dependence of activation of paranodal K^+ channels is the same as nodal channels, even though their pharmacological behavior is quite distinct. Are internodal and paranodal K^+ channels identical? To answer this question one needs to achieve good spatial control by clamping demyelinated internodes in a chamber where the width of the recording pool is 10–20 µm. Even so, such experiments would not be internally controlled and subject to criticism.

Frog nodes have several types of K^+ channels which differ in kinetics and pharmacological sensitivity, and vary in sensory and motor fibers[36]. In this study there were no significant differences in 4-AP and TEA sensitivity between sensory and motor fiber nodal or paranodal channels. Activation of nodal and paranodal K^+ channels appeared similar, but studies of K^+ channel kinetics in myelinated nerve is complicated by K^+ accumulation[36]. Since K^+ tail currents were not measured, it is possible that K^+ channel subpopulation density varies between nodes and paranodes and produces the pharmacological differences observed.

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1 Smith, K.J., and Schauf, C.L., Nature *293* (1981) 297.
2 Pelhate, M., Hue, B., and Chanelet, J., C. r. Séanc. Soc. Biol. *166* (1971) 1598.
3 Schauf, C.L., Colton, C.A., Colton J.S., and Davis, F.A., J. Pharmac. exp. Ther. *197* (1976) 414.
4 Ulbricht, W., and Wagner, H.H., Pflügers Arch. *367* (1976) 77.
5 Kirsch, G., and Narahashi, T., Biophys. J. *18* (1978) 507.
6 Dubois, J.M., in: Advances in Biosciences, vol. 35, pp. 43–51. Ed. P. Lechat. Pergamon Press, New York 1982.
7 Quinta-Ferreira, M.E., Rojas, E., and Arispe, N., J. Membr. Biol. *66* (1982) 159.
8 Horackova, M., Nonner, W., and Stämpfli, R., Proc. int. Un. physiol. Sci. *7* (1968) 198.
9 Chiu, S.Y., Ritchie, J.M., Rogart, R.B., and Stagg, D., J.Physiol., Lond. *292* (1979) 149.
10 Brismar, T., J. Physiol., Lond. *298* (1980) 171.
11 Chiu, S.Y., and Ritchie, J.M., Nature *284* (1980) 170.
12 Bostock, H., Sears, T.A., and Sherrat, R.M., J. Physiol., Lond. *313* (1981) 301.
13 Brismar, T., Acta physiol. scand. *113* (1981) 167.
14 Chiu, S.Y., and Ritchie, J.M., J. Physiol. *313* (1981) 415.
15 Targ, E.F., and Kocis, J.D., Brain Res. *328* (1985) 358.
16 Waxman, S.G., Kocis, J.D., and Eng, D.L., Muscle Nerve *8* (1985) 85.
17 Kocis, J.D., Bowe, C.M., and Waxman, S.G., Neurology *36* (1986) 117.
18 Ritchie, J.M., Proc. R. Soc., Lond. B *215* (1982) 273.
19 Kocis, J.D., and Waxman, S.G., Nature *304* (1983) 640.
20 Bowe, C.M., Kocis, J.D., and Waxman, S.G., Proc. R. Soc., Lond. B *224* (1985) 355.
21 Kocis, J.D., in: Ion Channels in Neural Membranes, pp. 123–144. Eds Ritchie, Keynes and Bolis. Alan Liss, Publ., New York 1986.
22 Kiloh, N., Harvey, A.L., and Glover, W.E., Eur. J. Pharmac. *70* (1981) 53.
23 Chiu, S.Y., and Ritchie, J.M., J. Physiol. *322* (1982) 485.
24 Pencek, T.L., Schauf, C.L., Low, P.S., Eisenberg, B.R., and Davis, F.A., Neurology *30* (1980) 593.
25 Jones, R.E., Heron, J.R., Foster, D.H., Snelgar, R.S., and Mason, R.J., J. neurol. Sci. *60* (1983) 353.
26 Stefoski, D., Davis, F.A., Faut, M., and Schauf, C.L., Ann. Neurol. (1986) in press.
27 Dubois, J.M., Proc. Biophys. molec. Biol. *42* (1983) 1.
28 Targ, E.F., and Kocis, J.D., Brain Res. *363* (1986) 1.
29 Dodge, F.A., and Frankenhauser, B., J. Physiol. *143* (1968) 76.
30 Pencek, T.L., Schauf, C.L., and Davis, F.A., J. Pharmac. exp. Ther. *204* (1978) 400.
31 Schmidt, H., and Stampfli, R., Helv. physiol. pharmac. Acta *22* (1964) C143.
32 Hille, B., Biophysics and Physiology of Excitable Membranes, pp. 230–246. Ed. W.J. Adelman. Van Nostrand Reinhold, New York 1971.
33 Sigworth, F.J., Ph. D. thesis. Yale University, New Haven 1979.
34 Bergman, C., Pflügers Arch. *317* (1970) 287.
35 Brismar, T., Pflügers Arch. *393* (1982) 348.
36 Dubois, J.M., J. Physiol. *318* (1981) 297.
37 Molgo, J., Lundh, H., and Thesleff, S., Eur. J. Pharmac. *61* (1980) 25.
38 Den Hertog, A., Pielkenrood, J., Biessels, P., and Agoston, S., Eur. J. Pharmac. *94* (1983) 353.
39 Kirsch G., and Narahashi, T., J. Pharmac. exp. Ther. *226* (1983) 174.

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Regional brain [Met]-enkephalin in alcohol-preferring and non-alcohol-preferring inbred strains of mice

K. Blum, A.H. Briggs, J.E. Wallace*, C.W. Hall** and M.A. Trachtenberg***

*Departments of Pharmacology and Pathology, *University of Texas Health Science Center, and **Southwest Research Institute, San Antonio (Texas 78284, USA), and ***Matrix Technologies, Inc., Houston (Texas 77058, USA), 19 June 1986*

Summary. Scrutiny of the data from these studies reveals that the C58/J alcohol-preferring mice have significantly lower baseline methionine-enkephalin levels in both the corpus striatum and hypothalamus compared to C3H/CHRGL/2 non-alcohol-preferring mice. In other brain regions in these two strains, specifically, pituitary, amygdala, midbrain, and hippocampus, analysis of methionine-enkephalin levels did not show any significant differences. This suggests that the hypothalamus may indeed be a specific locus involved in the regulation of alcohol intake, via the molecular interaction between neuroamines, opioid peptides, as they are influenced by genetics and environment.

Key words. Opioid peptides; methionine-enkephalin; alcohol-avoiding mice; alcohol-preferring mice; hypothalamus; corpus striatum.

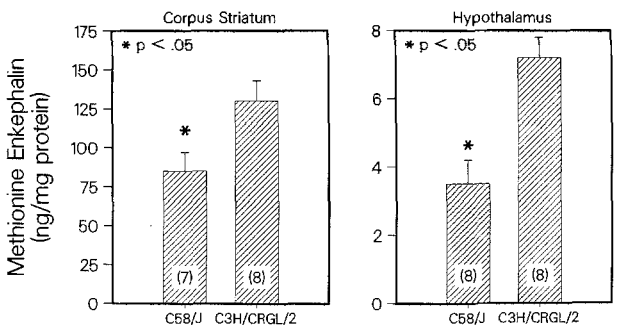
Numerous studies have attempted to establish a relationship between brain neurotransmitters and neuropeptides and alcohol-drinking behavior. Blum et al.[1] have previously shown that there is correlation between whole brain methionine-enkephalin ([Met]-enk) and amounts of alcohol consumed. Using a 14-day preference test, an estimated correlation of 0.909 was found between mouse whole brain [Met]-enk levels and alcohol consumption in alcohol-preferring (C57BL/6J, C58/6J) and alcohol avoiding strains (DBA2/J and C3H/CHRGL/2). In that study, C57BL/6J and C58/6J mice, which drank more alcohol than the DBA2/J and C3H strains, exhibited significantly less brain [Met]-enk. In later studies, Blum et al.[2] discovered that a substrain of the C57BL group, specifically C57BL/6N, supplied by the Simonsen laboratories, reverted to more normal alcohol consumption levels. Examination of [Met]-enk brain levels in this substrain revealed significantly higher levels as compared to C57B/6J mice[3]. C57BL/6J and DBA/2J exhibited individual differences in alcohol-drinking behavior when tested in a 1-day

acceptance experiment utilizing a 10% ethanol solution. After testing, ethanol was withdrawn for an 8-week period. There was an observable difference in alcohol consumption between the high preference and low preference animals. There was significant correlation (0.602) between mouse whole brain [Met]-enk levels and alcohol consumption in high and low preference inbred mouse strains[4]. One conclusion from these data, subsequently supported by others[5,6], is that craving for alcohol correlates with a genetically based decrease in brain and pituitary opioid peptides[7]. These observations, coupled with specific findings of differential amounts of neurotransmitters, such as serotonin, in particular brain regions, in alcohol-preferring and non-alcohol-preferring rats[8] prompted an investigation of regional brain [Met]-enk in inbred mice with different propensity for ethanol consumption.

Materials and methods. Male CH3/CHRGL/2 and C58/J mice (8 weeks old) were obtained from the Jackson laboratories, maintained at constant temperature (25 ± 2°C) and lighting (12 h light/12 h dark) conditions. A total of 32 mice were utilized in this investigation. The animals were acclimatized to the laboratory for 1 week and maintained on food and water ad libitum prior to sacrifice and evaluation of regional brain [Met]-enk levels. The animals were decapitated, the brains removed and dissected into various brain regions including pituitary, amygdala, midbrain, hippocampus, hypothalamus and corpus striatum. The brain regions were then frozen on dry ice. The samples were weighed and homogenized at 95°C in a solution of 2 ml acetic acid and the homogenate heated for 5 min at 95°C. The samples then were chilled to 4°C for 5 min and centrifuged at 14,000 × g for 15 min. The supernatant fractions were removed, shell-frozen in borosilicate tubes, and lyophilized overnight. The next day the residue was resuspended in 0.1 ml phosphate buffer, pH 6.8/0.1% bovine serum albumin and centrifuged at 1000 × g for 15 min. The supernatant fractions were then assayed for [Met]-enk (Immunonuclear Kit, Stillwater, MN). Duplicate samples were run, and a log/logit y/1-y graph was used to determine binding. A complete description of our procedure as well as validation tests has been reported[4].

Results and discussion. Examination of the data reveals that the C58/6J alcohol-preferring mice (estimate of 14-day preference ratio is 0.4–0.9) have significantly ($p < 0.05$) lower baseline [Met]-enk levels in both the corpus striatum, 84 ± 12.5, and hypothalamus, 3.5 ± 0.7 ng/mg protein, as compared to C3H/CHRGL/2, non-alcohol-preferring mice, (estimates of 14-day preference ratio is 0.05–0.57)[9,10]. The latter had [Met]-enk levels of 129.0 ± 13.0 and 7.2 ± 0.25 ng/mg protein, respectively (fig.). No significant differences in [Met]-enk levels were seen in other brain regions in these two strains, in particular, pituitary, amygdala, midbrain, and hippocampus.

For years the hypothalamus has been assigned the central and exclusive role in regulating ingestive behavior (compulsive disorders). However, there is experimental evidence suggesting that ingestive behaviors are profoundly influenced by the neuroamine pathways that pass through the hypothalamus. Electrical stimulation of the lateral hypothalamus elicits feeding or drinking; injections of neurohumoral transmitters into the hypothalamus elicits feeding or drinking, depending on the neurotransmitter used; and, hypothalamic lesions result in overeating and obesity[11]. It is further suggested that the effects of lateral hypothalamic lesions on ingestive behavior might be due to depletion of dopamine from the striatum[12].



Differences in specific content of methionine-enkephalin, expressed in ng/mg protein, as seen in two isolated brain regions of C58/J (alcohol-preferring mice) and C3H/CRGL/2 (non-alcohol-preferring mice). Number of animals per group shown in parentheses.

In terms of addictive behavior, the significance of dopaminergic neurons becomes evident since interruption results in a cessation of self-stimulation. Many researchers are convinced that not only dopamine[13], but also norepinephrine[14] and serotonin[15] are involved with dopamine in the mediation of reward. In considering behavioral specificity, it is important to link hypothalamic functionability with other neuroanatomical loci such as the striatum, in order to understand control of the complex behavior of ingestion of alcohol.

Involvement of various CNS neuroamines, such as dopamine and serotonin, in addictive behavior, specifically alcohol craving and reward, is becoming more firmly established[16]. Of interest is the work of Barbaccia et al.[17] who showed genetic differences in the relation between dopamine and endorphins. In this regard, Schwartz[18] reported that chronic treatment of rats with haloperidol (1 mg/kg daily) led to a 4-fold increase of pro-enkephalins (PE) in RNA, specific to the striatum. The increase in PE and RNA was paralleled by increases in PE and enkephalin-containing peptides, suggesting that blockade of nigro-striatal dopamine transmission relieved tonic inhibition and resulted in PE synthesis.

The role of serotonergic mechanism(s) in alcohol intake is receiving support from a number of investigators[19,20]. For example, Schwartz[18] reported that treatment of rats with drugs which deplete serotonin increased the content of both [Met]-enk and B-endorphin in the hypothalamus, but not in any other brain region. No changes were detected in either RNA or precursor content of PE or pro-opiomelanocortin. Thus, serotonergic transmission may regulate opiod peptide utiliation without affecting synthesis.

It is interesting to note that, thalamic and hypothalamic serotonin levels in alcohol-preferring rats (N/nih heterogenous stock) were significantly lower than those found in non-alcohol-preferring rats[8]. Since no other brain regions displayed this difference, the authors suggest a possible genetic role of the serotonergic system of the hypothalamus in the mediation of preference for alcohol. Additionally, Felten and Felten found decreases in medio-basal hypothalamic serotonin in rats consuming ethanol in a liquid diet[21].

The fact that there is a connection between nigro-striatal dopamine and hypothalamic control of ingestive behavior and that there is an intimate relationship between dopamine and endorphin synthesis provides support for the possibility that hypothalamic opioid peptides may, in part, mediate alcohol intake.

In humans there is evidence to support the concept that dopamine and serotonin are involved with one another[22]. It has been found that selective destruction of brain serotonergic pathways, by intraventricular injection of neurotoxins, induces hyperphagia[23]. It has been suggested that a serotonergic component of the brain that arises in the lower brain stem and ascends through the hypothalamus is involved in the regulation of ingestive behavior.

In the current report, analysis of [Met]-enk levels in the pituitary, amygdala, midbrain and hippocampus of both alcohol-preferring and non-alcohol-preferring mice, showed no significant differences. Only the hypothalamus showed significant differences. This suggests that the hypothalamus may indeed be a specific locus involved in the regulation of alcohol intake, via the molecular interaction between neuroamines and opioid peptides as they are influenced by genetics and environment. Additional research whereby the ratio of certain neurotransmitters (e.g.

serotonin) and neuropeptides (endorphins and ACTH) specifically in the hypothalmus[24] of alcohol-preferring and non-alcohol-preferring animals will provide important information with regard to the chemical control of uncontrollable drinking.

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1 Blum, K., in: Central and Peripheral Endorphins, p. 339. Eds E.E. Muller and A.R. Genazzani. Raven Press, New York 1984.
2 Blum, K., Briggs, A.H., Elston, S.F., and DeLallo, L., Toxic. Eur. Res. *3* (1981) 261.
3 Blum, K., Briggs, A.H., DeLallo, L., Elston, S.F., and Ochoa, R., Experientia *38* (1982) 1469.
4 Blum, K., Elston, S.F., DeLallo, L., Briggs, A.H., and Wallace, J.E., Proc. natn. Acad. Sci. *80* (1983) 6510.
5 Crabbe, J.C., Allen, R.G., Gaudette, N.D., Young, E.R., Kosobud, A., and Stack, J., Brain Res. *219* (1981) 219.
6 Crabbe, J.C., Keith, D.L., Kosobud, A., and Stack, J., Life Sci. *33* (1983) 1877.
7 Blum, K., Briggs, A.H., DeLallo, L., and Elston, S.F., Subs. Alc. Act/Mis. *1* (1980) 459.
8 Murphy, J.M., McBride, W.J., Lunens, J., and Li, T.K., Neurosci. Abstr. *11* (1985) 290.
9 McClearn, G.E., and Rodgers, D.A., Q. H. Stud. Alcohol *20* (1959) 691.
10 McClearn, G.E., Proc. VI. Int. Congr. Pharmac. *3* (1975) 59.
11 Hennessey, J.W., and Grossman, S.P., Physiol. Behav. *17* (1976) 103.
12 Stricker, E., and Zigarone, M. J., Progress in Psychobiology, Physiology and Psychology. Academic Press, New York 1976.
13 Bozarth, M.A., and Wise, R.A., Life Sci. *29* (1981) 1881.
14 Brown, Z.W., Amit, Z., Levitan, D.E., Ogrew, S.O., and Southerland, A., Archs int. Pharmacodyn. *230* (1977) 76.
15 Pradham, S.W., in: Brain Stimulation Reward, p. 171. Eds A. Waugier and E.T. Rolls. Elsevier North Holland Press, Amsterdam 1976.
16 Wise, R.A., and Bozarth, M.A., in: Neurotoxicology, p. 111 Eds K. Blum and L. Manzo, Marcel Dekker Inc., New York 1985.
17 Barbaccia, M.L., Reggiani, A., Spano, F.F., and Trabucchi, M., Psychopharmacology *74* (1981) 260.
18 Schwartz, J.P., IV World Congr. Biol. Psych. (1985) 418.
19 Navanjo, C.A., and Sellers, E.M., IV World Congr. Biol. Psych. (1985) 10.
20 Wu, P.H., Navanjo, C.A., and Fain, T., IV World Congr. Biol. Psych. (1985) 79.
21 Felton, S.Y., and Felten, D.L., Neurosci. Abstr. 11 Part I (1985) 298.
22 Agrew, H., IV World Cong. Biol. Psych. (1985) 240.
23 Grossman, S.P., Hosp. Prac. *12* (1977) 45.
24 Nash, J.F., and Markel, R.P., Neurosci. Abstr. 11 Part I (1985) 295.

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Tetanus intoxication causes an increment of serotonin in the central nervous system

J. Aguilera, J. Heredero and F. Gonzalez Sastre

Departamento de Bioquimica y Biologia Molecular, Facultad de Medicina, Universidad Autonoma de Barcelona, Bellaterra, Barcelona (Spain), 16 July 1986

Summary. Mice injected with tetanus toxin (TTx) showed an increase of 5-hydroxytryptamine (serotonin, 5-HT) levels in the central nervous system. The increment was not uniform thoughout the central nervous system. Particularly significant were the 25% and 80% increases observed, respectively, in whole brain and spinal cord. The levels of dopamine and norepinephrine remained unchanged. The subsequent studies of 5-HT turnover revealed a synthesis rate in the tetanic animals that was almost double that of controls. The degradation rate of the amine as well as the levels of 5-hydroxyindolacetic acid were unaffected.
Key words. Serotonin; 5-hydroxytryptamine; tetanus toxin; pargyline; alphamethyldopa; indolamine.

Tetanus is a grave condition produced by tetanus toxin and characterized by muscular rigidity and recurrent spasms leading to death in a considerable percentage of cases. Several reviews have been published dealing with the chemistry[1,2], physiological effects[1,3] and pathophysiology[4] of the toxin. The toxin is integrated by two polypeptidic chains of 100,000 and 50,000 daltons linked by a disulfide bridge[5,6]. As in the case of other bacterial toxins, one of the chains may have the toxic properties while the other mediates the binding to the target cells[7]. To exert its effects on the central nervous system (CNS), the toxin has to be bound by the peripheral nerve endings, probably through membrane gangliosides[8], incorporated inside them, and then retrogradely transported through the axon to the spinal cord, which is the level at which the toxin impairs the release of inhibitory amino acids from interneurons controlling the activity of the motoneurons. Conclusive evidence for or against a subsequent transport to higher levels of the CNS is currently lacking. However, this mobility of the toxin has proved very useful for studying the connections between different cerebral structures[9].

Both in vitro and in vivo experiments show that the toxin affects preferentially the synapses which use glycine or GABA as a transmitter[10]. Thus, the inhibition of glycine release at the spinal level plays a key role in the symptomatology of tetanus, in man as well as in experimental animals[11,12]. Nevertheless, as we mentioned above, the effect of the toxin on supraspinal regions is less well known, and the encephalic symptoms of tetanus infection have not been satisfactorily explained.

However, several direct effects of the toxin on certain brain structures have already been demonstrated. Thus, the intranigral injection of tetanus toxin suppresses the bicucculine-sensitive inhibition of neurons in the substantia nigra evoked from the striatum[13]. Certain encephalic symptoms of tetanus such as insomnia, parkinsonism, hyperthermia and hypertension seem to suggest the implication of different monoaminergic systems[1]. Moreover, TTx has been detected in the cerebrum of rats 24 h

Table 1. 5-HT levels in brain and spinal cord of the mouse after injecting tetanus toxin

Dose	Controls (nmol/g wet tissue)	Treated
	Brain	
1 MLD	4.12 ± 0.48 (10)	5.03 ± 0.59 (14)**
2 MLD	3.63 ± 0.34 (11)	4.52 ± 0.22 (11)***
10 MLD	3.94 ± 0.40 (6)	4.87 ± 0.53 (6)*
	Spinal cord	
2 MLD	3.85 ± 0.90 (6)	6.94 ± 2.60 (6)*

The results are expressed as means ± SD for the number of animals between brackets. The results have been analyzed using Student's t-test; * = $p < 0.05$, ** = $p < 0.025$, *** = $p < 0.005$, in comparison to control value.