CHRONOBIOLOGICAL ASPECT OF THE MECHANISM OF LITHIUM SALTS ACTION IN EXPERIMENTAL ALCOHOLISM

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After consumption of a 5% solution of ethanol for two months the diurnal rhythm of histophysiological activity of the rat pineal gland was found to be disorganized. A course of lithium chloride injections in doses corresponding to those used clinically reversed these changes and prevented their development if given during the two months' consumption of ethanol. This effect of lithium correlated with the suppression of preference for ethanol observed under these experimental conditions, or with the prevention of its development. The possible link between this property of lithium salts to reverse chronobiological disturbances of pineal activity and their clinical efficacy in alcoholism and other affective disturbances with a periodic course is discussed. KEY WORDS: lithium; preference for ethanol; diurnal rhythm of the pineal gland.

One aspect of the pathogenesis of alcoholism which has received little study is the chronobiological effect of ethanol, manifested as disorganization of normal biorhythms [9, 11]. However, the role of central synchronizers of biorhythms, the most important of which is the pineal gland [6], in the mechanism of this effect has not been proved. Meanwhile lithium salts, which have a marked normothymic (antiphase) action in manic-depressive psychosis and other mental diseases based on a pathological disturbance of biorhythms [7, 10], are regarded as promising substances for the treatment and prevention of alcoholism [2, 13].

It was accordingly decided to study the diurnal rhythm of histophysiological activity of the pineal gland by the use of a model of alcoholism and its experimental prevention and treatment by lithium salts.

EXPERIMENTAL METHOD

Alcoholism was produced in noninbred male albino rats weighing initially 120-150 g. For two months the animals received as sole source of fluid a 5% solution of ethanol [2]. Lithium chloride (LiCl) was used and the Li^+ concentration in the blood plasma was monitored by flame photometry [4]. In the experiments of series I (experimental therapy), after two months of ethanol consumption the experimental animals received intraperitoneal injections of a 5% solution of LiCl in a dose of 35 mg/kg, the effective dose for the suppression of alcohol preference in rats [2], were given twice a day, with an interval of 8 h, daily for two weeks. The control animals under identical conditions received injections of distilled water. In series II (experimental prophylaxis) together with ethanol solution, LiCl, was given to the experimental animals for 2 months in a concentration of Li^+ of 7.5 meq/liter which, as preliminary experiments showed, is effective for preventing the development of alcohol dependence in the animals of this species. The control rats received the equivalent dose of Na^+ (NaCl) instead of ethanol. The experiments were carried out in the spring and summer, with natural alternation of day and night. Experimental, control, and intact animals were decapitated during the daytime, when the amplitude of the normal diurnal rhythm of central neuroendocrine regulation is maximal for the animals of this species [3], 24 h after the end of two months' consumption of ethanol and also after the last injection of LiCl (series I) or the combined administration of ethanol with LiCl or of NaCl (series II). The pineal glands were fixed in Carnoy's fluid and embedded in paraffin wax. Sections 5-7 μ thick, obtained by cutting the pineal glands of the experimental, control, and intact animals of each series of experiments mounted in the same block, were stained with a mixture of gallocyanin and chrome alum by Einarson's method, to determine total nucleic acid (TNA), and also with hematoxylin-eosin. The functional state of the gland was judged by the usual histophysiological criteria: by measuring the nuclei of the pinealocytes with the MOV-1 screw ocular micrometer and

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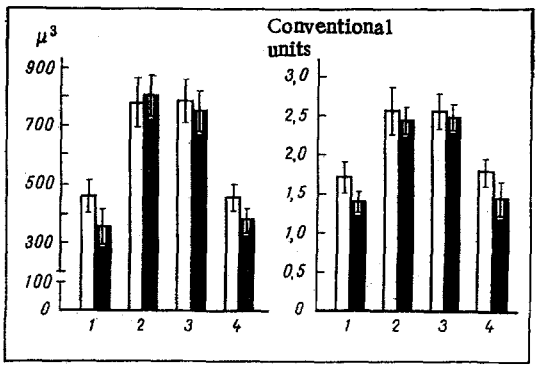

Fig. 1

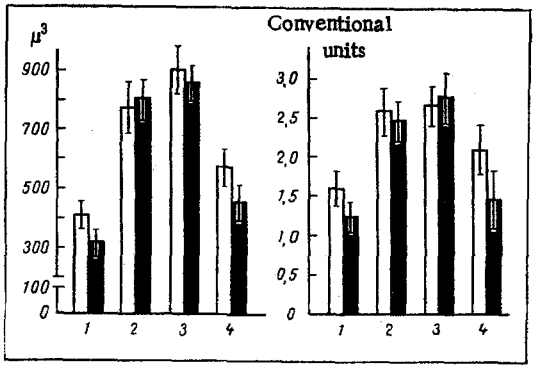

Fig. 2

Fig. 1. Effect of LiCl (35 mg/kg twice a day for 14 days) on nuclear volume (left) and TNA content in cytoplasm (right) of pinealocytes of rats receiving 5% ethanol solution for previous two months. Ordinate: left – nuclear volume of cells (in μ^3), right – TNA content (in conventional units); abscissa: 1) intact animals, 2) experimental animals before receiving LiCl, 3) experimental animals receiving water (control), 4) experimental animals receiving LiCl. White and black columns denote values determined at 3-4 and 9-10 p.m. respectively.

Fig. 2. Effect of LiCl when given for 2 months in conjunction with 5% ethanol solution on nuclear volume (left) and TNA content in cytoplasm (right) of rat pinealocytes. Abscissa: 1) intact animals, 2) experimental animals receiving ethanol, 3) experimental animals receiving ethanol and NaCl, 4) experimental animals receiving ethanol and LiCl. Remainder of legend as in Fig. 1.

calculation of their volume by the equation for an ellipsoid of rotation [1], and by studying the dynamics of the TNA content in their cytoplasm and calculation of the mean histochemical coefficient [5].

EXPERIMENTAL RESULTS AND DISCUSSION

Examination of the pineal glands of intact rats revealed clear changes in the indices chosen to reflect the histophysiological state of the gland during the 24-h period (Fig. 1). In the pineal glands of animals receiving a 5% ethanol solution for two months a sharp increase was found in the nuclear volume of the pinealocytes and in the TNA content in their cytoplasm. At the same time there was a marked decrease in the amplitude of the diurnal fluctuations of these indices, the differences between which ceased to be significant in the periods studied. These findings indicate that ethanol causes a marked increase in the activity of the pineal gland, in the course of which the normal chronobiological characteristics of the gland are lost.

In the experiments of series I, during the study of the pineal glands of rats treated experimentally with LiCl, under the influence of the compound the indices of the histophysiological state of the gland previously disturbed by ethanol were restored to normal and the normal rhythm of their changes during the 24-h period also reappeared (Fig. 1). This effect of LiCl is evidently not accidental, for the histophysiological characteristics of the pineal glands of the control rats were indistinguishable from those of animals examined immediately after cessation of their prolonged ethanol intake.

The results of the experiments of series II confirmed the presence of a regular diurnal rhythm of changes in the histophysiological state of the pineal gland in intact rats (Fig. 2). Examination of the pineal glands of the control animals receiving ethanol and NaCl for two months also revealed a considerable increase in the indices of histophysiological activity of the gland with loss of the normal rhythm of fluctuation of these indices during the 24-h period. The rise in the indices characterizing the degree of increased activity of the gland also exceeded (by 5-12%) that found in the animals receiving ethanol solution only. A moderate increase in the histophysiological indices of activity of the gland with preservation of the normal rhythm of fluctuation of these indices in the 24-h period characteristic of intact animals was found in the pineal glands of rats receiving LiCl, together with ethanol, in a dose substantially retarding the development of alcohol dependence.

Comparison of these results with those obtained previously [2] suggests definite correlation between the state of the pineal gland and the degree of development of alcohol dependence. For instance, during the formation of selective preference for ethanol in rats by administration of a 5% solution of ethanol for two months, a marked increase was observed in the activity of the pineal, accompanied by loss of the normal chronobiological characteristics of this neuroendocrine center. Continuation of the preferential consumption of ethanol and of

the above-mentioned changes in the state of the pineal gland of the control animals can evidently be taken to indicate that correlation between these phenomena is not accidental. On the basis of these facts it can tentatively be suggested that the disorganization of the normal biorhythms of the body as a whole, observed under the influence of ethanol [9, 11], may be due in turn to a disturbance of the activity of the pineal gland, which occupies a dominant place in the mechanism of their neuroendocrine regulation [6]. This hypothesis is supported by evidence [8] which indicates that an increase in pineal activity caused by constant darkness or by administration of melatonin leads to an increase in ethanol consumption in rats.

Depression of ethanol preference by LiCl and reversal of the previously formed motivation in the experiments of series I were accompanied by restoration of the normal histophysiological state of the pineal gland and of the original chronobiological characteristics of this center for biorhythm regulation. The same rule clearly also applied to the prophylactic administration of LiCl in the experiments of series II. Under these conditions the Li^+ concentration in the blood plasma of the experimental animals at the time of their sacrifice was 1.1 ± 0.18 and 0.6 ± 0.08 meq/liter respectively.

Hence it can be concluded that the chronopharmacological effect of LiCl revealed by this investigation may play an important role in the mechanism of the antialcoholic action of this lithium salt in experimental alcoholism.

Since chronobiological disturbances lie at the basis of manic-depressive psychosis and other psychiatric disturbances with a periodic course [10], and since the Li^+ concentration in the blood plasma of experimental animals was close to that generally accepted for the treatment and prevention of such disturbances [7, 12], it can be postulated that this effect of LiCl is one factor which determines the normothymic action of lithium salts in the above diseases.

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