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Dynorphin A (1-17), Met-Enkephalin-Arg^6-Phe^7 and Substance P (1-11) Levels in the Brain of Mice with Different Levels of Ethanol Consumption

R. Yu. Yukhananov, P. M. Klodt, A. Yu. Shemanov, A. E. Rat'kin, F. Nyberg and A. I. Maiskii

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There are several hypotheses connecting the development of ethanol dependence with the activity level of the endogenous opiate system. According to some of these hypotheses, a deficiency in this system provokes ethanol consumption and the formation of alcohol dependence [2,3]. In particular, such a deficiency may include disorders of the synthesis or processing of opioid peptides and acceleration of their hydrolysis. An alternative assumption is that the development of ethanol dependence is due to activation of the opiatergic system [10,11]. In the present study we determined the concentration of some opiates in the brain of three strains of mice, one of which, C57Bl10/D1, exhibits a high level of ethanol consumption in a free choice situation, whereas the other two, A/Sn and A.CA, practically do not consume ethanol under the same regime. Earlier, we used the same approach to measure the content of Met- and Leu-enkephalins and β-endorphin in rats with different levels of ethanol consumption [1,5]. Taking into account the fact that Met-enkephalin may be accumulated in the brain as a result of the processing of both proopimelanocortin and proenkephalin, while Leu-enkephalin may be a product of either proenkephalin or prodynorphin, we focused on measuring the levels of dynorphin and Met-enkephalin-Arg^6-Phe^7, which are formed only in the processing of prodynorph in and proenkephalin, respectively. In addition, we measured the concentration of substance P (1-11), the secretion and synthesis of which are controlled by

Institute of Pharmacology, Russian Academy of medical Sciences, Moscow; Department of Pharmacology, University of Uppsala, Sweden. (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

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TABLE 1. Level of Peptides (in fmole/mg) in the Brain of Rats with Different Ethanol Consumption

Peptide	Mouse Strain	Brain structure		
		striatum	hippocampus	spinal cord
Substance P (1–11)	C57Bl10/D1	41.5±4.37 (6)	12.5±1.89 (6)	42.2±1.47 (4)
	A/Sn	38.6±1.57 (5)	14.3±2.98 (5)	41.1±2.47 (5)
	A.CA	41.5±2.45 (4)	13.5±1.14 (4)	48.1±3.05 (4)
Dynorphin (1–17)	C57Bl10/D1	7.93±0.98 (6)	2.16±0.26 (6)	6.59±0.54 (4)
	A/Sn	8.75±0.91 (5)	2.7±0.36 (5)	6.09±0.45 (5)
	A.CA	6.98±0.12 (4)	2.4±0.17 (6)	7.17±0.53 (4)
Met–Enk–Arg^6–Phe^7	C57Bl10/D1	85±10.44 (4)	11.5±1.91 (5)	52.9±0.99 (4)
	A/Sn	133±13.67 (5)	10.3±2.83 (4)	82.7±7.5 (4)**
	A.CA	145±10.60 (4)*	10.5±2.20 (6)	84.2±4.7 (4)**

Note. Mean values and mean square errors are presented. Peptide concentration was determined in acid extracts by RIA following separation on a Sep–Pak cartridge. One asterisk – $p<0.05$; two asterisks – $p<0.01$ in comparison with C57Bl10/D1 mice. Number of mice is indicated in parentheses.

the opiatergic system [7,14]. This peptide can also modulate ligand binding to opiate receptors [8].

MATERIALS AND METHODS

Mice of the C57Bl10/D1, A/Sn, and A.CA strains were obtained from the Laboratory of Experimental Biological Models, Russian Academy of Medical Sciences. The animals were kept under natural light on a standard diet at 21-22°C. For the assessment of ethanol consumption, the mice were placed in individual cages and given water and 10% ethanol on a free choice schedule. Testing was performed over 5 days and consisted in daily measurement of liquid consumption in the interval from 10:00 to 11:00 h. Intact mice were used for determination of the peptide concentration in the brain. The brain and spinal cord were taken immediately after decapitation, divided into parts on ice, weighed, and frozen in liquid nitrogen for further determination of peptide concentration. The brain tissue was immersed in 1 M acetic acid containing 0.01% mercaptoethanol that was heated beforehand to 95°C and boiled for 5 min. Subsequently, the samples were homogenized by ultrasound and heated again for 5 min at 95°C. After centrifugation the supernatant was separated on Sep-Pak microcolumns (Water, Millipore) with 80% methanol. The eluate was collected and evaporated in a vacuum centrifuge (Savant vac., Savant, Hicksvile, USA). The pellet was dissolved in acetic acid and oxidized with hydrogen peroxide at 37°C to obtain Met-enkephalin-Arg^6-Phe^7-sulfoxide. The samples were evaporated again and the pellet was dissolved in MeOH-HCl (1:1) solution. The samples were used to determine peptide concentration by RIA. The method of preparing antisera and their characteristics were described earlier [9,12], and only minor modifications were introduced. The 25 μl samples were mixed in a tube with 100 μl corresponding antibody and 100 μl ^{125}I-labeled peptide (5000-6000 cpm); the antigen-antibody complex was precipitated with second antibodies (Pharmacia). Statistical processing of the data was carried out by the ANOVA method, with subsequent multiple comparison after Scheffe, using Logstat software.

RESULTS

The data on peptide concentration are summarized in Table 1. The nature of dynorphin and substance P distribution proved to be close to that described for other mouse and rat strains [6,12]. The level of these peptides is maximal in the striatum and spinal cord and is much lower in the hippocampus. We failed to detect any differences between the dynorphin and substance P concentration in any of brain structures. The Met-enkephalin-Arg^6-Phe^7 level was again the highest in the striatum and lowest in the hippocampus. A similar relative distribution of the peptide has been described in the rat brain [4,13]. We found that the Met-enkephalin-Arg^6-Phe^7 level was significantly reduced in the striatum and spinal cord in C57Bl10/D1 mice, whereas there were no reliable differences among the three mice strains in the hippocampus. Under the free choice regime, C57Bl10/D1 mice consumed a significant volume of ethanol even after the first contact with it (15±2.1 ml 96% ethanol/kg/day), whereas the A/Sn and A.CA mice practically did not drink ethanol under the same conditions (<2 ml 96% ethanol/kg/day). Thus, in the mice that did not consume ethanol under the free choice schedule the level of Met-enkephalin-Arg^6-Phe^7 was much higher in the spinal cord and striatum than in the strain characterized by a high level of ethanol consumption. Earlier, we showed

that the concentration of Mt- but not Leu-enkephalin was much lower in rats displaying a preference for ethanol [1,5]. However, those results did not allow us to clarify with which of the two precursors of Met-enkephalin these differences could be connected. The data obtained in the present study definitely point to a decrease in the synthesis, or an increase in the release, of products of proenkephalin but not of prodynorphin processing. Moreover, the fact that the substance P concentration is equal in the three mouse strains tested speaks for a certain specificity of these differences.

As is well known, Met-enkephalin-Arg^6-Phe^7 is the major form in which products of proenkephalin processing are accumulated [13]. We therefore assume that the decrease in its concentration is determined by stable changes in the synthesis or release of the enkephalins formed from proenkephalin A.

Thus, the data obtained indicate that the high level of ethanol consumption in C57Bl10/D1 mice is accounted for, at least partly, by a decrease in the synthesis or processing of opioid peptides from proenkephalin and, correspondingly, is connected with a decrease in the activity of the endogenous opiate system rather that with its activation.

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