BRAIN SEROTONIN TURNOVER IN ALCOHOLIC MICE

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Abstract—Mice were made alcoholic on an alcohol liquid diet. The rate of serotonin synthesis in the brains of chronically alcoholic mice was found to be almost half that of the control group. However, the effect of acute alcoholism demonstrated a tendency to increase the rate of serotonin synthesis. Brain levels of 5HIAA were decreased during chronic alcohol ingestion, however, the brain levels of serotonin in alcoholic mice were similar to those in the untreated mice. These data suggest that serotonergic units in the central nervous system are inhibited during chronic alcoholism.

Recently, attention has been directed towards a possible interrelationship between the biogenic amines and alcohol or morphine in order to define the pathogenesis of alcoholism and dependence on morphine alkaloids.

It has already been demonstrated that ethanol or acetaldehyde interacts on the metabolism of serotonin (1, 2, 3) or catecholamine (4, 5). Moreover, salsolinol (6) and tetrahydropapaveroline (7, 8) are formed from dopamine during alcohol metabolism in vitro. The latter alkaloid is of particular importance as it is a precursor of morphine alkaloids in biosynthesis in plants (9).

Furthermore, Way et al. have suggested that dependence on morphine is associated with an increase in brain serotonin turnover (10, 11). If such is the case, brain serotonin turnover would also be increased in chronic alcoholism. In the experiment reported herein, contrary to the above findings, a decrease in serotonin turnover in chronic alcohol ingestion was observed.

MATERIALS AND METHODS

1. Preparation of alcoholic mice

Alcoholic mice were prepared by a modification of the method according to Freund (12). Adult male and female ddN mice weighing 25 g or more were fed 1.5 g of lab chow per mouse per day until their wt. was reduced to 21 or 22 g. Then, the mice were housed three to a cage and only had access ad lib to ethanol or control solution for 10 days. The basic diet preparation selected was the equiweight mixture of modified milk and skim milk which contains the following components per 100 g: protein, 23.9%; fat, 9.5%; carbohydrate, 58.6%; vitamin A, 1000 U; vitamin B1, 0.45 mg; vitamin B2, 0.1 mg; vitamin B12, 2 μg; vitamin C, 20 mg; vitamin D, 250 IU; vitamin E, 3.0 mg; niacin, 2.0 mg; linoric acid, 225 mg; 415 calories.
TABLE 1. Liquid diets.

|                | Ethanol | Milk | Sucrose | Water added |
|----------------|---------|------|---------|-------------|
| Volume ml      | 5.0     | 26.2 | 0       | 95          |
| Calories ml    | 15.4    | 63.9 | 6.5     | 0.9         |
| Weight g       | 0.0     | 0    | 0.0     | 0.0         |

The composition of the liquid diets is listed in Table 1. Less than 30% of the calories was supplied in the form of ethanol. Protein content is 36.8 mg/ml and the calories are 16% of the total calories. Liquid diets were prepared every morning and the 24-hour volume of fluid consumed was measured. Acutely alcoholic mice were prepared by giving 5 g/kg of ethanol orally 20 min before drug injection.

2. Measurement of serotonin turnover

Synthesis rates of serotonin were estimated according to the method of Tozer et al. (13). The method is based on the assumption that the rate of serotonin formation is equal to the efflux rate of its metabolite, 5-hydroxyindoleacetic acid (5HIAA). After monoamine oxidase was blocked with tranylcypromine, the brain levels of 5HIAA decline exponentially. The rate of serotonin synthesis is calculated from the product of the rate constant of 5HIAA decline and the normal 5HIAA level. A plot of $\log_{10}[5HIAA]$ versus time yields a straight line, the slope of which is 1/2.3 times the rate constant of 5HIAA efflux. The slope was calculated by the method of least squares.

Mice were decapitated at intervals of 0, 30, 60 and 90 min after i.p. injection of tranylcypromine in a dose of 10 mg/kg. The brains (minus cerebellum) were quickly removed, weighed and homogenized in 9 volume of 0.4 N perchloric acid containing 0.01% of cysteine. Excess perchloric acid was removed by adjusting the supernatant to approx. pH 5 with 6 N potassium carbonate. The supernatant was removed into a 50 ml centrifuge tube, and 0.1 ml of 3 N hydrochloric acid, 3 g of sodium chloride and 20 ml of ethyl ether were added. After shaking for 15 min, 15 ml of ether layer was put into a 50 ml centrifuge tube containing 3 ml of 0.5 N phosphate buffer, pH 7. The rest of the sample was used to assay serotonin. The ether layer was removed by aspiration and the remaining extract was adjusted to approx. pH 10 by addition of solid sodium carbonate. Then, 4 ml of borate buffer, pH 10, saturated with sodium chloride, and 15 ml of n-butanol saturated with water were added. After shaking for 15 min, the buffer layer was removed by aspiration and the butanol layer was washed with 5 ml of the buffer. Ten ml of the butanol layer was transferred into a 50 ml centrifuge tube containing 20 ml of isoctane and 3 ml of 3 N hydrochloric acid. The phosphate buffer layer was measured for fluorescence at an activation wavelength 295 $m\mu$ and a fluorescence wavelength 340 $m\mu$ to estimate 5HIAA, and the hydrochloric acid layer was measured for fluorescence at an activation wavelength 295 $m\mu$ and a fluorescence wavelength 540 $m\mu$ to estimate serotonin.
RESULTS

1. Fluid consumption and body weight changes during liquid diet intake

As shown in Table 2, the mean daily alcohol liquid diet consumptions of male and female mice was 9.0 and 7.9 ml per animal, which is equivalent to 0.36 and 0.32 g of alcohol consumptions per animal, respectively. Since the mice consumed the alcohol liquid diet at the rates of 7.4 to 9.6 ml per animal per day, the intake of control liquid diet was restricted to 10 ml per animal per day, the main reason being that the brain levels of serotonin may be partly dependent on daily food intake. Mean reduction of body wt. of male and female mice on the alcohol liquid diet was 0.36 and 0.34 g per mouse per day, respectively, while that of the control mice was 0.23 and 0.06 g. Therefore, the mice on either the alcohol or control liquid diet for 10 days lost 0.6 to 3.6 g of body wt. However, they appeared to be healthy and no behavioral change was observed.

| Table 2. Daily fluid consumption and weight change. |
|-----------------------------------------------------|
| Male mice | Female mice |
| mean daily fluid consumption (ml) | mean daily weight decrease (g) | mean daily fluid consumption (ml) | mean daily weight decrease (g) |
| Alcohol diet | 9.0 | 0.36 | 7.9 | 0.34 |
| Control diet | 10 | 0.23 | 10 | 0.06 |

Each value represents a mean from 24 animals.

2. Brain serotonin and 5HIAA levels in alcoholic mice

As shown in Table 3 and Fig. 1, the brain level of 5HIAA in female mice of chronic alcoholism was 0.288 µg/g brain compared with 0.358 µg/g in control, and the level in male mice of chronic alcoholism was 0.262 µg/g compared with 0.301 µg/g in control. Therefore, as shown in Fig. 1, brain levels of 5HIAA decreased during chronic alcohol ingestion. However, acute administration of alcohol tended to increase brain levels of

| Table 3. Brain serotonin turnover in alcoholic mice. |
|-----------------------------------------------------|
| Acute | Chronic | Acute | Chronic |
| Female | Male | Female | Male |
| | Brain levels of 5HIAA µg/g ± S.E. | Rate constant of 5HIAA loss k (hr⁻¹) | Brain levels of 5HT µg/g ± S.E. | SHT turnover rate µg/hr nmol/g/hr | Rate constant of 5HT turnover k (hr⁻¹) |
| Control | 0.358 ± 0.014 | 0.755 | 0.517 ± 0.009 | 2.07 | 0.249 | 1.413 | 0.482 |
| Alcohol | 0.371 ± 0.018 | 0.757 | 0.534 ± 0.023 | 2.06 | 0.259 | 1.468 | 0.485 |
| Control | 0.358 ± 0.014 | 0.755 | 0.509 ± 0.010 | 2.04 | 0.249 | 1.413 | 0.489 |
| Alcohol | 0.288 ± 0.020 | 0.506 | 0.504 ± 0.022 | 3.76 | 0.134 | 0.762 | 0.266 |
| Control | 0.301 ± 0.006 | 0.757 | 0.456 ± 0.024 | 2.17 | 0.210 | 1.191 | 0.460 |
| Alcohol | 0.369 ± 0.039 | 0.677 | 0.450 ± 0.018 | 1.95 | 0.230 | 1.307 | 0.512 |
| Control | 0.301 ± 0.006 | 0.757 | 0.546 ± 0.011 | 2.60 | 0.210 | 1.191 | 0.385 |
| Alcohol | 0.262 ± 0.032 | 0.517 | 0.564 ± 0.026 | 4.52 | 0.125 | 0.708 | 0.221 |

Each value represents a mean of 6 determinations.
5HIAA. On the other hand, brain levels of serotonin in both acutely and chronically alcoholic mice were almost similar to those in control mice. After tranylcypromine was injected, the brain levels of serotonin in chronically alcoholic mice tended to increase with time, compared with that in control (Fig. 2), while those in acutely alcoholic mice tended to increase.

These data suggest that the decrease in brain levels of 5HIAA during chronic alcohol ingestion is due to depression of serotonin release and not to inhibition of monoamine oxidase activities.

3. Brain serotonin turnover in alcoholic mice

Turnover rates of brain serotonin in chronically alcoholic mice were roughly half those in control groups, as shown in Fig. 3. The results are summarized in Table 3. The calculated turnover rates of

![Fig. 1. Brain levels of serotonin and 5HIAA in alcoholic mice. Values are expressed as microgram per gram of wet tissue. Each bar represents the mean and standard error of six determinations.](image1)

![Fig. 2. Brain serotonin levels in alcoholic mice after injection of tranylcypromine. Ordinate shows mean microgram of brain serotonin per gram of wet tissue in six mice injected with 10 mg/kg of tranylcypromine at different times (abscissa).](image2)

![Fig. 3. Turnover rates of serotonin in alcoholic mice. Values are expressed as microgram per gram of wet tissue per hr.](image3)
serotonin in female and male mice with chronic alcoholism were 0.134 and 0.125 μg/g/hr compared with 0.249 and 0.210 μg/g/hr in control, respectively. These differences were statistically significant at the level of p<0.05. Turnover rates of serotonin in female and male mice of chronic alcoholism were 3.76 and 4.52 hours compared with 2.04 and 2.60 hours in control. Therefore, in spite of the consistent brain steady-state levels of serotonin, turnover rate or synthesis rate of serotonin decreased during chronic alcoholism. On the other hand, turnover rates of serotonin in acutely alcoholic mice tended to increase.

DISCUSSION

Brain levels of serotonin during alcohol administration have been examined by many investigators regarding the possible effect of alcohol on brain biogenic amines. Gursey and Olson claimed that the central depressant effect of ethanol has been explained on the basis of a reduced level of brain serotonin and norepinephrine (14). They also reported that chronic administration of ethanol resulted in a significant decrease in serotonin and norepinephrine in rabbit brain stem. However, other investigators have not demonstrated the shift of brain levels of serotonin (15, 16, 17). Duritz et al. claimed that this discrepancy appears to be related to the effect of acetaldehyde formed in the oxidation of ethanol (18).

As suggested by Tozer et al., variations in rates of the amine synthesis might be a function of the number of neurons (13). Should this be true, the synthesis rate of serotonin would reflect the number of the functional serotonergic units. The present authors demonstrated that although the rate of serotonin synthesis was decreased during chronic administration of ethanol the initial level of brain serotonin was maintained. Therefore, it is strongly suggested that serotonergic units in the central nervous system are inhibited during chronic alcoholism. Indeed this decrease in the synthesis rate of serotonin appears to be associated with chronic alcoholism, as the effect of acute alcoholism demonstrated the tendency of an increase in the synthesis rate of serotonin.

It has been demonstrated that tetrahydropapaveroline which is a condensation product of dopamine and its corresponding aldehyde was formed in vitro from dopamine in the absence of exogenous coenzymes with liver or brain homogenates (7). It has also been revealed that this alkaloid formation was facilitated in the presence of alcohol or acetaldehyde (8). This is most interesting because tetrahydropapaveroline is the requisite intermediate in the biosynthesis of a variety of poppy alkaloids (9). Accordingly, these data indicate a biochemical concept for the role of neuroamines in the potential addiction of alcohol (8). On the other hand, Way and his colleagues reported that the rate of serotonin synthesis in the brain of mice rendered tolerant to and physically dependent on morphine was increased and they extended the hypothesis that tolerance or dependence development to morphine is associated with changes in brain serotonin turnover (10, 11). These findings have not, however, been confirmed by other workers (19, 20, 21). From our results, it is suggested that tolerance to or dependence on alcohol, unlike morphine, may be associated with a decrease in brain serotonin turnover.
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