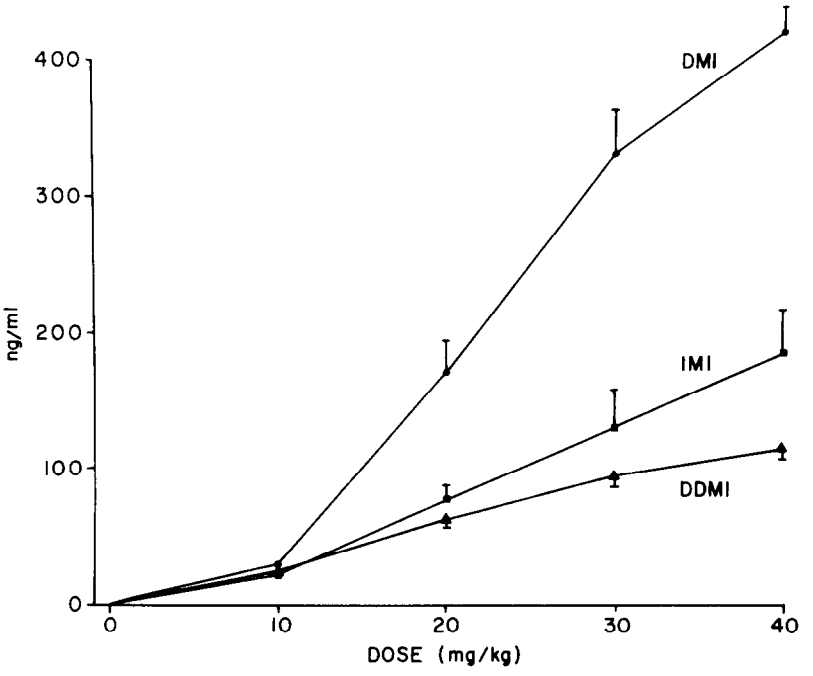


Fig. 2. Relationship between IMI dose and plasma concentrations of IMI, DMI, and DDMI. These assays were performed with the same samples used in Fig. 1 according to the experimental details provided in the legend.

Analyses of plasma drug levels for the dose–response study are shown in Fig. 2. The concentrations of IMI, DMI and DDMI 4.5 hr after a dose of 20 mg/kg of IMI (the dose used in all subsequent experiments) were 77 ± 11, 172 ± 22 and 63 ± 7 ng/ml respectively. At each dose between 20 and 40 mg/kg, the plasma level of DMI was slightly greater than twice the level of IMI (223–246%), whereas levels of DDMI were somewhat less than IMI (62–82%). As the dose of IMI rose above 20 mg/kg, the plasma levels of IMI, DMI and DDMI increased approximately in proportion or greater.

*Time–course of the effects of IMI on tyrosine and pHPG.* A time–course study was conducted for a dose of 20 mg/kg of IMI. In contrast to our other experiments, food was removed from the cage of each animal as it was injected. This was done to rule out the possibility that the decrements in pHPG and/or tyrosine were simply due to IMI reducing food intake.

The results reveal that both brain and plasma tyrosine concentrations were diminished 1.5 hr after injection, before there was a significant decline in brain pHPG, but tended to return to near control levels by 6 hr, at which time the depletion in pHPG persisted (Table 1). Therefore, it is clear from these data that both tyrosine levels (in plasma and brain) and pHPG levels (in brain) are reduced by IMI but with different time–courses.

*Influence of fasting on the effects of IMI.* Although the data in Table 1 apparently rule out the possibility that the decrements in pHPG and tyrosine caused by IMI were due to a decrease in food intake, it is still possible that IMI had inhibited the intestinal absorption of tyrosine originating from food consumed before the injection. Therefore, an experiment was carried out in which the influence of IMI was examined in animals fasted for 24 hr before being killed (19.5 hr before injection). The results shown in Table 2 indicate that fasting significantly lowered plasma tyrosine ($P < 0.01$, Newman–Keuls multiple

Table 1. Time–course of the effects of IMI on pHPG and tyrosine

| Time (hr) | Brain pHPG (ng/g) | Plasma tyrosine (μg/ml) | Brain tyrosine (μg/g) |
|---|---|---|---|
| 0 | 2.15 ± 0.15 | 15.4 ± 1.3 | 12.4 ± 0.8 |
| 1.5 | 1.89 ± 0.16 (88%) | 10.5 ± 1.1 (68%)* | 9.8 ± 0.3 (79%)* |
| 3 | 1.26 ± 0.06 (59%)† | 11.2 ± 0.7 (73%)* | 9.2 ± 0.3 (74%)† |
| 4.5 | 1.43 ± 0.08 (67%)† | 9.8 ± 0.6 (64%)† | 9.0 ± 0.6 (73%)† |
| 6 | 1.43 ± 0.19 (67%)† | 12.5 ± 0.6 (81%) | 10.6 ± 1.9 (85%)† |
| 4.5 (saline) | 1.89 ± 0.15 (88%) | 14.3 ± 1.4 (93%) | 11.5 ± 0.8 (93%) |

Rats (N = 5 per group) were fed regular laboratory chow *ad lib*. IMI (20 mg/kg, i.p.) or saline was injected at various times before the animals were killed. The times of injection were staggered so that all the animals were killed between 3:30 and 5:00 p.m. All values represent the mean ± SE.

*† The percentages as compared to the "zero" time group are in parentheses and are significantly different as follows: * $P \pm 0.05$; † $P < 0.01$ (one-way analysis of variance followed by a Newman–Keuls multiple comparison test).

Table 2. Effects of IMI in fasted rats

| Treatment | Brain pHPG (ng/g) | Plasma tyrosine (μg/ml) | Brain tyrosine (μg/g) | Brain MHPG (ng/g) | Brain DHPG (ng/g) |
|---|---|---|---|---|---|
| Non-fasted | | | | | |
| Saline | 1.88 ± 0.35 | 12.4 ± 0.7 | 9.84 ± 1.01 | 77 ± 4 | 93 ± 9 |
| IMI | 0.81 ± 0.04* | 10.7 ± 0.7 | 9.89 ± 0.39 | 62 ± 3* | 59 ± 4† |
| | (43%) | (86%) | (101%) | (81%) | (63%) |
| Fasted | | | | | |
| Saline | 1.55 ± 0.26 | 8.61 ± 0.77‡ | 8.63 ± 0.32 | 72 ± 3 | 84 ± 8 |
| (% non-fasted) | (82%) | (70%) | (88%) | (94%) | (90%) |
| IMI | 0.59 ± 0.14* | 8.65 ± 0.38 | 7.77 ± 0.41 | 64 ± 2 | 56 ± 2* |
| (% fasted saline) | (32%) | (100%) | (90%) | (89%) | (66%) |

Rats (N = 4–5 per group) were fasted for 24 hr before being killed or fed regular laboratory chow *ad lib*. IMI (20 mg/kg, i.p.) or saline was injected 4.5 hr before sacrifice.

*–‡ Statistical analysis: * $P < 0.05$, and † $p < 0.01$, IMI vs the corresponding saline-treated group; ‡ $P < 0.01$, fasted vs the corresponding non-fasted group (one-way analysis of variance followed by a Newman–Keuls multiple comparison test).

comparison test) but caused small but nonsignificant decreases in brain pHPG as well as in brain tyrosine, MHPG and DHPG levels. However, IMI lowered brain pHPG levels in fasted as well as in non-fasted rats to 32 and 43% of control ($P < 0.05$), respectively, even though IMI caused no further decline in plasma and brain tyrosine levels when measured 4.5 hr after injection. IMI also significantly lowered brain MHPG in the non-fasted rats and DHPG in both the fasted and non-fasted rats.

*Effects of IMI on plasma phenylalanine levels.* Since it is possible that IMI lowers tyrosine through an inhibition of hepatic phenylalanine hydroxylase, plasma levels of phenylalanine were determined for an experiment in which IMI or saline was injected 1.5 hr before sacrifice. The phenylalanine concentrations were not significantly different between IMI- and saline-injected rats (20.3 ± 1.3 vs 18.7 ± 1.0 μg/ml respectively).

*Effects of IMI on tyrosine-induced increases in brain pHPG.* To find out whether the decreases in pHPG caused by IMI could be due in part to an inhibition of the conversion of tyrosine to pHPG, an experiment was conducted to determine whether IMI could block the increase in pHPG produced by an injection of tyrosine. As shown in Table 3, the administration of tyrosine (200 mg/kg, i.p.) 1 hr before sacrifice (saline–tyrosine) elevated the brain concentration of pHPG by 82%, as compared to the saline–saline control group. IMI (20 mg/kg, i.p.) injected 4.5 hr before an injection of saline and 5.5 hr before sacrifice (IMI–saline) reduced brain pHPG to 52% as compared to controls. IMI produced a similar percentage reduction in brain pHPG in animals that also received an injection of tyrosine. Thus, brain pHPG in rats that received IMI and tyrosine (IMI–tyrosine) was only 46% of that in animals that received tyrosine alone. In contrast, when IMI was injected only 1 hr before tyrosine, it had a smaller, nonsignificant influence on brain pHPG.

Assay of tyrosine concentrations indicated that they were increased by almost 3-fold in plasma and 4-fold in brain 1 hr after tyrosine was administered. When IMI was injected 5.5 hr before death (IMI–saline), it had no significant effect on either plasma or brain endogenous tyrosine levels. Similarly, IMI injected 4.5 hr before tyrosine (IMI–tyrosine) did not alter significantly the elevations in plasma and brain tyrosine concentrations seen after tyrosine alone (saline–tyrosine), although the increases in tyrosine were slightly smaller in IMI-pretreated animals. However, plasma tyrosine tended to be lower in the animals that received IMI only 1 hr prior to tyrosine, reaching a level only 66% as high as that in the animals given tyrosine alone, but this difference did not reach statistical significance. On the other hand, brain tyrosine was significantly lower (by 46%, $P < 0.01$) in these rats than in those that received IMI 4.5 hr before tyrosine.

Tyrosine did not affect either brain NE or DHPG concentrations; however, it did cause a small (12%) but significant rise in brain MHPG. IMI, on the other hand, caused significant reductions in the brain concentrations of MHPG and DHPG (to 88 and 48% respectively). Interestingly, tyrosine did not raise brain MHPG levels in the rats that were pretreated with IMI (IMI–tyrosine vs IMI–saline, 99%). Brain DA and DOPAC were not changed significantly by any of the treatments.

Plasma levels of IMI and the two metabolites formed by demethylation, DMI and DDMI, revealed that 5.5 hr after an injection of IMI (in the IMI–saline group) the concentration of DMI (113 ng/ml) was nearly twice as high as the concentration of the parent drug (IMI = 69 ng/ml) and the didesmethylated metabolite (DDMI = 60 ng/ml). The plasma concentrations of all three compounds were virtually unchanged by an injection of tyrosine 1 hr prior to sacrifice (IMI–tyrosine group). However, in the group of rats that was treated with IMI 1 hr before tyrosine and 2 hr before sacrifice, the plasma concentration of IMI was about the same as or slightly higher than the concentration of DMI.

## DISCUSSION

Effects of IMI could be due to IMI itself or to one of its metabolites. When IMI is injected intraperitoneally, it is known to be converted rapidly to DMI [9]. This was also apparent from the plasma

Table 3. Effects of an acute IMI pretreatmen

| Treatment | Brain pHPG (ng/g) | Plasma tyrosine (μg/ml) | Brain tyrosine (μg/g) | Brain NE (ng/g) |
|---|---|---|---|---|
| Saline–Saline | 3.27 ± 0.32 | 18.4 ± 0.7 | 14.9 ± 0.8 | 264 ± 7 |
| Saline–Tyrosine | 5.94 ± 0.72 | 71.8 ± 3.9 | 73.8 ± 3.7 | 246 ± 8 |
| (% Saline–Saline) | (182%)* | (389%)* | (495%)* | |
| IMI–Saline | 1.71 ± 0.20 | 17.5 ± 0.3 | 16.8 ± 0.7 | 240 ± 5 |
| (% Saline–Saline) | (52%) | (95%) | (113%) | (91%) |
| IMI–Tyrosine | 2.76 ± 0.52 | 62.1 ± 11.9 | 59.9 ± 10.3 | 248 ± 12 |
| (% Saline–Tyrosine) | (46%)* | (86%) | (81%) | |
| IMI–Tyrosine (1 hr) | 4.29 ± 1.10 | 47.4 ± 11.2 | 40.0 ± 9.8 | 238 ± 16 |
| (% Saline–Tyrosine) | (72%) | (66%) | (54%)‡ | |

Rats were divided into five weight-matched groups of six animals each. Animals in the saline–saline group received two injections of saline 4.5 hr apart. Saline–tyrosine animals received 200 mg/kg of tyrosine in place of the second saline injection. The IMI–saline and IMI–tyrosine groups were treated as were the first two groups, except that they were administered 20 mg/kg of IMI instead of the first saline injection. The last group was injected the same as the IMI–tyrosine group, except that IMI was given only 1 hr before tyrosine. All animals were killed 1 hr after the second injection.

drug levels we observed, where the ratio of DMI:IMI was approximately 1:1, 1.5–2 hr after injection (Table 3 and data not shown), and approximately 2:1, 4.5 hr after injection (Fig. 2). Thus, it would be expected that DMI might account for at least part of the effects of IMI. Since our data show that plasma DDMI levels were almost equal to those of IMI, it is also possible that DDMI might contribute to these effects, but the pharmacological actions of this metabolite are largely unknown. However, the fact that DDMI is a primary amine like the catecholamines and trace amines suggests the possibility that it might be active in influencing neurotransmitter function. On the other hand, a direct influence by IMI cannot be ruled out. In fact, we have found that amitriptyline, another tricyclic antidepressant that is demethylated less rapidly than IMI [16, 17], has effects similar to those of IMI described in this paper [18]. Moreover, we have also found that subcutaneous and intraperitoneal injections of IMI have similar effects on brain pHPG (unpublished observation) even though IMI is demethylated far less when it is administered subcutaneously [9].

The present study confirmed the observation made by us earlier [1, 2] that IMI decreases the concentration of pHPG in the brain of rats. The data further suggest that at least two separate mechanisms contribute to this effect.

One mechanism for pHPG depletion appears to involve a deficit in tyrosine. By measuring tyrosine and pHPG in the same tissues, it was clear that the concentrations of both were lowered by IMI, although with different time–courses (Table 1). Thus, at 1.5 hr after IMI, plasma and brain tyrosine levels were near their maximal decreases, whereas brain pHPG levels had not yet declined significantly. On the other hand, plasma and brain tyrosine levels had returned to near control levels by 6 hr, whereas brain pHPG remained maximally decreased. Based on the results in Fig. 1, Table 2 and other studies (unpublished), it would appear that brain tyrosine levels generally are back to control by 4.5 hr. However, at 4.5 hr in the time–course study, tyrosine concentrations were lowered significantly in the brain and were reduced in the plasma more than in other studies. These discrepancies may be due to two differences in the experimental protocol of the time–course study. First, all of the groups were compared to uninjected controls ("zero" time) rather than to a saline-injected group. It appears from the one saline-injected group (4.5 hr) that was included in this experiment (Table 1) that an injection of saline alone slightly lowered both brain and plasma tyrosine levels (by about 7%). Although these decreases were not statistically significant, they were seen consistently in other experiments as well. However, this effect is insufficient to account for the apparent decreases in both plasma and brain tyrosine at 4.5 hr. When compared to the saline-injected control group, plasma and brain tyrosine concentrations were still reduced significantly to 69 and 78% respectively ($P < 0.05$). The second difference between the protocol of the time–course study and our other experiments was that food was removed from the cage of each animal as it was injected. While 24-hr fasting caused only small, nonsignificant reductions in both brain and plasma tyrosine levels (Table 2), it is possible that the absence of food may have prolonged the return of tyrosine levels to baseline, resulting in the small depletion in brain tyrosine.

The decreases in tyrosine at 1.5 hr after IMI are consistent with the work of Tagliamonte *et al.* [8], who reported that 10 mg/kg of DMI lowers tyrosine concentrations to 83% of control in plasma and to 66% in the brain 1.5 hr after injection. Rosloff and Davis [19] also noted that 20 mg/kg of DMI reduces endogenous tyrosine levels 1.25 hr later to 89 and 81% in brain and plasma, respectively, although the decrease in brain tyrosine was not statistically significant, except after chronic treatment. These investigators suggested that a reduction in food uptake may have accounted for the decreased plasma and brain tyrosine levels. However, this explanation can be ruled out by our experiments which showed that IMI decreased tyrosine levels even though food was removed from the cages at either the time of injection (Table 1) or 24 hr before sacrifice (Table 2). On the other hand, our finding that IMI administered 1 hr before an injection of tyrosine attenuated the elevation in brain tyrosine (Table 3) suggests that

tyrosine-induced changes in brain pHPG

| Brain MHPG (ng/g) | Brain DHPG (ng/g) | Brain DA (ng/g) | Brain DOPAC (ng/g) | Plasma IMI (ng/ml) | Plasma DMI (ng/ml) | Plasma DDMI (ng/ml) |
|---|---|---|---|---|---|---|
| 79 ± 1 | 73 ± 3 | 561 ± 9 | 97 ± 5 | | | |
| 89 ± 3 (112%)† | 72 ± 5 | 543 ± 23 | 106 ± 3 | | | |
| 69 ± 3 (88%)† | 35 ± 5 (48%)* | 528 ± 15 (94%) | 92 ± 10 | 69 ± 10 | 113 ± 11 | 60 ± 8 |
| 69 ± 4 (78%)* | 45 ± 4 (62%)* | 572 ± 20 | 94 ± 7 | 58 ± 8 | 107 ± 8 | 42 ± 3 |
| 79 ± 6 (89%) | 52 ± 9 (72%) | 571 ± 16 | 122 ± 12 | 208 ± 18 | 178 ± 18 | 61 ± 5 |

*–‡ All values represent the mean ± SE. The percentage given in parentheses are for comparisons with the group indicated. The first four groups were statistically compared with one-way analysis of variance and, where significant differences were found, Newman–Keuls multiple comparison tests were performed: * $P < 0.001$ and † $P < 0.05$ vs saline–saline. For the last group, IMI–tyrosine (1 hr), analysis was performed using Student's two-tailed $t$-test: ‡ $P < 0.01$ vs IMI–tyrosine.

IMI may inhibit tyrosine absorption. Whether the decrement in endogenous tyrosine caused by IMI can be explained at least in part by such a mechanism remains to be determined.

Another possible mechanism by which IMI might decrease tyrosine concentrations is via an interaction with adrenoceptors. Previous investigations have demonstrated that the β-adrenergic agonist, isoproterenol, reduces plasma tyrosine levels [20, 21]. Stimulation of β-receptors as a result of IMI or its metabolites blocking NE re-uptake similarly could diminish plasma tyrosine. However, since isoproterenol increases rather than decreases tyrosine in the brain [20], stimulation of peripheral β-receptors could not account for the decline in brain tyrosine levels that is produced by IMI. On the other hand, since unlike isoproterenol IMI readily crosses the blood–brain barrier, we cannot rule out the possibility that IMI lowers brain tyrosine by stimulation of central β-receptors. To our knowledge, nothing is known concerning the regulation of tyrosine by brain adrenergic receptors.

It is also possible that IMI lowers tyrosine by inhibiting phenylalanine hydroxylase. However, this possibility can be ruled out by our finding that IMI failed to increase plasma phenylalanine levels.

The reductions in tyrosine concentrations do not appear to be able to account entirely for the decreases in brain pHPG. First, pHPG was depleted by about 50% in the brain (Tables 1 and 3; Fig. 1a) and by about 75% in the 24-hr urine [1], as compared to a maximal decrease in brain and plasma tyrosine levels of only about 25% (Table 1). Second, the decreases in brain pHPG persisted for at least 24 hr [1], but the levels of tyrosine in brain had returned to control levels by 4.5 to 6 hr. The possibility cannot be ruled out, however, that larger and longer-lasting decreases in tyrosine may occur in key cellular or subcellular compartments regulating octopamine turnover.

The data in Table 3 suggest that a second mechanism of pHPG depletion involves an inhibition in the conversion of tyrosine to pHPG. One injection of tyrosine (200 mg/kg) raised the brain pHPG concentration by 2.7 ng/g after 1 hr. However, when animals were pretreated with IMI 4.5 hr before tyrosine (a time chosen because decreases in tyrosine had virtually disappeared by then), the increase in pHPG produced by tyrosine (i.e. IMI–tyrosine vs IMI–saline groups) was only 1.1 ng/g. Thus, IMI appears to have blocked the rise in brain pHPG without significantly changing plasma or brain tyrosine concentrations.

Previous studies on [$^3$H]tyramine metabolism in rat brain slices have indicated that either pretreatment of the rats with DMI (6–60 mg/kg, 1 hr before sacrifice) or adding DMI directly to the incubated slices ($10^{-7}$ to $10^{-6}$ M) decreases the synthesis of [$^3$H]octopamine [22]. Since DMI did not diminish the retention of [$^3$H]tyramine by the slices or the production of its acidic metabolite, [$^3$H]$p$-hydroxyphenylacetic acid, the authors interpreted these data to suggest that DMI inhibits the uptake of [$^3$H]tyramine at intraneuronal sites where dopamine-β-hydroxylase is located, thereby reducing the conversion of [$^3$H]tyramine to [$^3$H]octopamine.

If this mechanism is correct, it could explain the decreased conversion of tyrosine to pHPG that we have observed *in vivo* and also could account partly for the ability of IMI to lower endogenous brain pHPG concentrations. Further support for this mechanism is provided by the results in Table 3, which show an interaction between IMI and tyrosine in altering brain MHPG levels. When tyrosine was administered alone, it caused a 12% increase in brain MHPG, which is identical to the increase we previously reported [23]. While this increase could be due to tyrosine hydroxylase not being fully saturated at normal brain tyrosine concentrations [6], we believe it is more likely caused by an enhanced release of endogenous NE by the tyramine or octopamine that is newly synthesized from tyrosine [7]. On the other hand, IMI by itself produced a 12% decrease in brain MHPG. As mentioned earlier, this reduction, though small (9–14%), has been very consistent in eight separate experiments. However, animals pretreated with IMI 4.5 hr before tyrosine had brain MHPG levels identical to those of rats administered IMI alone, indicating that tyrosine failed to increase MHPG levels in IMI-pretreated

rats. These results could be explained by the hypothesis mentioned above, since IMI (perhaps via DMI) blocks the uptake of tyramine into synaptic vesicles, thereby preventing the release of NE and the concomitant elevation in MHPG.

Although tyrosine had no effect on brain DHPG as it did on MHPG, this apparent discrepancy may be explained by the fact that DHPG preferentially reflects intraneuronal and MHPG extraneuronal NE metabolism [24, 25]. This distinction in their production at separate sites of NE metabolism may also account for the greater decrease in DHPG than in MHPG which we found was caused by IMI (Table 3). Similar results were reported previously for DMI, which was also found to cause a greater reduction in DHPG than in MHPG [26] or a decrease in DHPG but not in MHPG [25].

The depletion of pHPG by IMI alternatively could be explained by an inhibition of one of the enzymatic steps involved in octopamine synthesis or degradation. *In vitro* studies, however, argue against this possibility. For example, Creveling *et al.* [27], reported that both IMI and DMI have little effect on purified dopamine-$\beta$-hydroxylase, even in concentrations up to 1 mM. We have found that tricyclic antidepressants inhibit reversibly type B monoamine oxidase (MAO-B) [28]; but since octopamine is deaminated principally by MAO-A [29], this should not affect octopamine metabolism. Finally, *in vitro* studies have indicated that aldehyde reductase is not inhibited by 0.5 mM IMI [30].

Another possible explanation for our results is that IMI or one of its metabolites might decrease the release of octopamine from either noradrenergic or perhaps specific octopamine-containing neurons. Since octopamine is synthesized by using the same enzymes as are used for NE (which additionally requires tyrosine hydroxylase), it would be expected that at least part of the octopamine in brain would be contained in NE neurons and therefore would be regulated to some extent by noradrenergic receptors. However, evidence against this possibility is given by our finding that a dose of yohimbine that increases brain MHPG to almost 300% of control failed to increase pHPG levels; instead, 6 hr after the drug was given, there was a decrease in pHPG that was explainable by an equal reduction in tyrosine levels [2].

It is also possible that octopamine is present in specific neurons which are regulated by "octopamine" receptors. Although octopamine has been identified as a putative neurotransmitter in several invertebrates (for review, see Ref. 31), there is at present only sparse, indirect evidence for octopamine serving as a neurotransmitter in the mammalian brain. One recent study suggested that octopamine turnover in the rat (as measured by an accumulation of octopamine following MAO inhibition) is regulated by octopamine receptors in the hypothalamus but by presynaptic adrenergic receptors in the striatum [32].

In conclusion, these studies suggest that at least two mechanisms are involved in the decrease in pHPG produced by IMI. The first is a reduction in tissue tyrosine concentrations. While the cause of this is not yet known, it may be due partly to an inhibition of tyrosine absorption. A second mechanism for pHPG depletion appears to be related to a block in the conversion of tyrosine to pHPG. The most likely explanation for this is the apparent ability of IMI or its metabolites to inhibit the uptake of tyramine into synaptic vesicles, thereby blocking the $\beta$-hydroxylation of tyramine to form octopamine. Further studies will be needed to find out whether a deficiency of either tyrosine or octopamine plays an important role in the antidepressant and/or side effects of IMI.

*Acknowledgements*—This work was supported by NIMH Grant MH28340 (D. J. E.). We thank CIBA-Geigy for providing imipramine hydrochloride, USV Pharmaceuticals for the desipramine hydrochloride, and Dr. J. Stephen Kennedy (Neurosciences Research Branch, NIMH, Rockville, MD) for the didesmethylimipramine and the authentic pHPG. We thank Josie Longanecker for typing and Thomas Peckyno for technical assistance.

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