NOREPINEPHRINE AND SEROTONIN METABOLISM AND CLINICAL RESPONSE TO COMBINED IMIPRAMINE AND AMITRIPTYLINE THERAPY IN DEPRESSION

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SUMMARY

The present study was aimed to find out the alteration of NE and 5HT systems in depression, if so in the same direction or not and to find out the clinical response to combined therapy of Imipramine and Amitriptyline, both tricyclic antidepressants. The study group consisted of 39 depressives as defined by I.C.D.-9 and 17 normal controls. Hamilton Depression Rating Scale was used to assess the severity of depression initially and after clinical improvement with drugs. Urinary MHPG and CSF 5HIAA were estimated for all depressives as per the standard procedure.

It was found that both urinary MHPG and CSF 5HIAA were lowered in depressives but when individual patients were analysed both were not altered in the same direction in a given individual. All the depressives showed equal therapeutic response to combined administration of Imipramine and Amitriptyline.

The Catecholamine (CA) hypothesis of affective disorder proposes that some, if not all, depressives are associated with absolute or relative decrease in catecholamines particularly Nor Epinephrine (NE), Indoleamine (IA) and Serotonin (5HT) (Schildkraut, 1965; van Praag, 1977; 1978). The previous studies led to two concepts—the concept that central amine metabolism can play a role in the pathogenesis of depression and the concept that the group of depressions is a heterogeneous one in biochemical terms. At least two types of depressions exist—5HT deficiency play a role in the pathogenesis of one, while NE deficiency is important in the pathogenesis of the other (van Praag, 1977b). This conclusion raises the new question—Can both 5HT and NE metabolism be disturbed in the same patient or are the depressions either chiefly 5HT or NE deficient. There are studies to show that there is interaction between 5HT and NE systems (Johnson and Kim, 1973). The general conclusion is that 5HT system of the raphe and NE system of Locus Coeruleus (LC) may reciprocally inhibit one another.

In the recent years based on the hypothesis of 5HT and NE involvement several different attempts have been made to develop laboratory tests that might predict the response of depressed patients to various tricyclic antidepressants (TCA). 5HT deficient depressives improve in response to compounds considered to increase the amount of 5HT available in the brain e.g. Cloimipramine and NE deficient group improve with drugs on NE system e.g. Imipramine (IMI) and Nortriptyline (NT). It was reported by Asbery et al. (1972) that low 5HIAA (metabolite of 5HT) depressed group did not respond to NT whereas high 5HIAA-low MHPG (metabolite of NE) did. Low 5HIAA group found to be non responders to IMI (Goodwin and Post, 1975). It was also reported

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that low 5AIAA group responded better to clomipramine which has marked inhibitory effect on the 5HT reuptake into neuron.

Many investigators used MHPG levels to differentiate subtypes of depression (Schildkrut et al., 1978; Palaniappan, 1980). It is believed that the urinary MHPG reflects brain metabolism and hence one approach has been to measure urinary excretion of MHPG. Maas et al. (1972) reported that low MHPG group responded better to IMI. It was found that all depressives with MHPG excretion below 1000 U/g/24 hrs. responded well to IMI and Desmethylimipramine (DMI). Beckman and Goodwin (1975) reported that responders to AMI excreted significantly higher MHPG than did the non responders. These findings led to the conclusions that there may be two groups—one with low MHPG excretion and the other with high level. The group with low level are associated with an alteration in NE system show favourable response to IMI or DMI and poor response to AMI, the group with high MHPG showed favourable response to AMI, failure to respond to IMI and associated with alteration in 5HT system (Maas et al., 1972). It is to be noted here that AMI is not an effective blocker of NE uptake but its active metabolite Nortriptyline (NT) is (Maas et al., 1972). If the therapeutic level of NT is maintained, the AMI would also give better result in depressive with low MHPG excretion. It is assumed that the group with 5HT deficiency where NT is less useful is NE deficient. It is of interest to note that Goodwin et al. (1977) showed inverse relationship between CSF 5HIAA and urinary MHPG. They reported that MHPG excretion in the low 5HIAA group was significantly higher than in the high 5HIAA group. However this has not been confirmed by other investigators. Moreover in most of the studies either IMI or AMI has been tried and there is no known study to find out the response to combined therapy of IMI and AMI. The facilities to measure the amine levels and to monitor the blood levels of antidepressants are not available in most of the places at least in developing country like India. Hence it is in practice to administer combined IMI and AMI therapy, IMI during day time and AMI during bed time as it has also sedative effect and there is no need to add another drug to induce sleep.

This study was undertaken to find out whether both 5HT system and NE system were involved in depressives and if so in the same manner or not to find out the clinical response to combined IMI and AMI therapy.

**Methods and Materials**

The study consisted of 39 depressives, 22 males and 17 females between the age group of 22 and 50 years, admitted to Institute of Mental Health, Madras. The patients were diagnosed by two Psychiatrists in a separate setting based on I.C.D. 9 criteria. Complete physical examination was done by a Physician and ruled out neurological and other somatic illnesses. Routine laboratory investigations were carried for all patients and few had CAT scan. All the patients were administered Hamilton Depression Rating Scale (HDRS-24 item version) to assess the severity of the illness.

**Laboratory Investigations**

All the patients were drug free for at least three weeks prior to the investigation. They were all asked not to take any drug and to avoid completely bananas, coffee, tea, cigarette etc. prior to the investigation as they might alter the level of the amine metabolite 5HIAA and MHPG. Lumbar puncture was done...
with extreme precaution as per the standard procedure and CSF collected. Ascorbic acid was added to CSF as preservative and immediately kept in deep freeze. Two 24 hrs. urine collection was done for all the patients for estimation of MHPG and N/6 HCL was added as preservative.

Follow-up and drug therapy
All the patients were started on combined Imipramine (IMI) and Amitriptyline (AMI) drugs. Initially IMI was started with 50mg during day time in divided doses and AMI 25mg during bed time. Slowly the dose was increased to maximum dosage of IMI during day time in divided dose and AMI at bed time. The dose was adjusted according to the patients need. Few patients had in addition benzodiazepines at bed time and none of the patients in this series had received electroconvulsive therapy. There were no side effects reported except minor side effects like dryness of mouth, constipation etc. Each patient was followed up regularly and interviewed by the same psychiatrists every week. They were taken up for biochemical study once there was clinical improvement and after assessing by administering Hamilton Depression Rating Scale. The interval period between initial and second study (after clinical improvement) was not same for all the patients as the criteria was the clinical improvement and improvement on Hamilton score and not the interval period. Collection of urine and CSF was done as for the initial study. Drug free period could not be maintained here due to ethical reasons.

Normal controls
The normal control consisted of 17 persons in whom no physical illnesses were detected and these were the persons admitted for minor surgical procedures under spinal anaeesthesia. All these persons were seen by physician and two psychiatrists and ruled out the possibility of any physical and psychiatric illness. None of their first degree relatives suffered from psychiatric illness. Hamilton Depression Rating Scale was administered as a second screening tool. CSF and 24 hours urine collection were done as described above.

Biochemical analysis
MHPG in urine was estimated essentially by the method of Ruthven and Sandler (1965) and 5HIAA in CSF essentially by the method of Korf et al. (1971). All estimations were carried out within next 48 hours after collection of specimen. Each patient was given code number and this was broken only at the end of the study for data analysis. Clinicians and laboratory personnel were blind to each others result.

Results
It has been reported that urinary MHPG and CSF 5HIAA were altered in depressives. To find out meaningful association between these amine metabolites and severity of depression, Hamilton score and the level of these metabolites were correlated. It was observed that there was negative correlation between total Hamilton score and urinary MHPG (Co-efficient-0.1160, NS) and CSF 5HIAA (Co-efficient-0.1250, NS) but not statistically significant. This did not suggest any strong association between these amine metabolites and severity of depression.

The urinary MHPG and CSF 5HIAA of all the depressives were compared with that of normal controls. It showed significantly lower level in depressives (p<.001, Table-I). It was of interest to note that MHPG and 5HIAA, metabolites of NE and 5HT were altered
in the same direction. But this did not give any idea whether they were altered in the same manner and in the same proportion in all the depressives studied. To find out this, all depressives were studied individually. All the depressives were split into groups with low or high MHPG excretors and with low or high 5HIAA in CSF. As it was reported by Maas et al. (1972) that patients with urinary excretion less than 1000µg/24 hours responded well to IMI therapy, this level was taken as criteria to divide high and low. Likewise for CSF 5HIAA the level of 15ng/ml. was taken as criteria to divide as Low and High 5HIAA group. This was based on the study of Asberg et al. (1972) who found that the therapeutic effect of Nortriptyline (NT) which is a relatively selective inhibitor of NE group was less pronounced in depressive patient with CSF 5HIAA level of less than 15ng/ml. When it was analysed they formed four groups—1. Low MHPG-Low 5HIAA (N-2); 2. Low MHPG-High 5HIAA (N-10); 3. High MHPG-High 5HIAA (N-14); and 4. High MHPG-Low 5HIAA (N-13). The total Hamilton score before and after treatment were compared and the mean differences were taken which obviously shows the degree of clinical improvement. The clinical improvement as reflected on Hamilton score was due to the impact of drug treatment mainly. The mean score of each group were compared to assess the differential clinical improvement (Table-II). It was observed that there were no difference in the

### Table I—Comparison of Amines Metabolites in Depression with Normals

| Group                  | Mean | S.D. |
|------------------------|------|------|
| Urinary MHPG, µg/24 hrs. |      |      |
| Depression (N=39)     | 1.46 | 0.24 |
| Normals (N=17)        | 2.96 | 0.11 |
| t=31.88, p<.001       |      |      |
| CSF 5HIAA ng/ml.      |      |      |
| Depression (N=39)     | 18.50| 5.07 |
| Normals (N=17)        | 41.99| 1.17 |
| t=26.88, p<.001       |      |      |

### Table II—Comparison between groups on mean increase in Hamilton score

| Groups               | Mean | S.D. | N  | t   | p   |
|----------------------|------|------|----|-----|-----|
| Low MHPG-Low 5HIAA  | 31.00| 5.00 | 2  | 1.86 | .10 |
| Low MHPG-High 5HIAA | 24.10| 3.67 | 10 |     |     |
| High MHPG-Low 5HIAA | 28.23| 6.01 | 13 | 1.94 | .10 |
| Low MHPG-High 5HIAA | 24.10| 3.67 | 10 | 0.01 | NS |
| High MHPG-High 5HIAA| 24.14| 9.12 | 14 |     |     |
| Low MHPG-Low 5HIAA  | 28.23| 6.01 | 13 |     |     |
| High MHPG-High 5HIAA| 24.10| 9.12 | 14 | 1.33 | NS |
| Low MHPG-Low 5HIAA  | 31.00| 5.00 | 2  | 0.78 | NS |
| High MHPG-Low 5HIAA | 28.23| 6.01 | 13 |     |     |
| Low MHPG-High 5HIAA | 31.00| 5.00 | 2  | 1.15 | NS |
mean increase Hamilton score between the groups except the difference between Low MHPG-High 5HIAA group and Low MHPG-Low 5HIAA group and between Low MHPG-High 5HIAA group and High MHPG-Low 5HIAA groups (p<.01). It was also observed that when the four groups were compared, the groups with low 5HIAA had more mean increase on Hamilton score.

Discussion

The differences in drug action can assume clinical importance when we define biological sub groups of depression which respond consistently to specific drug. The existence of biological sub group has been advocated earlier (van Praag, 1977; Asberg et al., 1976; Schioldkraut et al., 1978). The findings of lower level of urinary MHPG and CSF 5HIAA in this study were consistent with many earlier reports. The fact that urinary MHPG and CSF 5HIAA were altered in the same manner in all depressives propose the hypothesis that in all depressives both NE and 5HT systems were involved in the same manner. In 5HT deficient depressives the therapeutical effect of an antidepressant with most NE potentiating effect-NT was less marked than that in patients without demonstrable defect in central 5HT (van Praag, 1977c). It is assumed to expect these NT susceptible patients are NE deficient. Maas et al. (1972) proposed that depressives associated with high urinary excretion of MHPG who respond better to AMI and had abnormally associated 5HT system. It is noteworthy to point that Sacchetti et al. (1976) reported that in a small sample that low MHPG depressives responded to AMI. In the present study of 39 subjects, only 10 had low MHPG-High 5HIAA and 13 had high MHPG-low 5HIAA. It was assumed that the former group would respond to IMI or NT and the latter to AMI or Clo mipramine. In this series, the rest 16 subjects did not have the expected MHPG and 5HIAA alteration. Diagnostically all 39 form a homogenous group. If one would select a drug for the above mentioned 16 depressives, it might be difficult to choose whether AMI or IMI. AMI is probably not an effect blocker of NE uptake but its active metabolite NT is (Maas et al., 1972). Hence it might be true that AMI would be an ideal and would be effective in both 5HT and NE deficient groups, provided the plasma level of NT is within the therapeutic range. Here NT is an active metabolite of AMI and there is report to assess the dosage of AMI to have the therapeutic level of its metabolite. The depressives of this two groups (N=16) might or might not respond to one group of drug alone (either IMI or AMI). The rationale that all our patients were put on combined therapy of IMI and AMI is understood here and the drug started without knowing the levels of MHPG or 5HIAA and plasma levels of drugs were not measured.

The results showed equal improvement as reflected on Hamilton score. This means that all depressives responded equally to combined therapy of IMI and AMI. For NT, IMI and AMI, there were studies that have shown correlation between plasma levels and therapeutic effect (Burrows et al., 1972; Muscettola et al., 1978). The therapeutic range for plasma concentration in the case of IMI is confined by lower therapeutic and an upper toxic limit (Gram et al., 1976). These studies indicate a lower critical limit of plasma level of 200 to 250ng/ml for total concentration of IMI and AMI. Very high levels were not associated with good or poor response but to toxic effect. In the present study, the optimum dosage was maintained in all the depressives.
Even though dose/plasma concentration relationship might not be same for all patients, it was reasonably expected to have the therapeutic level. The altered NE system might also be acted by NT, the active metabolite of AMI. In the NT lower and upper therapeutic plasma concentration range has been established (Montgomery et al., 1978; Ziegler et al., 1977). AMI has been studied by several authors. Where plasma/effect relationship has been found, there has been a positive correlation (Braithwaite et al., 1972; Ziegler et al., 1977). The study by Kupfer et al. (1977) clearly indicated that the high level of AMI and/or NT were not associated with unfavourable therapeutic effect. Pharmacokinetic factors may be of some importance and for a drug like AMI one may speculate if the combination with other compounds may create new avenue. There is no known study to the authors knowledge which report about the interaction between the different drugs in Tricyclic antidepressant (TCA) and the dose/plasma concentration relations when they are given in combination. The clinical use of plasma monitoring of TCA raise the question of how the dose can be adjusted early in the course of treatment in order to bring the plasma concentration on an appropriate therapeutic level.

It was observed from the table II that the group which had more mean increase on Hamilton score in comparison, the level of 5HIAA were low. Probably the level of 5HIAA or the depressives with 5HT alteration towards lower was more significant than urinary MHPG. In comparison of two groups with high 5HIAA level in both groups, the mean increase on Hamilton were exactly similar (24.10 and 24.14). Here the level of MHPG varied. It might be noted that the depressives with Low MHPG-Low 5HIAA responded more than the other groups. The group which showed good improvement next to that was high MHPG-Low 5HIAA. The group with Low MHPG-Low 5HIAA responded better than the other may be explained that IMI acted upon NE system and AMI acted upon 5HT system and increased their levels. But this interpretation should be given cautiously since the sample in this group is very small (N-2). Moreover plasma concentration of these drugs were not measured. The group with high MHPG-low 5HIAA level might be in support of the hypothesis of involvement of 5HT system rather than NE system and might have been acted upon by AMI. The interpretations are highly hypothetical and further studies are needed with larger samples with estimations of plasma concentration of drugs.

The reason for not finding out the alterations in the assumed manner were not clear. However, there was one flaw in this method of defining low or high MHPG or 5HIAA. There is no universally accepted criteria by which it may be categorised as low or high. It was our own criteria arrived at, based on some previous studies. What we called as high MHPG or 5HIAA might be still lower than that of the normals. It was only relative term. It was also noted that no single patient had normal or higher than normal urinary MHPG or CSF 5HIAA. The mode of administering these combination was also to be noted. Both of them were not combined together during day time or night but IMI was administered during day time in divided doses and AMI at bed time as single dose. The obvious reason for administering AMI at bed time was that it has sedative effect also.

The present investigation led to the following conclusions. The depressives excreted less MHPG and had less CSF
5HIAA than that of normals, their levels were negatively correlated with the severity of depression though not up to statistically significant level, both were not altered in the same direction in all depressives and all the depressives showed equal therapeutic response to combined therapy of IMI and AMI as shown on Hamilton score.

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