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Contents of Monoamines in Forebrain Regions of Alcohol-Preferring (P) and -Nonpreferring (NP) Lines of Rats[1]

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MURPHY, J. M., W. J. McBRIDE, L. LUMENG AND T.-K. LI. *Contents of monoamines in forebrain regions of alcohol-preferring (P) and -nonpreferring (NP) lines of rats.* PHARMACOL BIOCHEM BEHAV **26**(2) 389–392, 1987.— The contents of monoamine neurotransmitters and metabolites were assayed in the frontal cortex, nucleus accumbens and anterior striatum of rats from the selectively bred alcohol-preferring P and nonpreferring NP lines. Lower levels of serotonin (20–30%) in all three brain regions of P as compared with NP rats lends support to the hypothesis that a decreased metabolic activity and/or innervation by serotonin neurons is associated with the abnormally high volitional intake of ethanol. Of additional interest, however, were the approximately 25% lower contents of dopamine and its major metabolites in the nucleus accumbens of the P rats. This observation may indicate that P rats have a specific deficiency in the dopaminergic projections from the ventral tegmental area to the nucleus accumbens and, since the accumbens is an important structure in brain reward circuitry, it might also be an important determinant of the excessive volitional intake of alcohol by P rats.

Dopamine Norepinephrine Serotonin 3,4-Dihydroxyphenylacetic acid Homovanillic acid 5-Hydroxyindoleacetic acid Frontal cortex Nuclues accumbens Anterior striatum Alcohol-preferring rats Alcohol-nonpreferring rats

THE voluntary oral intake of alcohol by laboratory animals and by humans is influenced by genetic factors [4, 6, 9, 29]. However, the genetically determined biochemical abnormalities that predispose an individual to consume excessive quantities of alcohol are unknown [4, 12, 28]. Brain monoamine systems have been hypothesized as neurochemical substrates for various behaviors, including ethanol preference, sensitivity, tolerance and dependence, but findings have been contradictory [11–13, 16, 20, 21, 30]. Serotonin, in particular, has been implicated in ethanol preference and the maintenance of the volitional ingestion of alcohol [7, 18, 23]. Recently, some consistent findings have emerged with the pharmacological use of serotonin uptake inhibitors. The inhibition of 5-HT uptake decreased the volitional intake of ethanol in standard laboratory rats [1, 26, 27], in rats selectively bred for their alcohol drinking behavior [22], and possibly in humans [24]. The presumed mechanism of action of the 5-HT uptake inhibitors is to increase the concentration of 5-HT at the postsynaptic site. The modulatory effect of 5-HT uptake inhibitors on excessive alcohol drinking behavior is particularly important in light of findings that selectively bred rats of the alcohol-preferring P line have lower contents of serotonin in the cerebral cortex, hippocampus, corpus striatum, thalamus and hypothalamus as compared with rats of the alcohol-nonpreferring NP line [21]. Recent studies also demonstrated that post-absorptive effects of ethanol are reinforcing in the P-line of rats, indicating that they do not consume ethanol only for its taste or smell [32].

Although the contents of monoamines in several brain regions (e.g., hypothalamus, hippocampus) considered as important areas in the brain reward systems had been determined in the P and NP rats [21], one potentially important region, the nucleus accumbens, was not examined individually. Because of its probable importance in motivated behaviors and in the brain reward system [25,35], the present study was undertaken to compare the contents of monoamines in this structure between the P and NP lines of rats. The dissection procedure employed [10] enabled the rapid and accurate dissection of the frontal cortex, which may also play a part in the brain reward circuitry, and the anterior striatum. Hence, the monoamine levels in these regions were also determined.

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TABLE 1

CONTENTS OF SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID IN THE NUCLEUS ACCUMBENS, FRONTAL CORTEX AND ANTERIOR STRIATUM OF ALCOHOL PREFERRING (P) AND NONPREFERRING (NP) RATS

	nmoles/g wet wt. (mean ± S.E.M.)	
CNS Region	5-HT	5-HIAA
Nucleus Accumbens		
NP (N=10)	10.0 ± 0.4	4.0 ± 0.1
P (N=8,9)	7.9 ± 0.2‡	3.7 ± 0.2
Frontal Cortex		
NP (N=9,10)	6.1 ± 0.1	2.54 ± 0.09
P (N=8,9)	5.0 ± 0.2†	2.24 ± 0.07*
Anterior Striatum		
NP (N=10)	3.8 ± 0.3	3.7 ± 0.2
P (N=9)	2.7 ± 0.1†	3.1 ± 0.2

Statistical significance determined with the Student t-test.
*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

METHOD

The selectively-bred alcohol-preferring (P) and nonpreferring (NP) lines originated from a randomly bred, Wistar (Wrm: WRC (WI) BR) colony at the Walter Reed Army Institute of Research [17]. The methods used to develop these lines have been described [17]. The present study utilized adult male NP (N=10) and P (N=12) rats, which weighed 325–400 g, from the S23–24 generations. All animals had been tested for ethanol preference at puberty by established procedures [15]. The minimum criteria for classification as a P rat were the average intake, with food, water and 10% (v/v) ethanol freely available, of greater than 5 g ethanol/kg body wt./day and an ethanol to water preference ratio (v/v) of greater than 2:1. The criteria for the NP rats were the free-choice intake of less than 0.5 g ethanol/kg/day and a preference ratio of less than 0.2:1. Following the preference test, the animals were ethanol free for at least four weeks.

All rats were housed individually with ad lib food (Purina Rat Chow No. 5001) and water. A normal 12 hour light-dark cycle was maintained beginning with lights on at 0800 hours. The animals were handled daily for at least seven days prior to killing and were adapted to the killing apparatus to minimize stress. The rats were killed by the method of near-freezing in liquid nitrogen [31]. The brains were rapidly removed and the nucleus accumbens, frontal cortex and anterior striatum were dissected out at −20°C or lower [10, 21, 31]. The brain parts were stored at −70°C until assayed for the contents of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and norepinephrine (NE) by HPLC with electrochemical detection, as previously described [20,21]. Statistical differences between the NP and P groups were determined with Student's t-test.

RESULTS

The contents of 5-HT were 20–30% lower in the nucleus accumbens, frontal cortex and anterior striatum of the P as compared with the NP rats (Table 1). The level of 5-HIAA also tended to be lower in all three brain regions of the P rats, but only in the case of the frontal cortex did this difference reach statistical significance (Table 1).

The contents of DA, DOPAC and HVA in the nucleus accumbens were approximately 25% lower in the rats of the P as compared with the NP line (Table 2). In the anterior striatum, there was no difference in the content of DA between the P and NP lines, and the approximately 16% lower levels of DOPAC and HVA in the P relative to the NP group proved statistically significant only in the case of HVA (Table 2). No differences in the levels of DA, DOPAC and HVA were found in the frontal cortex between the two lines.

There were no statistically significant differences in the contents of NE in the nucleus accumbens and frontal cortex between the P and NP rats (Table 2). Because of technical limitations and the relatively low quantity present, it was not possible to obtain values for NE in the anterior striatum.

DISCUSSION

The lower levels of DA and 5-HT in the nucleus accumbens and of 5-HT in the frontal cortex and anterior striatum (Tables 1 and 2) of the P as compared with the NP rats could be a consequence of a relatively slower rate of synthesis and/or lower proportion of 5-HT and DA axon terminals in these brain areas of P animals. Since DA inputs to the nucleus accumbens arise from cell bodies in the ventral tegmental area, the lower DA content in the nucleus accumbens of the P rats may reflect a difference from the NP line in this distinct group of DA neurons. By contrast, the finding that the contents of DA in the anterior striatum (Table 2) and whole striatum [21] of the P and the NP rats were similar indicates no difference between the P and NP lines in nigrostriatal DA innervation and supports the contention that the nigrostriatal DA system is not involved in the volitional intake of ethanol [3]. Other investigators have found that low doses of ethanol (0.125–0.5 g/kg) activated the firing of DA neurons in the ventral tegmental area and stimulated the release of DA in the nucleus accumbens, whereas similar effects in the nigrostriatal system required as much as a five

TABLE 2

CONTENTS OF DOPAMINE, 3,4-DIHYDROXYPHENYLACETIC ACID, HOMOVANILLIC ACID AND NOREPINEPHRINE IN THE NUCLEUS ACCUMBENS, FRONTAL CORTEX AND ANTERIOR STRIATUM OF ALCOHOL PREFERRING (P) AND NONPREFERRING (NP) RATS

Region	nmoles/g wet wt. (mean ± S.E.M.) DA	DOPAC	HVA	NE
Nucleus Accumbens				
NP (N=10)	67 ± 2	6.2 ± 0.2	2.22 ± 0.10	3.2 ± 0.3
P (N=9)	51 ± 2†	4.7 ± 0.2†	1.53 ± 0.08†	4.4 ± 0.5
Frontal Cortex				
NP (N=8,9)	1.3 ± 0.3	0.19 ± 0.04	0.32 ± 0.02	2.4 ± 0.2
P (N=8,9)	1.4 ± 0.4	0.29 ± 0.04	0.30 ± 0.03	2.3 ± 0.2
Anterior Striatum				
NP (N=10)	98 ± 4	6.7 ± 0.5	3.7 ± 0.2	—
P (N=9)	96 ± 5	5.6 ± 0.2	3.1 ± 0.2*	—

Statistical significance determined with the Student t-test.
*$p<0.05$; †$p<0.001$.

fold higher dose of ethanol [5,8]. In light of previous findings that doses of ethanol up to 0.5 g/kg stimulated locomotor activity in P but not NP rats and produced blood alcohol concentrations in the range that is rewarding for P rats [14, 33, 34], the present observation of a selective difference in the DA content of the nucleus accumbens between the P and NP rats supports the hypothesis that this mesolimbic system is an important neurochemical/neuroanatomical substrate for the locomotor activating and reinforcing properties of ethanol [5, 8, 35].

The frontal cortex receives input from both the ventral tegmental area and the substantia nigra [2]. The absence of a difference between the P and NP groups in DA content of the frontal cortex could be due to: (a) the DA neurons in the ventral tegmental area that project to the frontal cortex are different from those that project to the nucleus accumbens, and/or (b) the contribution of the DA projections from the substantia nigra may be greater than that from the ventral tegmental area, thus masking any innate differences.

In the case of 5-HT, there was a 20–30% lower content in all three brain regions of the P as compared with the NP line (Table 1). These data may indicate widespread differences throughout the dorsal raphe nucleus which sends it serotonergic projections to the nucleus accumbens via the dorsal raphe bundle and to the striatum and frontal cortex via the dorsal cortical tract. A previous study [21] also indicated a lower level of 5-HT in several CNS regions (cerebral cortex, striatum, thalamus, hypothalamus and hippocampus) of the P rats as compared with the NP animals, and a similar trend was observed in heterogeneous stock rats found to have low and high preferences for ethanol [19]. In addition, the lower content of 5-HT appears to be one possible cause of excessive alcohol intake by P rats since 5-HT uptake inhibitors reliably attenuated their high volitional intake of ethanol [22].

The observed differences between the P and NP rats are not likely due to exposure of the animals to alcohol during preference testing, since a four week alcohol-free period had elapsed prior to brain dissection. Moreover, studies dealing with the chronic consumption of alcohol have not indicated any differences in these regional brain monoamines between control and alcohol-exposed P rats [20], and there was good agreement between the differences observed in the present study and previous observations of brain monoamines in alcohol-naive P and NP rats [21].

In general, stock rats will avoid drinking ethanol solutions greater than 5% (v/v). In our preference test, the selectively-bred rats are given a free and unlimited access to 10% (v/v) ethanol and water. Since the NP rats avoid the 10% ethanol, they appear to behave more like the general population of stock rats. Therefore, the differences observed in the levels of DA and 5-HT in the nucleus accumbens between the P and NP lines probably reflect an imbalance in the neuronal systems involved in the brain reward circuitry that regulates alcohol intake in the P rat.

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