ESTIMATION OF ACETYLCHOLINESTERASE ACTIVITY IN ADDICTION

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SUMMARY

Clinical and experimental evidence suggests that the use of morphine and its major derivative (heroin) plays a role in the alteration of acetylcholinesterase (Ach E) activity. We studied Ach E activity in 80 heroin addicts hospitalized for detoxification. The activity of the enzyme was estimated by the method of Ellman GL. at 30°C and pH 7.4 using acetylthiocholine iodide as substrate. The Michaelis Menten constant aKm and aVm were used for the estimation of activity in pre, during and post treatment. The results showed that Ach E activity was suppressed during addiction, showing denaturation of the enzyme. The activity was regained after detoxification.

INTRODUCTION

Acetylcholinesterase is an integral part of the membrane of human erythrocyte. Its location at or near the outer cell surface gives it special significance in studies of cellular membrane. Since the kinetic properties of the enzyme change under many clinically abnormal conditions. The alterations in Ach E activity in hemolytic diseases like ABO type of the newborn and paroxymal nocturnal hemoglobinuria (PNH), specially in autoimmune-haemolytic anemia, may be of important value in understanding certain disease processes at the cellular and membrane level. The behaviour of Ach E seems to be correlated with the membrane. It is of interest that the erythrocyte membrane which contains more than a dozen enzymes, abnormality has been reported only in Ach E which tells that bio-

chemical status of Ach. E is more closely related to that of the erythrocyte membrane than the other. The cyclic changes within or on either sides of the membrane environments may define the nature of the changes as countered in kinetics of the enzyme. Huge diurnal fluctuations were also reported in different individuals menstrual cycle and in pregnancy. Moreover the abnormality of Ach E has been reported by Tanaka, in the membrane microenvironment. The activities were also reported in tropical splenomegaly, PNH and other diseases. Such huge oscillations focussed our interest to characterize the enzyme, as a probe for membrane changes in heroin addiction. Certain sedative drugs, also causes inhibition of Ach E which tends to decrease the activity of the enzyme. We estimated the activity of Ach. In heroin addiction, at the time of admission,
after six days of treatment and finally at the time of discharge i.e. after 10-15 days of treatment.

MATERIAL AND METHODS

All reagents (Analar Grade) were obtained from E. Merck, (West Germany) Blood samples were collected from patients in Drug Abuse Treatment Center (DATC) at the time of admission. The blood samples (4 to 5 ml) were immediately mixed with ACD anticoagulant and then centrifuged (5000 G, 5 min) at room temperature. The plasma, the top buffy coat and one third upper portion of the packed cells were sucked off, and the remaining packed cells of reticulocytes, leukocytes and thrombocyte were washed 3 times with 10 volumes of ice-cold 0.9% NaCl. Haemolysate (enzyme) was prepared by adding 0.02 ml of washed cells to 50 ml of ice-cold distilled water. After about 15 min. This preparation was diluted with an equal volume of ice-cold potassium phosphate buffer (0.2 M, pH 7.4).

Enzymic Assay

The enzyme was assayed in replicate by the method of Ellman at 30°C and pH 7.4 using acetylthiocholineiodide as substrate, and 5,5 dithiobis, 2 nitrobenzoate as colour reagent. 2-3 ml of the enzyme preparation was added to 50 ml of colour reagent and then, after a 15 min. pre-incubation period, 25 ml of substrate was added. The change with time in the extinction at 412 nm was noted spectrophotometrically and reading was taken at one, two and three minutes interval in triplicates per min per gram haemoglobin.

Enzyme Parameters

A total of 80 replicate assays were run by the same observer at each of two concentrations of substrate, one was much lower (s1=10 nm) and the other much higher (s2=200 nm) than a provisional estimate of Michaelis Constant (aKm or aVm) of the enzyme were calculated. The enzyme parameters were computed by fitting the corresponding linear regression equations, which were derived from S/V versus S plot to the data.

\[ aKm = \left[ \frac{[(S_1/V_S1) - (S_2/V_S) - S_1]}{(S_2/V_S2 - S_1/V_S1)} \right] - S_1 \]
\[ aVm = \left[ \frac{1}{(S_2/V_S2 - S_1/V_S1)S_2 - S_1} \right] \]

RESULTS

The pre and post activity determination in heroin addicts along with treatment Ach E normals are given in the Table I. The table includes akm, aVm, Hb and percentage activity of the enzyme. The activity is increased during treatment, while high concentration of heroin and its metabolite, monoacetyl morphine decrease the activity of the enzyme. Tranquilizers reduce pathological anxiety, tension and agitation, most of them inhibit phosphorylation in the neurones and thus the formation of ATP is inhibited. Activity effect during the treatment is represented by bars in Fig. 1 while improvement of Ach E during treatment is given in Fig. 2.

DISCUSSION

Previously it was reported, that most of the drugs specially tranquilizer and other sedatives inhibit enzyme activity. In the present work, we have been able to detect significant difference in the activity of Ach E in pre and post treatment of addiction of heroin as compared to normal. As heroin is chemically diacetyl morphine and it is converted into monoacetyl-morphine, morphine and some time elute such as diacetylmorphine after metabolism, these metabolites decrease the activity of the enzyme, Ach E. Secondly the low
TABLE-1
ESTIMATION OF ACH. E ACTIVITY IN HEROIN ADDICTION

<table>
<thead>
<tr>
<th>S.No</th>
<th>Condition</th>
<th>Duration</th>
<th>aKm(um)</th>
<th>aVm(units)</th>
<th>Hb(g/dl)</th>
<th>Activity%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td></td>
<td>22± 1.8</td>
<td>77± 2.09</td>
<td>12.4± 0.52</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>At the time of admission</td>
<td></td>
<td>40± 0.42</td>
<td>61± 0.51</td>
<td>8.3± 0.41</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(57)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Heroin addiction</td>
<td>5th day of treatment</td>
<td>39± 0.63</td>
<td>63± 0.52</td>
<td>8.8± 0.67</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(10-15) days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>At the time of discharge</td>
<td></td>
<td>30± 0.9</td>
<td>74± 1.9</td>
<td>9.95± 0.33</td>
<td>77</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. Number of patients are given in parenthesis.

haemoglobin content in pre treatment patient, tells us, about the anaemic state of the patients due to suppressed Ach.E activity of the red cells, when the patients are detoxified, the activity is regained and haemoglobin content increases. It has been suggested that the change could be due to oxidation of membrane lipids, formation of mixed sulphide, splitting of membrane

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Fig. 1. Activity Representation of Ach. E during treatment.

- Normal (N)
- At the time of admission
- At 5th day of admission
- At the time of discharge

Fig. 2. Improvement of Ach. E activity after prolong treatment (0-14 days).

Although the Patient had failed to improve Ach. E activity during days 0-6. But activity Improvement started over six days treatment.
## TABLE – 2

**ESTIMATION OF ACH. E ACTIVITY DURING DETOXIFICATION OF HEROIN ADDICTION**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Duration</th>
<th>$aKm$ (um) (days)</th>
<th>$aVm$ (units)</th>
<th>Hb (g/dl)</th>
<th>Activity%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>40± 0.46 (25)</td>
<td>62± 0.6</td>
<td>8.2± 0.55</td>
<td>60</td>
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<tr>
<td>2</td>
<td>2-4</td>
<td>39± 0.71 (25)</td>
<td>63± 0.72</td>
<td>8.0± 0.8</td>
<td>60</td>
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<tr>
<td>3</td>
<td>4-6</td>
<td>37± 0.72 (25)</td>
<td>63± 0.65</td>
<td>8.0± 0.8</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>6-8</td>
<td>39± 0.51 (25)</td>
<td>60± 0.7</td>
<td>8.0± 0.78</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>8-10</td>
<td>33±0.49 (25)</td>
<td>68±0.66</td>
<td>8.8± 0.35</td>
<td>69</td>
</tr>
<tr>
<td>6</td>
<td>10-12</td>
<td>31±0.39 (25)</td>
<td>70± 0.51</td>
<td>9.4± 0.41</td>
<td>72</td>
</tr>
<tr>
<td>7</td>
<td>12-14</td>
<td>29±0.52 (25)</td>
<td>76±0.9</td>
<td>991±0.45</td>
<td>78</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. Number of individuals are given in parenthesis.

Disulphide bonds or both. The present study was carried out to observe the effects of heroin and its metabolite in vivo. The results showed that pre treatment activity alternation is significant from the normal and about 40% activity is lost by the use of heroin. Only those patients who were using heroin for the last one to five years were studied.

The activity lost by using heroin is regained after treatment of the patient. The activity change after six days of treatment is not significant. This could be due to certain other sedative and painkillers given to the patients. Secondly the patient had failed to improve clinically from 0-6 days treatment, suggesting that there is no significant Ach.E improvement. It must be emphasized that such a drug induced activity would be expected to ultimately resolve spontaneously after six days continuously during treatment. The enzyme is reactivated at the time of discharge due to detoxification of patient and this is why the activity of Ach E goes up.

In conclusion, the present work presented a characterization of Ach E in heroin addicts and we have been able to show significant differences in the activity of the enzyme in both normal and diseased state, especially during the course of the treatment of a disease. So we can use Ach E activity as a probe for diagnosing diseases and its prognostic.

### REFERENCES


