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ACTIVE PLATELET 5-HT UPTAKE IN DEPRESSIVES TREATED WITH IMIPRAMINE AND ECT

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ABSTRACT

Several studies have reported decreased platelet 5-HT uptake in patients of major depression. The present study was undertaken with the aim to study the active platelet 5-HT uptake in depressed patients and effect of treatment with imipramine/ECT on platelet 5-HT uptake in these patients. 30 patients of major depression and equal number of age and sex-matched controls were included in the study. The depressives received imipramine (N=15) or ECT (N=15). Pretreatment active platelet 5-HT uptake was lower in depressives. Treatment with imipramine resulted in significant decrease in 5-HT uptake while with ECT there was significant increase. The serotonergic mechanisms are discussed.

Key Words : Active platelet 5-HT uptake, major depression, imipramine, ECT

Considerable evidence has accrued in the last two decades to support the hypothesis that alterations in serotonergic neuronal function in the central nervous system occur in patients with major depression (Owens & Nemeroff, 1994). These findings include : (a) reduced CSF concentrations of 5-HIAA, the major metabolite of serotonin, in drug -free depressed patients, (b) reduced concentrations of 5-HT and 5-HIAA in postmortem brain tissue of depressed and (or) suicidal patients, (c) decreased plasma tryptophan concentrations in depressed patients, (d) in general, all clinically efficacious antidepressants augment 5-HT neurotransmission following chronic treatment, (e) clinically efficacious antidepressant action by all inhibitors of 5-HT uptake (f) increases in the density of $5HT_2$ binding sites in postmortem brain tissue of depressed patients and suicide victims as well as in platelets of 5-HT transporter (determined with ^3H imipramine) sites in platelets of drug-free depressed patients.

Numerous studies have shown a low number of ^3H imipramine binding sites (Bmax) in platelets of major depression (Briley et al., 1980; Asarch et al., 1980; Paul et al., 1981; Tny et al., 1994; Gronier et al., 1994; Lawrence et al., 1993). Although it has been suggested that decreased platelet imipramine binding may be a putative biological marker of depressive illness, some studies have not confirmed this finding. Ellis & Salmond (1973) performed a meta analysis of published reports on imipramine binding in groups of depressed and healthy control subjects and found that there was a highly significant decrease in (Bmax) maximal binding values in the depressed subjects. This finding remained highly significant even when only high affinity binding studies (Kd<1 nmol/L) were considered, although the absolute size of this decrease was smaller.

The mechanism of antidepressant action of electroconvulsive therapy (ECT) is still a matter of controversy. Shapira et al. (1992) showed that ECT enhances central serotonergic responsivity. Subramanyam (1975) reported

that ECT produced a sustained increase in the synthesis and turn over of amines in the brain. Jori et al. (1975) reported that ECT caused significant increase in CSF 5-HIAA at the time of recovery than at the beginning of the treatment. Costain et al. (1979) reported that ECT potentiates 5-HT effects in test animals (rats).

The present study was undertaken with the aim to study active platelet 5-HT uptake in depressed patients and effect of imipramine and ECT on active platelet 5-HT uptake in these patients.

MATERIAL AND METHOD

Subjects for the study consisted of a depressive group and a control group. All consecutive drug-naive patients of depression of both sexes between 17-60 years of age who were admitted in the department of psychiatry, K.G.'s Medical College. Lucknow were screened. DSM-III-R criteria (APA, 1987) was used for diagnosis of major depression-single episode or recurrent. Hamilton Rating Scale for Depression (Hamilton, 1960) (HRSD) was used to rate the severity of depression and those patients scoring 17 points or more on 17 item scale were included in the study. Exclusion criteria were presence of physical illness requiring active medication, papilloedema, epilepsy, mental retardation, organic brain syndrome, pregnancy, and drug/alcohol dependence (Dalal, 1997).

Control group comprised of normal healthy volunteers matched for age and sex with depressives. Controls were screened on Cornell Medical Index (CMI) (Broadman et al., 1949) and those who gave thirty or more "yes" responses on entire CMI and/or ten or more 'yes' responses on M-R sections of CMI were excluded. Controls with past or family history of depression were also excluded. Other exclusion criteria were same as for the depressive group.

All the subjects included in the study were kept drug free for seven days except tablet lorazepam on s.o.s. basis because antidepressants or antipsychotics may alter platelet 5-HT uptake (Todrick & Tait, 1969; Boullin et al., 1976). The subjects were kept on a diet free of pineapples, bananas, plums and nuts for the entire duration of study Controls were also kept on a similar dietary restriction for a period of 7 days before taking blood sample This specific restriction on the diet was imposed especially for tryptophan containing food items as tryptophan a precursor of 5- hydroxytryptamine (5-HT), can alter the levels of 5-HT and its metabolites in serum and CSF (Wurtman & Fernstrom, 1974; Sneddon, 1973; Knott & Curson, 1972) and this may alter the results.

The depressives were divided randomly into imipramine group (N=15) and ECT group (N=15). Subjects in the imipramine group received tablet imipramine on a fixed dosage of 225 mg/day i.e. 75 mg three times daily which was achieved within a week starting from 75 mg/day on first three days followed by 150 mg/day on next three days and 225 mg/day thereafter, which was continued till the end of 5 weeks study period provided there were no serious side-affects of imipramine which were assessed on Asberg side effect scale which was administered at weekly interval. The treatment was stopped at any assessment point at which clinical improvement occurred i.e. HRSD score fell below 5 points.

Subjects in the ECT group were given only modified electroconvulsive therapy first three on alternate days and on every fourth day thereafter to a maximum of ten at the end of five weeks treatment phase. Atropine (1mg I.V.) was administered two minutes before the anaesthetic medication. Thiopental (2-3 mg/kg body weight I.V.) was used as the anaesthetic agent and succinylcholine (0.5-1 0 mg/kg body weight I.V.) as muscle relaxant. Sine wave ECT was administered by "Electrocon" model manufactured by Associated Electronic Engineers Bangalore using bitemporal electrodes. 90 to 120 volt electrical stimulus for 0.5-1.0 seconds was given to induce seizures.

Severity of depression and response to imipramine/ECT was assessed on HRSD which

was administered on the day of hospitalization and then at weekly interval till the end of the treatment phase.

Routine haemogram, SGOT, serum bilirubin, serum creatinine, serum proteins, blood urea, blood sugar, urine examination and bilateral fundus oculi examination were done in all subjects and those with abnormal test results were excluded. For platelet 5-HT uptake estimation, subjects were kept overnight fasting and in the morning (between 8 to 9 a.m.) 10 ml venous blood was taken by disposable syringe rinsed with 3.8% sodium citrate and transferred into polypropylene tube containing 1 ml of 3.8% sodium citrate as anticoagulant (Mills & Robert, 1967). Blood samples were drawn in fasting condition in the morning (Meltzer et al., 1981) to control for the diurnal rhythm of platelet 5-HT uptake. The sample tubes were immediately (within one hour) sent to Industrial Toxicology Research Centre, Lucknow for assessment.

The platelet 5-HT uptake was estimated by following the principles of the method described by Scott et al. (1979). The counting was done in a liquid scintillation counter (LKB Wallac). In order to assess the active uptake, the passive uptake was substracted from the total uptake. The data is represented in terms of picomoles of ^{3}H (tritiated) 5-HT uptaken / 10^{8} platelet/5 minutes.

For depressives the platelet 5-HT uptake estimation was done prior to starting the treatment and at the end of treatment. For controls only one sample was estimated.

Student's "t" test was used to find the level of significance between two mean values. Paired "t" test was used to determine the level of significance of mean of difference where the observation were paired.

RESULTS

The sample for the study consisted of 30 patients of major depression and equal number of age and sex-matched controls. Mean age of depressives and controls was 43.93 and 42.60 years respectively and there was no significant difference between the two.

HRSD scores in depressives receiving imipramine was 28.26±3.94 which decreased significantly to 5.0±4.23 after treatment. HRSD scores in depressives receiving ECT's was 28.47±4.41 which decreased significantly to 5.07±4.23 after treatment.

TABLE
COMPARISON OF ACTIVE PLATELET 5-HT UPTAKE* IN DEPRESSIVES AND CONTROLS

	Imipramine Group (N=15)	ECT Group (N=15)	Controls (N=30)
	A	B	C
Pre-treatment	1.27±0.43	1.32±0.40	1.795±0.45
	D	E	
Post-treatment	0.28±0.19	1.97±0.38	

*Uptake values in picomoles of ^{3}H 5-HT uptaken/10^{8} platelets/5min

A vs C t=3.66; df=43; p<.001
B vs C t=3.38; df=43; p<.01
A vs D t=7.52; df=14; p<.01
B vs E t=3.87; df=14; p<.01

Pretreatment active platelet 5-HT uptake in depressives in imipramine group was 1.27±0.43 (pmol/10^{8} platelets/5min) and 1.32±0.40 in ECT group which were significantly lower as compared to controls (1.795±0.45). There was no significant correlation between active platelet 5-HT uptake and severity of depression. There was also no significant correlation between active platelet 5-HT uptake between the subjects with first episode depression and recurrent depression.

After treatment with imipramine, there was a significant decrease in active platelet 5-HT uptake (0.28±0.19) from pretreatment levels. Mean percentage blocking (decrease) in active platelet 5-HT uptake after treatment with imipramine was 76.47±16.87.

After treatment with ECT, there was a significant increase in active platelet 5-HT uptake (1.97±0.38) from pretreatment levels. Mean number of ECTs given to depressives was 8.87±2.45. Percentage increase in active

platelet 5-HT uptake after ECTs was 47.06±34.71.

DISCUSSION

This study was undertaken with the aim to study the active platelet 5-HT uptake in depressed patients and the effect of imipramine and ECT on active platelet 5-HT uptake in these patients. The major finding of our study was that platelet 5-HT uptake was lower in patients of major depression as compared to controls and imipramine treatment resulted in further decrease in active platelet 5-HT uptake while ECT treatment resulted in increase in active platelet 5-HT uptake.

The uptake of 5-HT in both brain and blood platelet requires active uptake process against a considerable concentration gradient (Blackburn et al., 1967; Chase et al., 1969). The process of passive uptake of 5-HT in both brain and blood platelets is of little or no physiological importance, only contributing to uptake at high substrate concentrations and not influenced by drugs such as imipramine which block the active transport process (Fusk et al., 1964). The 5-HT concentration in whole blood or platelets and the total 5-HT uptake (both active & passive) lack the specificity which the active platelet 5-HT uptake has and do not parallel with the CNS parameters so closely as the active platelet 5-HT uptake does. Hence, active platelet 5-HT uptake was studied and for this the estimation was done at a low concentration of 5-HT and for a short incubation period which is necessary for accurate results (Sneddon, 1973).

Active platelet 5-HT uptake was significantly lower in depressed patients as compared to controls prior to treatment (table). Similar findings have been reported by Hallstrom et al. (1976), Scott et al. (1979), Ehsanullah (1980) and Born et al. (1980). However, Shaw et al. (1971) found no difference in the uptake of platelet 5-HT in depressed patients, but the excessive concentration of 5-HT and the long incubation time used in that study precluded accurate determination of active 5-HT.

Lingjaerde (1983) hypothesized that there is a lowered turnover of 5-HT in atleast a subgroup of depressed patients which is reflected in lowered concentration of 5-HIAA in the CSF or lowered concentration of 5-HT in blood or in platelets or lowered active 5-HT uptake in blood platelets of these patients (uptake can be taken as a reflection for turnover). Moreover, as platelet can be taken as neuronal model for 5-HT, it can be interpreted that akin to platelets, in the pre-synaptic neurons the active 5-HT uptake is lowered in depressed patients which may be a compensatory mechanism, the function of which is to "make the best" out of the reduced amount of 5-HT in the synaptic cleft.

Treatment with imipramine resulted in significant decrease in active platelet 5-HT uptake as compared to the pretreatment levels. It has been reported that patients receiving imipramine gradually loose upto 80% of the original 5-HT content of their platelets (Yates et al., 1963, Tuomisto, 1994). Todrick & Tait (1969) reported that tertiary amines were more potent inhibitors than their demethylated derivatives to inhibit the uptake of 5-HT by human platelets. Several investigators have reported increase in Km but Vmax remaining same, i.e., the affinity of the transport is decreased, but the number of hypothetical molecules remains the same and concluded a purely competitive inhibition of imipramine on platelet 5-HT uptake (Lingjaerde, 1979; Tuomisto et al., 1979; Meltzer et al., 1981).

Tricyclic antidepressants exert their antidepressant action by the way of blockade of monoamine (5-HT or norepinephrine) uptake at presynaptic axoplasmic membrane (Kessel & Simpson, 1995; Rafaelsen, 1980; Lewis, 1974). The dimethylated tertiary amines (e.g. imipramine and amitryptiline) are more potent in blocking serotonin uptake than the monomethylated secondary amines (e.g. desipramine and nortryptiline) which block norepinephrine more potently (Kessel & Simpson, 1995).

ECT successfully ameliorated the depressive symptoms. The mean duration of treatment with ECT was 3.99 weeks. The response rate was 82.77% which is in keeping with the reports that most of the depression show good therapeutic response to ECT (Dubovsky, 1995).

As stated earlier that uptake could be taken as a reflection for turnover, the findings of the present study suggests that ECT might increase the turnover of 5-HT in platelets. Gayford et al. (1973) found increased 5-HT blood levels after ECT in depressed patients. Subramanyam (1975) reported that ECT produced sustained increase in synthesis, utilization and turnover of the amines in the brain of depressives. Jori et al. (1975) reported that ECT determined significant increase in CSF 5-HIAA at the time of recovery than at the beginning of the treatment. Costain et al. (1979) found that ECT potentiates 5-HT effects in test animals (rats). Although no firm conclusions can be drawn, it is possible that by ECT increased turnover of 5-HT in blood platelets (findings of the present study), in blood (Gayford et al., 1973) and increased turnover of CSF-5HIIA (Subramanyam, 1975; Jori et al., 1975; Costain et al., 1979) may be relevant to the antidepressant activity of ECT.

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