volume leads to a decrease in the intracranial volume of fluid and to a decrease in CSF pressure. The presence of correlation between the fall of CSF pressure and the rise of diuresis is evidence that the effect of furosemide on the kidney can be regarded as a criterion of the magnitude of the effect of the drug on the intracranial pressure.

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CHANGES IN LIVER CYTOCHRONE P-450 CONCENTRATION IN CONGENEIC RESISTANT MICE DURING CHRONIC ALCOHOLIZATION

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UDC 616.89-008.441.13-092.9-07:616.36-008.931:577.152.112]-07

KEY WORDS: congeneic resistant mice; principal mouse histocompatibility system (H-2); cytochome P-450; ethanol.

The adaptive increase in ethanol oxidation during chronic alcoholization takes place mainly through induction of a microsomal ethanol-oxidizing system (MEOS). Activation of MEOS is accompanied by qualitative and quantitative changes in its terminal component, namely cytochrome P-450, on which ethanol is oxidized to acetaldehyde [5, 8, 9, 12, 14]. Despite the important role of MEOS in adaptation of the organism to ethanol, no investigations devoted to the study of its particular features in animals with marked differences in their response to alcohol have been undertaken.

In the investigation described below the concentration of cytochrome P-450 was studied in the liver of two pairs of congeneic resistant (CR) mice, with simultaneous recording of differences in their level of ethanol consumption. The use of CR mice, differing genotypically only in relation to the H-2 system, as the model was determined by existing observations indicating functional unity of the immune and metabolic systems [2] and participation of the principal histocompatibility system in the regulation of metabolic, endocrine, and neurotransmitter processes when disturbed by chronic alcoholization [2, 3, 6, 13].

EXPERIMENTAL METHOD

Experiments were carried out on male mice of two pairs of CR lines (B10.R111 and B10.R107; A/Sn and A.SW) weighing 20-35 g and kept on a standard laboratory diet. The pure-strain ancestors of these lines of mice were generously provided by the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR. During chronic alcoholization the animals received a 10% ethanol solution as the sole source of fluid; control animals received water. The level of alcohol consumption was expressed as the ratio of the quantity of alcohol consumed by the experimental animals to the quantity of water consumed by the control mice. The animals were kept in cages six at a time. The duration of alcoholization was 69 days. From the 42nd through the 48th days of the experiment ethanol

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 685-687, December, 1984. Original article submitted June 8. 1983.

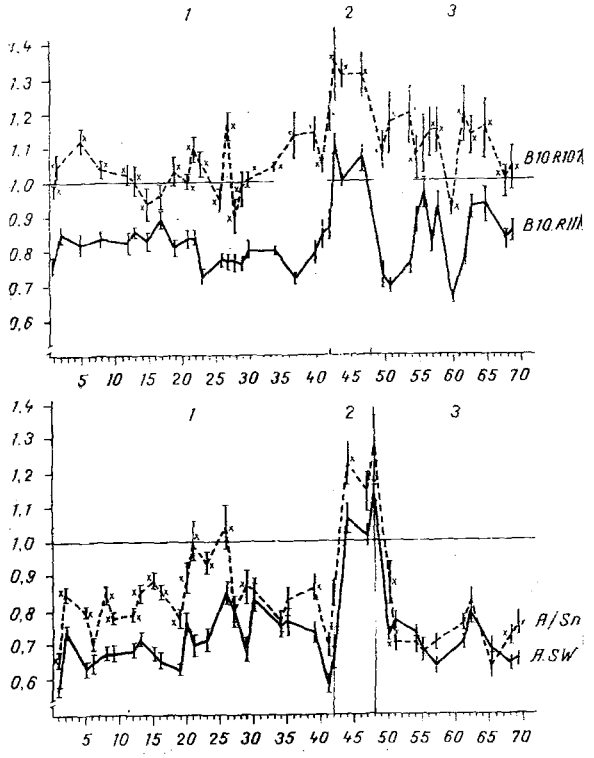

Fig. 1. Ethanol consumption by CR mice. Abscissa, period of alcoholization (in days); ordinate, ethanol consumption (experiment/control). Here and in Fig. 2: asterisk indicates statistically significant differences between lines. 1 and 3) 10% ethanol, 2) water.

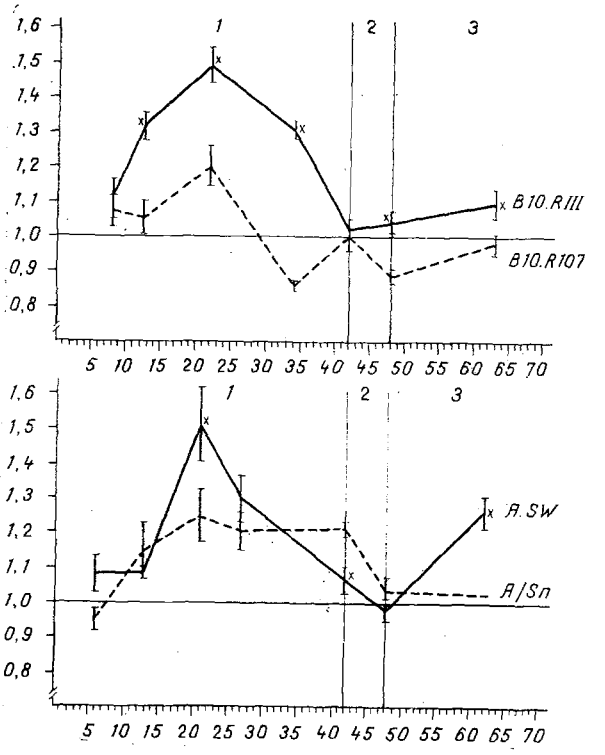

Fig. 2. Changes in cytochrome P-450 concentration in liver of CR mice during chronic alcoholization, calculated per milligram protein. Abscissa, period of alcoholization (in days); ordinate; concentration of cytochrome P-450 (experiment/control).

in the drinking vessels of the alcoholized mice was replaced by water, and this time interval was designated the withdrawal period. The concentration of cytochrome P-450 in liver homogenates of control and experimental mice was determined on a DW2 spectrophotometer (from "Aminco," USA) by the method in [11]. The animals were decapitated, the liver was cut into small pieces in medium containing 150 mM KCl and 50 mM Tris-HCl buffer, pH 7.5, in the ratio of 1:10, and then homogenized in a glass homogenizer with Teflon pestle at 3000 rpm for 1 min. All operations were carried out at 4°C. The concentration of the hemoprotein was calculated per milligram protein and per gram tissue. Protein was determined by Lowry's method [10] in the presence of 0.1% sodium deoxycholate. The results were processed by computer in the automatic control of Medical-Biological Experiments Department, Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute.

EXPERIMENTAL RESULTS

In the course of chronic alcoholization differences in ethanol consumption were found in the test pairs of CR mice, and in particular, lines B10.R111 and B10.R107 (Fig. 1). These differences remained throughout the period of the experiment. B10.R107 mice were characterized by a higher level of ethanol consumption than B10.R111 mice. Stable differences between lines A.SW and A/Sn were found only during the first 29 days of alcoholization, and subsequently they were preserved only at individual points of the experiment. In this pair of CR mice, line A/Sn was the one which actively consumed ethanol. Consequently, the results are evidence that differences affecting a limited region of chromosome 17 (the H-2 complex) may be realized phenotypically as different levels of ethanol consumption.

Analysis of the cytochrome P-450 concentration showed that the maximum of induction of the hemoprotein during chronic alcoholization was recorded in the 3rd week of the experiment (Fig. 2). Subsequently there was a gradual fall in the cytochrome P-450 level to its initial values by the 42nd day of alcoholization. This fall may be regarded as a reflection of adaptation of the animal to the chronic action of ethanol. During the withdrawal period an increase in the cytochrome P-450 concentration to the control values was found. Despite the whole general character of the changes, the intensity of cytochrome P-450 induction during alcoholization varied sharply among animals of the various lines tested. It was increased the most in lines of mice with low tolerance to ethanol (B10.R111 and A.SW), whereas the increase in concentration of the hemoprotein in the liver of B10.R107 and A/Sn mice was less marked. During repeated alcoholization of the animals for 2 weeks after the withdrawal period the cytochrome P-450 concentration in B10.R107 and A/Sn mice was at the control level, whereas for B10.R111 and A.SW lines an increase in concentration of the enzyme was observed. Incidentally, similar principles governing the change in hemoprotein level were found whether calculated per milligram protein or per gram tissue. Very small fluctuations in the cytochrome P-450 level in the liver of intact animals throughout the experiment enabled averaged values of this parameter to be compared in the lines of CR mice. Statistically significant differences in the hemoprotein level (in nanomoles/g tissue) were found in mice of line B10.R107 and B10.R111: B10.R107 31.1 ± 0.52, B10.R111 27.5 ± 0.86 ($P < 0.01$); expressed in nanomoles per gram protein: B10.R107 0.244 ± 0.004, B10.R111 0.199 ± 0.004 ($P < 0.01$). Consequently, the higher level of consumption of 10% ethanol solution and the weaker induction of cytochrome P-450 in mice of line B10.R107 than in those of line B10.R111 during chronic alcoholization were combined with an increased initial concentration of the hemoprotein in these animals.

Similar principles concerning the initial cytochrome P-450 level also were found for standard inbred mice of lines DBA/2 and BALB/c [4], which differ in their ethanol consumption [7]. No significant differences as regards enzyme concentration were found between A/Sn and A.SW mice, which have smaller differences in ethanol consumption (in nanomoles/mg tissue): A/Sn 29.2 ± 2.4, A.SW 26.6 ± 1.7 ($P < 0.05$); (in nanomoles/mg protein): A/Sn 0.163 ± 0.019, A.SW 0.146 ± 0.011 ($P < 0.05$). Consequently, definite correlation was observed between the level of ethanol consumption and the original cytochrome P-450 concentration, on the one hand, and the degree of induction of the hemoprotein on the other hand, during chronic alcoholization. Genetically determined differences in cytochrome P-450 concentration in the liver evidently are responsible for the predisposition of mice of the lines studied to ethanol consumption. The higher initial level of cytochrome P-450, the central component of MEOS, indicates increased powers of adaptation of the animal when the need has passed for intensive induction of cytochrome P-450 during alcoholization. These observations confirm the previous view of the important role of metabolism in the level of ethanol consumption. This view was based on the high rate of alcohol elimination from the blood stream in rats with marked alcohol motivation [1]. Considering that the results described above were obtained on CR mice, differing genotypically with respect to the principal histocompatibility system, that system can be regarded as responsible for the differences obtained in these experiments.

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EFFECT OF β-ENDORPHIN, ENKEPHALINS, AND THEIR SYNTHETIC ANALOGS ON UNIT ACTIVITY IN THE BULBAR RESPIRATORY CENTER

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UDC 612.828.014:423.014.46:615.35:547.95:547.943

KEY WORDS: endogenous opioid peptides; respiratory center neurons; microiontophoresis.

Endogenous peptides with morphine-like activity inhibit respiration [2, 7, 9, 10] as a result of the direct action of opioid peptides on the bulbar respiratory center and on suprabulbar structures concerned in the regulation of respiration [8]. Data in the literature on the direct effect of endogenous opioid peptides and their synthetic analogs on neurons of the bulbar respiratory center are scarce [6, 11].

It was accordingly decided to study the effect of microiontophoretic application of β-endorphin, enkephalins, and their synthetic analogs on single unit activity in the bulbar respiratory center. The observed effects of the opioid peptides were compared with the action of morphine.

EXPERIMENTAL METHOD

Unit activity in the bulbar respiratory center was recorded extracellularly in rabbits and cats anetsthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) [1, 4, 5], and the test substances were applied by microiontophoresis through multibarreled glass microelectrodes [3]. In the course of the experiment an "Élektronika DZ-28" mimicomputer, coupled to the apparatus for recording unit activity, processed information on unit activity and plotted it graphically. Parallel with unit activity, the pneumogram was recorded (to identify respiratory neurons). The following freshly prepared solutions were used for microiontophoresis: β-endorphin 0.001 M; Met- and Leu-enkephalin, Tyr-D-Ala-Gly-Phe-NH_2, Tyr-D-Ala-Gly-Phe-D-Leu, Tyr-D-Ala-Gly-(Me)Phe-Gly-ol 0.02 M (all opioid peptides were synthesized at the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, under the direction of Doctor of Chemical Science M. I. Titov); naloxone hydrochloride (from "Endo Laboratories," USA) 0.1 M; morphine hydrochloride 0.05 M. The solvent was 0.03 M NaCl, which also was used (3 M solution) to fill the recording and compensating barrels of the microelectrode. The substances were applied by currents of 10-50 nA with positive polarity.

To study the effect of the above-mentioned opioid peptides on respiration a series of experiments was carried out on waking rabbits. The frequency and volume of respiration were

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 0007-4888/84/9812-1666$08.50