# Interactions of imipramine and synthesis inhibitors on biogenic amines

The clinical antidepressant effects of imipramine have been explained by its inhibiting action on membrane uptake systems in catecholamine- and 5-hydroxytryptamine-containing neurons (Schanberg, Schildkraut & Kopin, 1967: Carlsson, Corrodi & others, 1969). These effects suggest that both catecholamines and indoleamines may be involved in depression. In an attempt to define more clearly the specific biogenic amine responsible for the antidepressant effect of tricyclic drugs, we treated patients showing clinical improvement on imipramine concurrently with either α-methyl-tyrosine (α-MPT) or *p*-chlorophenylalanine (PCPA) so as selectively to inhibit catecholamines or 5-HT synthesis, respectively (Shopsin & Gershon, 1973). Depression that had improved in patients treated with imipramine recurred when they were treated with PCPA but not with α-MPT. This suggested that serotonergic rather than adrenergic neuronal systems are involved in the antidepressant effects of imipramine.

We have now assessed the changes in brain amines in rats on a treatment schedule comparable to the clinical schedule above.

Groups of 8 to 11 male Sprague-Dawley rats, 200–250 g, were treated daily with imipramine, enzyme inhibitors, or vehicle as summarized in Table 1. Animals were killed by a blow on the head and brain catecholamines determined by the method of Neff, Sparo & others (1971), while 5-HT and 5-hydroxyindolacetic acid (5-HIAA) were estimated by the method of Curzon & Green (1971).

Chronic treatment with imipramine did not alter the endogenous concentrations of brain noradrenaline, dopamine, or 5-HT (Table 2). The concentrations of brain 5-HIAA, however, were significantly ($P<0.05$) lowered by the treatment schedule.

α-MPT treatment resulted in a significant depletion of brain noradrenaline ($P<0.001$) and of dopamine ($P<0.001$), but not of 5-HT or 5-HIAA. Imipramine before and during treatment with α-MPT provided some protection ($P<0.005$) against this depletion. However, the depletion of noradrenaline and dopamine remained statistically significant at $P<0.02$ and 0.001 respectively. The degree of protection was significantly less effective against dopamine depletion which was still 55% of control values.

Table 1. *Schedule of drug treatment.*

| Days 1–7 | Days 8–10 (30 min) | Day 11 (30 min) |
|---|---|---|
| Saline | Saline——→Methyl cellulose (0·25%) | Saline——→Methyl cellulose——→Death |
| Imipramine | Imipramine+++——→Methyl celulose (0·25%) | Imipramine——→Methyl cellulose——→Death |
| Saline | Saline——→α-MPT+ or PCPA++ | Saline——→α-MPT or PCPA——→Death |
| Imipramine | Imipramine——→α-MPT or PCPA | Imipramine——→α-MPT or PCPA——→Death |

+α-MPT: α-methyl-tyrosine, 200 mg kg$^{-1}$ daily, i.p. injection.
++PCPA: *p*-chlorophenylalanine, 150 mg kg$^{-1}$ on day 1 and 100 mg kg$^{-1}$ on subsequent days.
+++Imipramine: Administered at daily i.p. doses of 10 mg kg$^{-1}$.

Table 2. *Effects of imipramine on α-MT or PCPA induced amine changes in rat brain.*

| Treatment* | Noradrenaline (μg g$^{-1}$) | Dopamine (μg g$^{-1}$) | 5-HT (μg g$^{-1}$) | 5-HIAA (μg g$^{-1}$) | 5-HT (μg g$^{-1}$) | 5-HIAA (μg g$^{-1}$) |
|---|---|---|---|---|---|---|
| 1) Saline | 0·446 ± 0·03 | 0·537 ± 0·04 | 0·372 ± 0·02 | 0·276 ± 0·01 | 0·389 ± 0·01 | 0·298 ± 0·01 |
| 2) Imipramine | 0·462 ± 0·03 | 0·590 ± 0·04 | 0·365 ± 0·01 | 0·228 ± 0·01[c] | 0·368 ± 0·02 | 0·221 ± 0·01[b] |
| 3) α-MT | 0·186 ± 0·02[a] | 0·153 ± 0·02[a] | 0·361 ± 0·01 | 0·265 ± 0·01 | | |
| 4) Imipramine + α-MT | 0·325 ± 0·03[b,d] | 0·298 ± 0·03[a,d] | 0·360 ± 0·02 | 0·229 ± 0·01[c] | | |
| 3) PCPA | | | | | 0·188 ± 0·02[a] | 0·114 ± 0·004[a] |
| 4) Imipramine + PCPA | | | | | 0·320 ± 0·01[b] | 0·234 ± 0·01[c,d, e] |

*Treatment schedule as summarized in Table 1.
a: $P<0.001$; b: $P<0.02$; c: $P<0.05$; d: Comparison of α-MT PCPA with imipramine + α-MT PCPA; $P<0.005$; e: comparison of imipramine with imipramine+PCPA not significant.

Brain concentrations of 5-HT were not affected by combined imipramine-α-MPT treatment. Imipramine treatment alone and imipramine-α-MPT treatment resulted in a depletion of 5-HIAA concentrations ($P<0.05$).

Treatment with PCPA was accompanied by a significant decrease in 5-HT ($P<0.001$) and 5-HIAA ($P<0.001$) in brain. Prior and simultaneous injections of imipramine significantly, but incompletely, protected against the reduction in 5-HT and 5-HIAA concentrations. Brain concentrations of both amine and metabolite remained significantly below control values; this holds with greater significance for 5-HT ($P<0.02$).

The imipramine may have exerted its protective effects on catecholamine depletion by blocking the entry of α-MPT into presynaptic adrenergic neurons, thus sparing the intraneuronal synthesis of catecholamine from inhibition. Alternatively, it might have retarded catecholamine metabolism.

Imipramine treatment protected against the depletion of 5-HT and 5-HIAA caused by PCPA more than against catecholamine depletion by α-MPT. This may be partly due to the inhibition by imipramine of 5-HT turnover. That it can inhibit 5-HT synthesis is supported by the lowering of brain 5-HIAA concentrations in animals receiving it (Table 2). Modigh (1973) has reported a lowering of brain 5-HT turnover with other tricyclic drugs. Imipramine (Asberg, Bertilson & others, 1974). nortriptyline (Bowers, Heninger & Gerbode, 1969), and amitriptyline (Papeschi & McClure, 1971) have also been shown to lower the mean concentration of 5-HIAA in the cerebrospinal fluid of man. Bruinvels (1972) observed that imipramine inhibited the accumulation of 5-HIAA which occurred after pretreatment with probenecid and also of 5-HT after L-tryptophan. Changes in 5-HIAA after treatment with tricyclic drugs are unlikely to be due to an effect on the adrenergic neuron, since concurrent treatment with α-MPT did not alter this effect in the rats.

In clinical studies (Shopsin & Gershon, 1973), PCPA treatment resulted in a reversal of the antidepressant effects of imipramine so in rats imipramine partially blocked the 5-HT/5-HIAA depletion to a greater extent than it blocked α-MPT-induced noradrenaline and dopamine depletion. Psychiatric patients continually ingesting imipramine showed a return of depressive symptoms on small doses of PCPA given for only a few days, whereas α-MPT given over several weeks at doses up to 4 g did not alter one antidepressant effect of imipramine. This suggests that serotonergic mechanisms are involved in the antidepressant effect of imipramine and that more discreet change in the functional concentrations of this brain amine may be responsible for the appearance of depressive symptoms in man.

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June 17, 1974

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# Glucose absorption from the rat jejunum during acute exposure to metformin and phenformin

The mode of action of the blood glucose lowering biguanides is complex and not clearly understood. Relatively high concentrations of biguanides inhibit several metabolic enzymes *in vitro* but it is uncertain whether these actions contribute significantly to their action *in vivo* (Segre, 1969). Therapeutic concentrations of metformin, in the presence of insulin, increase glucose uptake by isolated diaphragms from alloxan diabetic rats. This effect may be due to an action on glycogen metabolism (Frayn & Adnitt, 1972). *In vitro* studies of rat small intestine indicate that high concentrations of biguanides inhibit glucose absorption (Love, 1969). These findings were confirmed in man *in vivo* (McColl, 1971) and *in vitro* (Wingate & Hadley, 1973). We have measured glucose absorption from the rat jejunum *in vivo* in the presence of intraluminal metformin and phenformin.

Male albino Wistar rats (350 g) were anaesthetized with pentobarbitone sodium (75 mg $kg^{-1}$ subcutaneously) and the lumen of 50 cm of proximal small intestine was gently washed through with saline at 37 °. In the control group the loops were perfused with 20 ml of normal saline (0·9% w/v) containing 10mM D-(+)-glucose for 20 min. Treatment perfusions were similar but also included the drug in the saline-glucose solution. The solutions were initially pH 5·6 and were maintained at 37 ° while continuously recirculated through the lumen by gas lift using 5% carbon dioxide in oxygen (Nissim, 1965). At the end of the experiments the rats were killed by bleeding, and the perfusion fluid volume was measured and its glucose concentration estimated by the glucose oxidase-peroxidase method (Boehringer Corporation Ltd.) on an Auto Analyser. The loops were weighed and results are expressed as the amount of glucose absorbed per unit weight of wet tissue during the 20 min perfusion ($\mu$ mol $g^{-1}$, in 20 min). As there was a negative correlation between glucose absorption and loop weight (regression coefficient — 1·55) the treatment means were corrected for weight and were also tested for significant differences by analysis of co-variance. The significance of individual differences in treatment means compared to the control group was estimated by the product of the Student Range, "Q", and the effective residual standard error (Snedecor & Cochran, 1967).

The results are summarized in Table 1. Phlorizin is a potent inhibitor of glucose absorption (Jervis, Johnson & others, 1956) and caused significant inhibition at $5 \times 10^{-5}$ and $2 \times 10^{-4}$M ($P<0{\cdot}005$). However, metformin ($10^{-4}$M) and high concentrations of phenformin (up to $10^{-2}$M) did not alter glucose absorption significantly ($P> 0{\cdot}05$).

The relatively high concentrations of biguanides necessary to inhibit sugar absorption *in vivo* (Czyzyk, 1969) and *in vitro* (Love, 1969) may indicate that they have a slow onset of action. Biguanides have been shown to accumulate in the intestinal wall after oral and parenteral administration at higher concentrations than in other