# Ethanol ingestive behavior as a function of central neurotransmission

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*Summary.* Uncontrollable alcohol ingestive behavior has been linked to deficits of central neurotransmission. The pineal gland plays an important role in modulating ethanol intake in numerous animal species. The opioidergic (i.e. β-endorphin, enkephalin, and dynorphin) system is involved in both the actions of alcohol and opiates, as well as craving and/or genetic predisposition towards abuse of these two agents. Furthermore, there is significant evidence to link ingestive behaviors with the ventral tegmental accumbens-hypothalamic axis, whereby the biogenic amines dopamine and serotonin are reciprocally involved. Evidence is presented which implicates the striatum and the hypothalamus as possible specific loci for regional differences between alcohol-preferring and alcohol-nonpreferring mice. We believe that photoperiod-induced alcohol ingestive behavior may involve alterations in both pineal and hypothalamic opioid peptides.

*Key words.* Ethanol; monoamines; ventral tegmental accumbens-hypothalamic axis; opioid peptides; pineal gland.

## *Darkness-induced ethanol intake*

In 1971, Geller[34] reported that laboratory rats maintained in total darkness developed a propensity to drink ethanol. The established preference for ethanol was not reversed under conditions of constant illumination. Similarly, administration of the pineal hormone, melatonin, to rats maintained under "normal" conditions of constant photoperiods also induced ethanol drinking.

Additional work by Sinclair[88] supported Geller's original findings. This early work clearly indicates that N-acetyl-methoxytryptamine (melatonin) and 1-methyl-6-methoxy-1,2,3,4-tetrahydro-1-carboline are implicated in the control of ethanol preference in rats[34,35]. Prolonged periods of darkness will also induce ethanol preference in the rat[20]. It is well established that dark exposure stimulates pineal metabolic and secretory activity[6,57,107]. The observation that light-deprived animals with intact pineal glands have a greater ethanol preference than pinealectomized controls is further supported by experiments utilizing congenitally blind rats[75]. Further, in the Syrian hamster, light deprivation, which stimulates the enzymes responsible for melatonin production in the pineal[7,58,108], enhances the animal's ethanol preference[76]. The pineal in the Syrian hamster is particularly active physiologically[48,77], to the extent that this species exhibits a propensity to drink ethanol at the expense of water[5]. From the work of Burke and Kramer[26], it appears that melatonin may be involved in the acquisition of ethanol preference rather than maintenance of alcohol consumption[20].

## *Opioid peptides and alcohol actions*

Increasing evidence from both animals and humans supports the interaction between ethanol or condensation amine metabolites and opiates[2,17,60,65]. Much evidence supports the involvement of endogenous peptidyl opiates in the actions of ethanol[49,64]. Opiate agonists reduce the volitional consumption of ethanol in rats[45,47] and in Syrian hamsters[80]. Ethanol consumption is increased after opiate withdrawal[47]. With the characterization of multiple opioid receptors (polyclonal receptor fragments) and stereospecific interactions of ethanol[51,100] and its condensation products[33,60], it is reasonable to conclude that certain endogenous opioids alter ethanol consumption in a way similar to opiates[46]. Long-term ethanol exposure increases the expression of δ-opioid binding sites in cultured neural cells[29]. Furthermore, ethanol induces the expression of functional opioid receptors, rendering these cells 3–5-fold more sensitive to opiates. The concomitant increase in opiate efficacy suggests that ethanol also alters receptor-effector coupling. Furthermore, it has been proposed that ethanol-seeking behavior is genetically linked to a deficiency of endogenous peptidyl opiates[14].

Experiments in our laboratory indicate that C57 BL/6J (alcohol-preferring) mice had lower levels of whole brain [Met]-enkephalin (MENK) as compared to DBA/2J (alcohol-nonpreferring) mice[15]. The finding of an inverse correlation between ethanol consumption and brain peptide opioids supports, in part, the concept of a deficiency of endogenous opioid peptides genetically linked to alcohol intake[13,14,19]. Considerable animal evidence suggests involvement of peptidyl opiates in mediating alcohol-seeking behavior[11]. Similarly, human addicts have one-third the level of β-endorphin (BED) and significantly more adrenocorticotrophic hormone in their cerebrospinal fluid than do normal volunteers[36]. Additional work in humans[31] and in animals[18] indicates that the opioid peptides may act as critical determinants for volitional intake of alcohol.

## *Brain reward systems, neurotransmitters, and neuropeptides*

Brain reward systems are integral to the function of endorphins and opiate receptors in the CNS. The reward

system extends from the brainstem via the hypothalamus and pituitary to telencephalic parts of the limbic system and further to the neocortex[81]. Various functions of these brain areas include analgesia, osmotic and thermal balances, and hormonal regulation. Lesions of telencephalic parts of the limbic system result either in aggressive behavior or in frustration. The system helps to control the center of complex movements (striatum) and to the "memory store" in the neocortex. The limbic system, comprising the hypothalamus and functionally important telencephalic regions, is the "core area" of the reward system which also is known to mediate drug effects[43, 81]. Tabakoff, for example, proposed that opiates and alcohol use these same pathways[99]. Addictive drugs use and affect those endogenous substrates which mediate the reward experience.

The medial forebrain bundle functions as a kind of "relay station" of the reward system. Interestingly, fibers passing through this bundle are dopaminergic, noradrenergic, and serotonergic[70]. While the role of serotonin (5HT) in the reward system is unclear, norepinephrine (NE) innervates essentially the entire brain from brainstem to neocortex[81, 82]. In contrast, DA has very defined locations. According to Moore and Bloom[66, 67] there are different dopaminergic systems, each with unique functions. While striatal dopamine controls complex movements, different dopamine systems in the hypothalamus suggest a role for the transmission of emotions regulating eating and drinking behavior, as well as reproduction.

For years, the hypothalamus has been assigned the central and exclusive role in regulating ingestive behavior (compulsive disorders). However, experimental evidence indicates that ingestive, as well as other behaviors, are profoundly influenced by neural tracts that pass through the hypothalamus. Electrical stimulation of the lateral hypothalamus elicits feeding or drinking; injections of neurohumoral transmitters into the hypothalamus elicit feeding or drinking, depending on the neurotransmitter used; and, systemic injections of gold thioglucose, which concentrates in the ventromedial hypothalamus resulting in a lesion, lead to overeating and obesity[42]. Furthermore, evidence from electrophysiologic experiments indicates that single cells in the hypothalamus change their firing rate in response to changes in blood glucose or to compounds, such as insulin or 2-deoxy-D-glucose, which modify cellular glucose utilization[40]. Lesions of the lateral hypothalamus produce their effects not by destroying a hypothalamic regulatory center, but by interrupting the pallidofugal fiber system, the major efferent outflow from the globus pallidus (GP) which runs through the lateral hypothalamus[40]. In addition, the GP projects to the lower brainstem, particularly to the substantia nigra (SN). The GP, SN, and caudate nucleus form a feedback loop that has been implicated in the organization of complex behaviors. The dopaminergic nigrostriatal pathway is a critical link in this system. Lesions of the SN, as well as the more selective interruption of the nigrostriatal pathways, produce aphagia and adipsia[39]. It has even been suggested that the effects of lateral hypothalamic lesions on ingestive behavior might be due to the depletion of striatal DA. In fact, severe DA depletion produces an initial period of complete unresponsiveness that results in aphagia and adipsia[95]. All these results support the conclusions that the complex feedback system between the GP, SN, and caudate nucleus must be intact if normal regulatory control over food and water intake is to survive.

In terms of addictive behavior, the significance of dopaminergic neurons becomes evident, since their interruption causes a cessation of self-stimulation. Many researchers are convinced that not only DA, but also NE[23, 24] and 5HT[71] are involved in conjunction with DA in the mediation of reward.

In considering behavioral specificity, it is important to link hypothalamic function with other neuroanatomical loci such as the accumbens. Together, the entire 'complex network', including sensory and motor functions and arousal, must be taken into account when one is attempting to understand control of the complex behavior of ingestion of alcohol.

Catecholamines are also of significant importance in addition research. For example, NE is responsible for the development and maintenance of functional tolerance because elimination of noradrenergic neurons abolishes tolerance without affecting physical dependence[78]. DA not only mediates reward, it may also mediate the euphoria of certain drugs (opiates and alcohol). In this regard, Wise and Bozarth[105] stated that the blockade of DA receptors interferes with all reward experiences and that opiates clearly stimulate dopaminergic systems.

While it is clear that opiate-mediated reward has as a substrate DA, there is no good agreement with regard to what mediates ethanol-induced reward.

Wise and Bozarth[105] proposed that DA is the catecholamine critically involved in the central mediation of reward by alcohol. These authors suggested that ethanol may act by inhibiting noradrenergic activity in the locus coeruleus. This activity has been proposed as a correlate of anxiety. Accordingly, inhibition of locus coeruleus neurons would relieve anxiety and release DA neurons from tonic noradrenergic inhibition at the locus coeruleus level.

However, Amit and Brown[3] refute the claim made by Bozarth and Wise[105] and point out that ethanol, per se, is not a very good anxiolytic drug. Furthermore, ethanol has been shown to stimulate neuronal activity and to increase noradrenergic functioning. Other studies by Amit's group[4, 25] show that ethanol consumption is markedly suppressed in animals treated with FLA-57, a dopamine-$\beta$-hydroxylase inhibitor. It is noteworthy that in these studies, brain NE levels were significantly depressed, whereas in the same brain tissue, there was only a small change in DA content. Additionally, a number of investigators have shown brain NE turnover in labora-

tory animals to increase following ethanol injections[27, 28, 30, 50]. Other work revealed that the DA receptor blocker haloperidol had no effect on ethanol self-administration[105].

Thus, it can be agreed that the NE system must be intact for the direct administration of ethanol reinforcement and that DA may play an intermediate role. However, for this review, we will focus on DA and neuropeptide interactions because it is our belief that these interactions are requisite for maintenance of ethanol consumption.

A large number of opiate receptors are found on dopaminergic nerve endings, more than on the endings of other neurons. Natural ligands, opioid peptides (endorphin, MENK) bind to these endogenous opioid receptors. Herz's group[9] suggest a link between dopaminergic and endorphinergic mechanisms on the one hand and the effects of alcohol and opiates on the other.

To summarize, it is apparent that both neurotransmitters and neuropeptides play a role in consummatory behavior. Anatomically, the hypothalamus and other closely associated neuropathways may form a complex network which constitutes specific loci or targets for mediation and control. It is our intent here to propose that, in the specific condition of alcohol ingestive behavior, that the opioid peptides at the level of the hypothalamus and the accumbens serve as a significant determinant, especially in relation to photoperiod-induced (darkness) 'aberrant' alcohol intake.

*Interrelatedness of neuroamines, opioid peptides, and alcohol-seeking behavior*

Generally, certain types of addictive behavior, such as obesity, may have common mechanisms which are mediated by opioid peptides. Obese, drug-free humans have a set of symptoms resembling those that opiate drugs induce in lean humans[63]. According to Margules[63], both show the following symptoms: (1) passivity in thoughts and deeds; (2) excessive interest in food and drink; (3) aversion to exercise such as running and other physical activities; and, (4) enhancement of certain immunological defense mechanisms.

There is evidence for the existence of an anti-opioid system in the body[61, 62] which counteracts all of these opioid actions, including activeness in thoughts and deeds, anorexia, urge to run, to have sex, and repair of certain immune functions. Accordingly, this system utilizes anti-opioid substances that could counteract the opiate withdrawal symptoms.

Boublik and co-workers[22] provide interesting evidence for the existence of a naturally occurring anti-opioid ligand in coffee that binds specifically to opioid receptors, acting as an opioid antagonist. This raises the question of whether the human brain could also have the capacity to synthesize anti-opioid ligands. In fact, Grevel and Sadee discovered a unique binding site in rat brain known as the lambda ($\lambda$) receptor[38]. This site is distinct from all other known opioid receptor sites and has a high affinity for naloxone. Furthermore, the regional distribution of $\lambda$ sites in the brain differs from other binding sites. The existence of these sites and the possible existence of a natural anti-opioid most likely serves to reinforce the genetic predisposition of the anti-opioid state, particularly as regards alcohol-seeking behavior. Since the pineal gland is involved in ingestive behaviors, as well as reproduction, it is possible that endorphins play a role in pineal activity as it relates to these behaviors. The interrelatedness of the opioid peptides and photoperiod will be described in a later section of this paper.

Certain drugs, including alcohol, induce changes in opioid levels of the central nervous system. Herz and Höllt[44] discovered that long-term morphine treatment of rats inhibited the synthesis of BED. Thus, the reduced BED levels are not a consequence of increased release, but of a reduced synthesis. Furthermore, it has also been found that 50% less proopiomelanocortin (POMC), the precursor of BED, is synthesized in comparison to controls. According to Herz and co-workers[44], long-term morphine treatment reduces the formation of BED in the rat by lowering the activity of the mRNA code for the precursor of BED. Of great interest is the finding that following long-term ingestion of alcohol in Syrian hamsters, [Leu]-enkephalin (LENK) in the basal ganglian is markedly reduced relative to nondrinking control hamsters[16]. Changes in endorphin content similar to those observed in long-term morphine treatment[72] have also been observed after long-term alcohol treatment of rats and guinea pigs[87].

Selective changes of different opioid systems after chronic alcohol treatment have been observed. In the pituitary, an $\alpha$-N-acetylation of BED was observed, rendering this endorphin opiate inactive, and reductions of up to 67% of dynorphin and $\alpha$-neoendorphin were found in the hippocampus. As to the hypothalamic functions of dynorphin and $\alpha$-neoendorphin, it has been shown that $\alpha$-neoendorphin participates in the control of eating behavior[86], while dynorphin is involved with the biological clock of the circadian rhythm[83]. Furthermore, following alcohol consumption there is an increase in hypophyseal BED, which is devoid of opiate activity[73]. Thus, new BED may be of the $\alpha$-N-acetylation form. In such circumstances, increases in brain opioids may not reflect opioid function, but may be due to a reduced brain utilization of the pseudo-endorphin substance[85].

In regard to the changes in the synthesis of $\alpha$-endorphin precursors, Goldstein stated[37], "the possibility should be entertained that a preexisting genetically determined endorphin deficiency could predispose to opiate addiction." Additionally, Bohus[21] stated that an excess or a deficiency of one of the potent endorphins can profoundly alter the interpretation of environmental stimuli. Van Ree[103] suggested that disturbance in neuropeptide systems may be a critical factor in the development of addic-

tive behavior. With regard to alcohol intake, Blum and co-workers[10,11] developed a "genotype theory" and found that mice with low innate enkephalin levels consume much alcohol, whereas mice with a high innate enkephalin level avoid alcohol. These experiments provided the first evidence to follow up the hypothesis of genetic endorphin deficiency in relation to increased alcohol consumption.

Involvement of various neuroamines, such as DA and 5HT in addictive behavior, specifically alcohol craving and reward, is becoming more firmly established. In this regard, Kiianmaa and Tabakoff[53] showed in alcohol-loving mice, a tolerance to the inhibitory effect of alcohol on DA release. These observations led to the idea that certain animals have a genetic resistance to the inhibitory influences of alcohol on DA release. In alcoholic humans, higher DA levels in CSF suggest a special tolerance in DA metabolism; Barbaccia and his colleagues[8] demonstrated genetic differences between DA and enkephalins that supported this linkage. Where dopaminergic nerve endings are rich in endorphin receptors, as shown in the C57/BL strain of mice, DA metabolism is increased considerably.

Interestingly, Schwartz[84] reported that chronic treatment of rats with haloperidol (1 mg/kg daily) led to a 4-fold increase of proenkephalin (PE) mRNA specific to the striatum. This increase in PE mRNA was paralleled by increases in PE and in enkephalin-containing peptides, suggesting that blockade of nigrostriatal DA transmission relieved a tonic inhibition and turned on PE synthesis. High DA levels with simultaneous reduction in endorphin content seem to be a paradox in explaining alcohol euphoria. The theory that alcohol exerts its euphoric effect by release of BED is not tenable in the development of alcoholic disease[104]. In several experiments, release of BED is evidenced after acute alcohol consumption, but with chronic alcohol consumption, BED is either reduced[31] or deprived of its opiate characteristics. Summers[96] reported on the condensation of acetaldehyde, a by-product of alcohol consumption and MENK. The resultant condensate, N-methyl derivative of MENK, is considerably less potent than the parent pentapeptide on the standard opiate assay system, but, nonetheless, is still about 3 times to 70 times more potent than the corresponding acetaldehyde adduct. This suggests that the ethyl moiety (coming directly from ethanol) at the $\alpha$-amino group is mainly responsible for the observed loss of potency, most likely due to imidazolidinone ring formation affecting the conformational mobility of the adduct compared to that of MENK[96,98]. Certainly, derivatization of enkephalins and endorphins with acetaldehyde to give products that fail to bind to their target receptors would presumably affect a variety of processes implicated in the normal physiological roles for these peptides[97,101,102].

As DA seems to be the key substance, it is important to point out that the development of addiction has, as its anatomical substrate, the reward system of the brain, particularly its 'core area', the limbic system. The transmitter mediating the reinforcing effect in the reward system is most probably DA. The euphorigen among the endorphins is suggested to be hypothalamic BED. The motivation for misuse of chemical substances is probably a genetic opioid deficiency. Opiate effects could be explained by an interaction of ligands with the opiate at the opiate receptor with subsequent release of DA, which could mediate the reinforcing effect. In brain slice experiments, it has been reported that DA, cocaine, or the selective dopamine $D_2$ receptor agonist (WO437) activate rewarding $CA_1$ target cells[93]. Additionally, evidence is now accumulating that opioid peptides may be rewarding by themselves without involvement of other neurotransmitter activation. Dynorphin-containing terminals have been demonstrated along with $\kappa$-opioid receptors in the $CA_3$ area of the rat hippocampus. Dynorphin is self-administered in experimental animals. Iontophoresis into single neurons of dynorphin induces firing of $CA_3$ neurons, suggesting that dynorphin (or possibly other opioids) may be a natural 'reinforcement neurotransmitter' in the $CA_3$ area of the hippocampus[94]. However, for alcohol, the condensation of acetaldehyde with DA to form tetrahydroisoquinolines (TIQ's) may act as an opiate[12], whereby alcohol effects, like opiates, could be explained by an exchange of endogenous ligands at the $\delta$-opioid receptor sites. A genetically-mediated increase in DA has been suggested in alcoholics. Consequently, via formation of condensation products, higher salsolinol (TIQ) levels were observed in alcoholics rather than in control subjects or nonalcoholics[89,91].

The fact that there is a connection between tegmental-accumbens DA and hypothalamic control of ingestive behavior and that there is an intimate relationship between DA and endorphin synthesis provides support for the possibility that hypothalamic BED may, in part, mediate alcohol intake.

In humans, there is evidence to support the concept that DA and 5HT are closely involved with one another[1]. A correlation is recognized between behavioral effects and depletion of the major biogenic amine 5HT in the forebrain. Selective destruction of serotonergic pathways in the brain, by ventricular injection of neurotoxins, induces hyperphagia. Results suggest that a serotonergic component of the brain that arises in the lower brainstem and ascends through the hypothalamus is involved in the regulation of ingestive behavior.

The role of serotonergic mechanism(s) in alcohol intake is receiving recent support from a number of investigators[69]. Naranjo et al.[69] evaluated ethanol drinking in humans by utilizing drugs known to enhance serotonergic transmission. Experiments in rats show a decrease in ethanol intake and preference after using agents which enhance serotonergic function. The administration of intraventricular serotonin or injection of its precursor, 5-hydroxytryptophan, intraperitoneally, attenuates alco-

hol consumption and preference in rats. Similar results are observed after the administration of several 5HT reuptake inhibitors (zimelidine, fluoxitine, citalopram, indalpine) and similarly, when a 5HT agonist (MK-212) is administered. In humans, administration of zimelidine to nondepressed, heavy drinkers is associated with a significant increase in the number of abstinent days and a decrease in the number of drinks consumed. Since ethanol significantly alters serotonergic neurotransmission [32], these results suggest that serotonergic enhancers like zimelidine may act centrally, possibly by interfacing with the neurobiological mechanisms regulating ethanol intake.

In terms of the regulation of ethanol intake, it is of great interest that Schwartz [84] reported that treatment of rats with drugs which deplete 5HT (fenfluramine, parachlorphenylalanine, or 5–7 dihydroxytryptamine) increased the content of both enkephalin and BED in the hypothalamus, but not in any other brain regions. However, no changes were detected in either mRNA or precursor content of PE or POMC. Thus, serotonergic transmission regulates opioid peptide utilization without affecting synthesis.

Once again, it is important to make a clear distinction between the amount of brain enkephalin and/or endorphin and it's function. For example, ethanol may induce 'aberrant' or opioid-devoid endorphins. It is also possible that alterations of serotonergic transmission may result in a reduced endorphinergic release leading to a lack of deficiency of opioid peptides and enhanced alcohol intake. This defect could be overcome by administration of drugs which enhance serotonergic activity, specifically in the hypothalamus.

In this regard, Murphy et al. [68] found differences in regional brain serotonin content in alcohol-preferring and nonpreferring rats from the H/Nih heterogeneous stock. Compared with low preference rats, the high preference animals had a significantly lower content of serotonin in the thalamus and hypothalamus. Since no other brain region displayed this difference, the authors suggest a possible role of the serotonergic system of the hypothalamus in the mediation of preference for alcohol. Further support is derived from the recent findings of Felten and Felten [32], who observed decreases in 5HT in the MBH in rats which consumed ethanol in a liquid diet. The authors suggest that the reduced serotonergic input to MBH may be related to the altered endocrine state of animals consuming alcohol, specifically the hypothalamic-pituitary axis related to ACTH.

*Brain regional specific opioid peptides as a function of genotype and photoperiod*

To further characterize the relationship between MENK levels of C58/6J (alcohol-preferring) and C3H/CRGL/2 (alcohol-nonpreferring), select brain regions of mice were measured by both RIA and HPLC analysis. The resultant data reveal that the C58/6J alcohol-preferring mice (estimates of 14-day preference ratio: 0.4–0.9) have significantly ($p < 0.05$) lower baseline MENK levels in both the corpus striatum, $85 \pm 11.2$, and hypothalamus, $32.5 \pm 12.8$ ng/mg protein, as compared to C3H/CRGL/2 alcohol-nonpreferring mice (estimates for 14-day preference ratio: 0.05 to 0.57), which had $129.0 \pm 14.4$ ng/mg protein, respectively.

Systematic study of MENK levels in different brain regions of several alcohol-preferring strains did not reveal significant differences. Specifically, pituitary, amygdala, midbrain, and hippocampal MENK levels did not differ between C58/6J and C3H/CRGL animals. Since the hypothalamus and corpus striatum represent areas with relatively high amounts of MENK, and in previous work we found significant strain-related differences in MENK in whole brain [11, 18], we infer that both the corpus striatum and hypothalamus may be involved in opioid peptide mediation of alcohol intake.

In terms of photoperiod involvement in alcohol intake, a recent paper by Roberts et al. [79] may shed additional light on this subject. These authors found that changes in photoperiod alter the daily rhythms of pineal melatonin and the hypothalamic BED content in the Syrian hamster. Their study examined the possible involvement of BED in the photoperiodic control of reproduction in the Syrian hamster. BED in the MBH, anterior hypothalamus (AHA), and preoptic area (POA), as well as the pineal melatonin content, were measured in male Syrian hamsters exposed to long day (LD) [16-h light; 8-h dark; lights on 07.00–23.00] or short day (SD) [8-h light; 16-h dark; lights on 07.00–15.00] for 8 weeks.

The marked increase in BED levels in the AHA and MBH in animals kept in SD coincided with the early increase in pineal melatonin content during the dark phase in these animals. As pointed out by Roberts et al. [79], it is not possible to propose a direct causal relationship between the secretion of melatonin and the rhythm of BED. It is known that destruction of cell bodies in the AHA can prevent the pineal-mediated effects of short photoperiod in Syrian hamsters [44]. Daily rhythms of several neuropeptides, including BED, have been reported in the rat [52]. Of great interest is the finding by Kumar et al. [59] who reported that pinealectomy in the rat tends to 'normalize' darkness-induced hypothalamic increases in MENK. This normalization might have a direct causal relationship to darkness-induced alcohol intake, since we have shown in our earlier papers that pinealectomy suppressed alcohol drinking in Syrian hamsters exposed to total darkness [20, 75, 76].

Kumar and co-workers [101] suggest, however, that common controlling mechanisms other than pineal may be responsible for generating daily changes in the activity of peptidergic neurons in the basal hypothalamus. In light of this, it is important to realize also that content of a neuropeptide may not reflect its functional activity. As

noted above, interference with serotonergic transmission leads to an increase in hypothalamic MENK, with a probable decrease in its utilization (i.e., decrease release, etc.). Also, ethanol has been shown to decrease 5HT specifically in the hypothalamus[32].

In the pineal gland, the rate of melatonin production most probably reflects 5HT turnover[55]. The rate-limiting step in the synthesis of rat pineal melatonin may be the N-acetylation of 5-hydroxytryptamine (5HT). This step is catabolized by the enzyme serotonin N-acetyltransferase (NAT) (NAT; E.C.2.3.1.5 acetyl CoA; arylamine-N-acetyltransferase). The rat pineal gland contains inordinately high concentrations of the NAT substrate 5HT relative to other tissues in the CNS. The rate of 5HT synthesis appears to be higher at night than during the daytime in the rat pineal gland. However, King et al.[54] provide evidence for the pineal storage of inactive levels of 5HT at night. In this regard, 5HT is inactivated by oxidative deamination, which is catalyzed primarily by the activity of MAO type A localized within the adrenergic nerve fibers and their endings in the pineal gland.

The 24-h rhythm in pineal 5HT levels appears to be a function of a similar rhythm in the availability of this amine to catabolism, primarily by means of oxidative deamination. Snyder and co-workers[92] have suggested that the availability of 5HT is related to daily changes in 5HT binding such that the nocturnal decline in 5HT levels reflects greater release of bound 5HT with subsequent catabolism, principally oxidative deamination. King and co-workers[54] postulated that 5HT within the pinealocytes is bound, stored, or in some other way made unavailable for further metabolism. With an appropriate physiological cue, e.g., the onset of darkness, 5HT is unbound, some of which is released extracellularly to be taken up by the adrenergic nerve endings where the amine is catalyzed via oxidative deamination. The remaining 5HT within the pinealocytes would be subject to N-acetylation, O-methylation, and deamination. Decrease of serotonergic activity at night could be due to a decrease in 5HT concentrations, a decrease in 5HT binding within the pinealocytes and/or an increased release of 5HT from the pinealocytes. A serotonergic deficit would tend to influence the production and utilization of pineal and hypothalamic opioid peptides[56]. It is logical that total darkness may result in an increased oxidative deamination of 5HT, leading to impairment of serotonergic transmission in specific brain regions (i.e., hypothalamus, striatum, and pineal gland) with a concomitant deficit in functional BED and/or MENK. Endorphinergic deficit seems to result in enhanced alcohol craving, even in light of the fact that small doses of synthetic opiates have been reported to enhance ethanol intake[106]. These facts, taken together, may provide the neuroendocrinological explanation for altered photoperiod as a determinant in alcohol intake in animals, as well as in humans.

*Conclusion*

The pineal gland plays an important role in modulating alcohol intake in numerous animal species. The endorphinergic system is intimately involved in both the actions of alcohol and opiates, as well as craving and/or genetic predisposition towards abuse of these two agents. Furthermore, there is significant evidence to link ingestive behaviors with the tegmental-accumbens-hypothalamic axis, whereby the biogenic amines DA and 5HT are reciprocally involved. In this paper we presented evidence that the striatum and hypothalamus might be specific loci for regional differences between alcohol-preferring and alcohol-nonpreferring mice. Evidence is also presented to suggest that the pineal gland and hypothalamic opioid peptides are influenced quite dramatically by alterations in photoperiod. Since it has been demonstrated that the biogenic amines DA and 5HT affect the synthesis and utilization of striatal and hypothalamic opioid peptides, respectively, it is conceivable that day-night differences of these amines are a direct cause of fluctuations of brain endorphinergic function.

We postulate here that: (1) during the day there is an increase in 5HT concentration via enhanced 5HT binding within the pinealocytes or decreased release of 5HT from the pinealocytes with a reduced catabolism through oxidative-deamination; (2) during the day there is an enhanced 'normal' utilization of hypothalamic BED; (3) diurnal hypothalamic BED, as well as MENK, are lower than nocturnal opioid peptides in the hypothalamus, but are more active compounds; (4) 'normal' or adequate day-time active opioid compounds lead to 'normal' or reduced volitional alcohol consumption; (5) alternately, during the night there is a decrease in 5HT concentrations via reduced 5HT binding within the pinealocytes or increased release of 5HT from the pinealocytes with enhanced catabolism through oxidative-deamination; (6) during the night there is an 'abnormal' utilization of hypothalamic BED; (7) nocturnal hypothalamic BED, as well as MENK, are higher than diurnal opioid peptides in the hypothalamus, but are more inactive components; and, (8) 'abnormal' or inadequate night-time inactive compounds lead to 'abnormal' or increased volitional alcohol consumption.

*Abbreviations used*: MENK = methionine-enkephalin; BED = β-endorphin; NE = norepinephrine; DA = dopamine; 5HT = serotonin; POMC = proopiomelanocortin; PE = proenkephalin; GP = globus pallidus; SN = substantia nigra; MBH = medio-basal hypothalamus; AHA = anterior hypothalamus; POA = preoptic area; LD = long day; SD = short day; NAT = N-acetyltransferase; CNS = central nervous system; TIQ = tetrahydroisoquinolines; CSF = cerebral spinal fluid; MAO = monoamine oxidase.

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# Mini-Review

## Vanadium biochemistry: The unknown role of vanadium-containing cells in ascidians (sea squirts)

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*Summary.* This article reviews several new developments in vanadium biochemistry, as elucidated from studies of ascidians. A hypothesis correlating ascidian blood cell function to anaerobiosis, via two prominent redox constituents, namely vanadium(III) and the tunichromes, a family of metal ion complexing/reducing hydroquinonoid peptides, is presented.
*Key words:* Vanadium; ion pump; tunichrome; tunic; ascidian; respiration; oxygen; anaerobe.

### *Introduction*

Organisms possess a variety of mechanisms for assimilating the particular transition metals needed for normal metabolic activity. Ascidians are one such case, for they display a remarkable ability to sequester and reduce vanadium in specialized blood cells termed vanadocytes; analogously, iron-accumulating species possess ferrocytes[1]. However, the function of this bioinorganic process has remained an enigma since 1911[2]. To date, a physiologic role for vanadium in animals remains conspicuously absent, even though it is considered to be an essential trace element[3]. In comparison, vanadium is integral to several algal bromoperoxidases[4] and to certain bacterial dinitrogenases[5]. Unlike previous attempts to explain the existence of oxygen sensitive V (III) in a living organism, the bioinorganic chemistry of vanadocytes is reviewed here in light of *anaerobic adaptation* as the crucial issue.

### *Background*

Ascidians (class Ascidiaceae) are sessile, filter-feeding chordates which inhabit all of the oceans[6]. Two traits of these prolific creatures are their resilient mantle (i.e., tunic; hence tunicates), and their powerful siphons (hence sea squirts). It has been estimated that species such as *Ascidia ceratodes* store vanadium in cell vacuoles (vanadophores) at concentrations up to 1 M[7], while vanadium levels in vanadocytes approach 10 million times that of sea water[8]; the element is often found associated with intracellular membranes and granules[9]. In general, at least five varieties of circulating blood cells have been identified: lymphocytes, stem cells, leucocytes, pigment cells, and vacuolated cells[10]. Three types of the latter are commonly seen: green/grey signet ring cells (SRC), characterized by one large vacuole; green compartment cells (CC), containing several angular vacuoles; and bright yellow to yellow/green, mulberry-shaped