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# Effects of Acute Ethanol Administration on Monoamine and Metabolite Content in Forebrain Regions of Ethanol-Tolerant and -Nontolerant Alcohol-Preferring (P) Rats[1]

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MURPHY, J. M., W. J. McBRIDE, G. J. GATTO, L. LUMENG AND T.-K. LI. *Effects of acute ethanol administration on monoamine and metabolite content in forebrain regions of ethanol-tolerant and -nontolerant alcohol-preferring (P) rats.* PHARMACOL BIOCHEM BEHAV 29(1) 169–174, 1988.—The contents of dopamine (DA), serotonin (5-HT) and their metabolites in the frontal cortex, anterior striatum, nucleus accumbens and hypothalamus of alcohol-tolerant and -nontolerant rats of the alcohol-preferring P line were determined one hour after the IP administration of 2.5 g ethanol/kg body wt. Compared with saline-injected controls, nontolerant P-rats injected with ethanol had (a) 60% higher levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the frontal cortex; (b) 30–60% higher levels of DOPAC and HVA in the anterior striatum and nucleus accumbens; and (c) 20% higher levels of 5-HIAA in all three forebrain regions. In the tolerant group, the effects of IP ethanol on DOPAC and HVA were markedly attenuated or completely eliminated in these three forebrain regions. However, in the case of 5-HIAA, an attenuated response was observed only in the nucleus accumbens of the tolerant group. The IP administration of ethanol had little effect on the contents of DA or 5-HT in any of these three forebrain regions, with the exception that 5-HT levels were elevated in the anterior striatum of both the tolerant and nontolerant groups. In the hypothalamus, there were no significant differences for the contents of DA, 5-HT or their metabolites between the nontolerant or tolerant P rats after IP ethanol. The data indicate that both acute ethanol administration and chronic alcohol intake by the P line of rats alters certain DA and 5-HT systems that may be involved in the brain reward circuitry and in DA pathways involved in motor functions.

Alcohol-preferring rats Chronic ethanol tolerance Dopamine Serotonin 3,4-Dihydroxyphenylacetic acid Homovanillic acid 5-Hydroxyindoleacetic acid Frontal cortex Nucleus accumbens Anterior striatum Hypothalamus

RECENT behavioral studies from our laboratory have demonstrated that the selectively bred alcohol-preferring P line of rats develops tolerance to an intoxicating dose of ethanol when allowed chronic free-choice consumption of 10% (v/v) ethanol, as well as following the forced administration of ethanol in a liquid diet [7]. The development of tolerance to the intoxicating effects of ethanol is a prominent feature of alcoholism [30], and the demonstration of tolerance development through chronic free-choice drinking in the P rats was one of the last remaining requirements to establish the P line of rats as a suitable animal model for studying the genetic/biological basis of "alcoholic" behavior. Other studies from our laboratory have demonstrated that the P rats (a) consistently consume greater than 5.0 g ethanol/kg/day in a free-choice situation [15, 17, 18, 34], (b) work through operant responding to obtain ethanol [26], (c) drink to intoxication as indicated by blood alcohol concentrations [18,21], (d) develop physical dependence with chronic free-choice drinking [34], (e) consume ethanol for its rewarding post-ingestive effects [33], and (f) develop acute tolerance to ethanol [35].

Neurochemical studies have demonstrated that the P rats

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TABLE 1

LEVELS OF DOPAC, HVA AND 5-HIAA IN THE FRONTAL CORTEX, ANTERIOR STRIATUM AND NUCLEUS ACCUMBENS OF NONTOLERANT P RATS ONE HOUR AFTER THE IP INJECTION OF SALINE OR 2.5 g ETHANOL/kg

| | nmol/g wet wt. (mean ± SEM) | | |
|---|---|---|---|
| Region | DOPAC | HVA | 5-HIAA |
| Frontal Cortex | | | |
| Saline (N=5) | 0.22 ± 0.05 | 0.27 ± 0.03 | 1.9 ± 0.1 |
| 2.5 g EtOH/kg (N=10) | 0.44 ± 0.05* | 0.54 ± 0.06* | 2.6 ± 0.1† |
| Anterior Striatum | | | |
| Saline (N=5) | 5.0 ± 0.5 | 2.7 ± 0.1 | 2.9 ± 0.1 |
| 2.5 g EtOH/kg (N=10) | 7.5 ± 0.4† | 4.4 ± 0.3† | 3.3 ± 0.1† |
| Nucleus Accumbens | | | |
| Saline (N=5) | 4.8 ± 0.5 | 1.5 ± 0.1 | 3.4 ± 0.1 |
| 2.5 g EtOH/kg (N=10) | 6.8 ± 0.3† | 2.7 ± 0.1† | 4.3 ± 0.1† |

Data obtained from nontolerant P rats on a normal diet of rat chow with $H_2O$ available as the sole drinking fluid.
*$p<0.05$; †$p<0.01$ with Student $t$-test.

TABLE 2

LEVELS OF DOPAC, HVA AND 5-HIAA IN THE FRONTAL CORTEX, ANTERIOR STRIATUM AND NUCLEUS ACCUMBENS OF NONTOLERANT AND TOLERANT P RATS, CONSUMING A LIQUID DIET, ONE HOUR AFTER THE IP INJECTION OF 2.5 g ETHANOL/kg

| | nmol/g wet wt. (mean ± SEM; N=6) | | |
|---|---|---|---|
| Region | DOPAC | HVA | 5-HIAA |
| Frontal Cortex | | | |
| Nontolerant | 0.56 ± 0.05 | 0.64 ± 0.04 | 2.9 ± 0.1 |
| Tolerant | 0.48 ± 0.03 | 0.57 ± 0.04 | 2.5 ± 0.1* |
| Anterior Striatum | | | |
| Nontolerant | 9.1 ± 0.5 | 5.2 ± 0.3 | 3.3 ± 0.1 |
| Tolerant | 6.9 ± 0.7* | 3.7 ± 0.4* | 3.0 ± 0.2 |
| Nucleus Accumbens | | | |
| Nontolerant | 6.8 ± 0.2 | 2.9 ± 0.1 | 4.4 ± 0.1 |
| Tolerant | 5.2 ± 0.2* | 2.2 ± 0.1* | 3.6 ± 0.1* |

Data obtained from nontolerant and tolerant P rats on the liquid diet. The tolerant rats were given ethanol in their liquid diet and consumed 9.9 ± 0.4 g ethanol/kg/day. Both nontolerant and tolerant groups received a single IP injection of 2.5 g ethanol/kg body wt. 60 minutes before killing.
*$p<0.05$ with Student $t$-test.

have lower contents of serotonin (5-HT) in several CNS regions [23] and lower contents of both dopamine (DA) and 5-HT in the nucleus accumbens [25] compared with rats of the NP line. In addition, the IP administration of 2.5 g ethanol/kg to alcohol-naive P rats increased the contents of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the cerebral cortex and striatum [24]. These observations suggest that some specific deficiencies in DA and/or 5-HT systems may mediate the abnormal alcohol-seeking behavior of the P rats, and alcohol consumption may produce an activation within these monoaminergic systems. On the other hand, chronic ethanol consumption by P rats, either after 15 weeks of free-choice drinking of ethanol or after seven weeks of forced ethanol administration in a liquid diet, had no effect on the steady-state levels of DA, 5-HT or their metabolites in several CNS regions [24]. One reason for the lack of any changes in the monoamine systems after chronic ethanol intake could be that neuronal tolerance had developed.

The objective of the present study was to determine if there are CNS neurochemical changes which could be related to the behavioral tolerance observed in the P-line of rats [7]. After the chronic consumption of ethanol, the behavioral tolerance was observed following an acute sedative-hypnotic challenge dose of 2.5 g ethanol/kg. Since the same dose had previously been demonstrated to elevate DOPAC and HVA in forebrain regions of alcohol-naive P rats [24], the present study examined the effects of 2.5 g ethanol/kg on the contents of DA, 5-HT and their metabolites in selected forebrain regions of nontolerant and alcohol tolerant P rats.

## METHOD

Adult male rats of the alcohol-preferring P line were from the S-23 generation and were approximately 8–10 months old. The selectively bred P line 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 this line have been described [15]. Animals were individually housed in a temperature- and humidity-controlled environment with a normal 12 hour day-night cycle beginning at 0600 hours.

Many of the P rats utilized for the present neurochemical study are the same animals used in the already reported behavioral experiments [7] which established that they develop chronic tolerance. These included: (a) 6 rats consuming a liquid diet containing ethanol (tolerant) and 6 rats pair-fed a control liquid diet (nontolerant); (b) 11 animals on the normal rat chow diet with $H_2O$ available as the sole fluid (nontolerant) and 11 P rats on the normal diet with free-choice between 10% ethanol and $H_2O$ (tolerant). An additional 16 P rats (nontolerant) were used in the present study. These animals had also been employed in behavioral experiments in which they had received IP ethanol injections. However, they were alcohol free for at least one month before the neurochemical experiments and were maintained on a normal rat chow diet with $H_2O$ during this period.

All rats consuming alcohol, either in the liquid diet or by free-choice drinking of a 10% ethanol solution, exhibited tolerance to an IP dose of 2.5 g ethanol/kg when tested after two weeks of ethanol exposure, while the corresponding control groups did not exhibit tolerance [7]. A descending jumping platform apparatus, used to assess the development of behavioral tolerance, has been previously described in detail [19, 32, 35]. Tolerance was demonstrated when the groups on two weeks of chronic alcohol intake exhibited shorter recovery times following an IP challenge dose of ethanol (a decrease from 155–170 minutes to 80–90 minutes) and higher blood alcohol concentrations at recovery (an increase from 220 mg% to 275–285 mg%) relative to control values [7]. The four groups were maintained on their respective diets for an additional two or three weeks before killing

TABLE 3

LEVELS OF DOPAMINE, SEROTONIN AND THEIR METABOLITES IN THE FRONTAL CORTEX OF ALCOHOL-TOLERANT AND ALCOHOL-NONTOLERANT P RATS ONE HOUR AFTER THE IP INJECTION OF SALINE OR 2.5 g ETHANOL/kg BODY WEIGHT

| | | nmoles/g wet wt. (mean ± SEM) | | | | |
|---|---|---|---|---|---|---|
| Chronic Conditions | IP | DA | DOPAC | HVA | 5-HT | 5-HIAA |
| Nontolerant | Saline (N=11) | 1.3 ± 0.2 | 0.31 ± 0.04 (9) | 0.39 ± 0.06 (9) | 4.7 ± 0.1 | 2.2 ± 0.1 |
| Nontolerant | EtOH (N=22) | 1.4 ± 0.2 | 0.49 ± 0.03* | 0.61 ± 0.04* | 4.9 ± 0.1 | 2.7 ± 0.1* |
| Tolerant | EtOH (N=17) | 1.2 ± 0.1 | 0.40 ± 0.04 | 0.48 ± 0.04† | 5.1 ± 0.1* | 2.6 ± 0.1* |

Statistical significance determined with a one-way ANOVA followed by the Newman-Keuls test. *$p<0.05$ vs. saline-injected group and †$p<0.05$ for differences between the two ethanol-injected groups.

for the neurochemical experiments and were not tested in the jumping apparatus during this period. It was necessary to allow at least 2 weeks to elapse between acute injections so that any tolerance developed to a single dose of ethanol would have dissipated [8]. On the day of killing, food and ethanol were removed as described in the behavioral tolerance experiments [7]; i.e., ethanol was removed at 0300, control diet was given to both nontolerant and tolerant liquid diet groups between 0300–0600, and food (but not $H_2O$) was removed at 0600 from nontolerant and tolerant rats on the normal diet. Rats were injected IP with either saline or 2.5 g ethanol/kg and killed 60 minutes later (between 1200–1500 hours) by the near-freezing technique [31]. The frontal cortex, nucleus accumbens, anterior striatum and hypothalamus were dissected in a cold box at −20°C and stored at −70°C until assayed for the contents of DA, DOPAC, HVA, 5-HT and 5-HIAA by HPLC methods, as previously described [23].

Blood samples were collected in heparinized capillary tubes from trunk blood at the time of killing, 60 minutes after the IP injection of 2.5 g ethanol/kg. Blood alcohol determinations were by a gas chromatography procedure previously described [19].

Statistical differences for multiple comparisons were determined with a one-way ANOVA and Newman-Keuls *post-hoc* test. A Student *t*-test was used for comparison of two groups.

## RESULTS

Table 1 shows the effects of the IP administration of 2.5 g ethanol/kg body wt. on the levels of DOPAC, HVA and 5-HIAA determined in the frontal cortex, anterior striatum and nucleus accumbens of nontolerant P rats. Compared with saline-injected controls, the ethanol-injected group had elevated levels of DOPAC (40–100%), HVA (60–100%) and 5-HIAA (15–35%) in all three brain regions.

Two experiments comparing tolerant and nontolerant P rats were performed. The first used 6 tolerant animals that had consumed ethanol contained in the liquid diet, and 6 nontolerant rats that were pair fed the isocaloric control liquid diet. These animals provided the opportunity to obtain neurochemical data which might indicate whether neuronal tolerance had developed in certain DA and/or 5-HT systems. Since there were so few tolerant P-rats available, both the nontolerant and tolerant groups were injected with 2.5 g ethanol/kg and killed after 60 minutes. The rationale for this approach was that if chronic ethanol intake produced neuronal tolerance in either the DA or 5-HT systems of the nucleus accumbens, frontal cortex or striatum, then the neurochemical response for the tolerant animals following the challenge dose of ethanol should be blunted when compared with nontolerant rats. This was a valid assumption since a previous study indicated that chronic alcohol consumption by the P rats, either in the liquid diet or in a free-choice situation between 10% ethanol and $H_2O$, did not alter the levels of DA, 5-HT or their metabolites in any of the CNS regions examined [24].

Sixty minutes following the challenge dose of ethanol, DOPAC and HVA levels in the anterior striatum and nucleus accumbens were approximately 25% lower in the tolerant than in the nontolerant group (Table 2). The tolerant rats also had significantly lower levels (15–20%) of 5-HIAA in the frontal cortex and nucleus accumbens than did the nontolerant P rats (Table 2). The tolerant P rats had slightly lower levels of DOPAC and HVA in the frontal cortex and slightly lower levels of 5-HIAA in the anterior striatum than the nontolerant group (Table 2). These differences, however, were not statistically significant.

The second experiment comparing the tolerant and nontolerant animals, used the P rats which were demonstrated to have developed behavioral tolerance with free-choice drinking of 10% ethanol [7]. These rats consumed 6.8 g ethanol/kg body wt./day and exhibited chronic tolerance within the same 2-week period as did the P rats on the liquid diet regimen containing ethanol [7]. Results very similar to the data observed in the first experiment were obtained (Tables 3–5).

Compared with the nontolerant saline-injected controls, the IP injection of 2.5 g ethanol/kg body wt. elevated the levels of DOPAC and HVA approximately 30–60% in the frontal cortex (Table 3), anterior striatum (Table 4) and nucleus accumbens (Table 5) of the nontolerant animals. However, comparison of the two ethanol-injected groups revealed that, with one exception (DOPAC in the frontal cortex), the contents of DOPAC and HVA were statistically lower (15–20%) in the 3 brain regions of the tolerant animals

TABLE 4

LEVELS OF DOPAMINE, SEROTONIN AND THEIR METABOLITES IN THE ANTERIOR STRIATUM OF ALCOHOL-TOLERANT AND ALCOHOL-NONTOLERANT P RATS ONE HOUR AFTER THE IP INJECTION OF SALINE OR 2.5 g ETHANOL/kg BODY WEIGHT

| | | nmoles/g wet wt. (mean ± SEM) | | | | |
|---|---|---|---|---|---|---|
| Chronic Conditions | IP | DA | DOPAC | HVA | 5-HT | 5-HIAA |
| Nontolerant | Saline (N=11) | 82 ± 6 | 5.8 ± 0.1 | 2.8 ± 0.2 | 2.2 ± 0.1 | 2.8 ± 0.1 |
| Nontolerant | EtOH (N=22) | 79 ± 4 | 7.6 ± 0.3* | 4.5 ± 0.2* | 2.5 ± 0.1* | 3.2 ± 0.1* |
| Tolerant | EtOH (N=16) | 80 ± 5 | 6.5 ± 0.1† | 3.7 ± 0.2*† | 2.7 ± 0.1* | 3.3 ± 0.1* |

*$p<0.05$ vs. saline-injected group and †$p<0.05$ for differences between the two ethanol-injected groups. See legend for Table 3.

than in the nontolerant group (Tables 3–5). The content of DA did not differ among the groups in any of the three brain regions (Table 3–5).

The level of 5-HIAA was higher (8–23%) in the three brain areas of both the tolerant and the nontolerant groups given IP ethanol than in those of the saline-injected group (Tables 3–5). However, only in the nucleus accumbens was the content of 5-HIAA statistically lower in the tolerant rats than in the nontolerant group following IP ethanol administration (Table 5). Compared with the saline-injected nontolerant animals, the content of 5-HT was significantly elevated in the frontal cortex for the ethanol-injected tolerant group (Table 3) and anterior striatum for both the ethanol-injected tolerant and nontolerant groups (Table 4). No statistically significant differences were observed among the three groups for the levels of 5-HT in the nucleus accumbens (Table 5).
three groups for the levels of 5-HT in the nucleus accumbens

The contents of DA, 5-HT and their metabolites were also determined in the hypothalamus of nontolerant and tolerant P-rats following injection of 2.5 g ethanol/kg. In the nontolerant P-rats, acute ethanol administration did not alter the levels of DA, 5-HT or their metabolites relative to saline values (data not shown). These data replicate results already published for the hypothalamus from nontolerant P rats [24]. In addition, there were no differences in the levels of DA, 5-HT or their metabolites in the hypothalamus of tolerant P rats compared with nontolerant animals following ethanol administration (data not shown).

Trunk blood samples taken at the time of killing (60 minutes after the IP injection of 2.5 g ethanol/kg) indicated that the alcohol tolerant rats had slightly lower BACs than did the nontolerant group (250±15 vs. 286±14 mg% for free-choice of 10% ethanol vs. normal diet control; and 251±12 vs. 291±15 mg% for liquid diet with ethanol vs. liquid diet control), but these differences were not statistically significant.

## DISCUSSION

One hour after the IP injection of 2.5 g ethanol/kg body wt., the contents of DOPAC, HVA and 5-HIAA were significantly increased in the frontal cortex, anterior striatum and nucleus accumbens of the nontolerant P rats compared with the saline group (Tables 1 and 3–5). The results for DOPAC and HVA are in agreement with previous findings for the P rat where elevated levels of these two metabolites were found in the cerebral cortex and striatum following acute ethanol administration [24]. Elevated levels of HVA and DOPAC in whole brain [14] and of DOPAC in the striatum [2,5] have also been reported following the acute administration of ethanol to animals not selected for alcohol-drinking preference. On the other hand, acute ethanol had no apparent effect on the levels of DOPAC or HVA in the frontal cortex [5] or nucleus accumbens [29] of stock rats. Therefore, the present findings for DOPAC and HVA may represent an unique response to acute ethanol in the frontal cortex and nucleus accumbens of the P line of rats. However, it is unclear if the elevated levels of 5-HIAA observed in the three forebrain regions is a response unique to the P-rats since, in whole brain of stock rats, some investigators have reported an increased content of 5-HIAA following acute ethanol [1, 6, 28], whereas others have reported no effect or even decreased synthesis of 5-HT [10,12].

A general effect on metabolism (unrelated to neuronal activity) or a general inhibition of metabolite efflux by the high dose of ethanol is not a likely explanation for the increased levels of DOPAC, HVA and 5-HIAA (Tables 1 and 3–5) since previous studies [24] demonstrated that the effects of 2.5 g ethanol/kg on DOPAC and HVA levels were limited to only two (cerebral cortex and striatum) of the 8 CNS regions examined, while no changes were found for 5-HIAA levels in any of the regions. Therefore, one possible interpretation of the present findings is that acute ethanol increased the activity of specific DA and 5-HT pathways. Furthermore, by examining more discrete regions in the present study (i.e., frontal cortex and anterior striatum), it proved possible to detect changes in the levels of 5-HIAA following acute ethanol, which had not previously been observed.

Electrophysiological data support the contention that acute ethanol can increase neuronal activity in two dopaminergic nuclei, namely the substantia nigra [20] and ventral tegmental area [9]. Recent biochemical experiments using an *in vivo* dialysis technique indicated enhanced release of DA in the striatum and nucleus accumbens of Sprague-Dawley rats following the IP injection of 2.5 g ethanol/kg [13]. On the other hand, electrophysiological evidence to date does not support a similar effect of ethanol on serotonergic neuronal activity in the dorsal raphe nucleus of stock Sprague-Dawley rats [3]. However, biochemical data from *in vivo* dialysis experiments suggest that enhanced release of 5-HT occurs in the striatum of Sprague-Dawley rats

TABLE 5

LEVELS OF DOPAMINE, SEROTONIN AND THEIR METABOLITES IN THE NUCLEUS ACCUMBENS OF ALCOHOL-TOLERANT AND ALCOHOL-NONTOLERANT P RATS ONE HOUR AFTER THE IP INJECTION OF SALINE OR 2.5 g ETHANOL/kg BODY WEIGHT

| | | nmoles/g wet wt. (mean ± SEM) | | | | |
|---|---|---|---|---|---|---|
| Chronic Conditions | IP | DA | DOPAC | HVA | 5-HT | 5-HIAA |
| Nontolerant | Saline (N=11) | 47 ± 3 | 5.2 ± 0.3 | 1.8 ± 0.1 | 7.1 ± 0.3 | 3.6 ± 0.1 |
| Nontolerant | EtOH (N=21) | 43 ± 1 | 6.8 ± 0.2* | 2.8 ± 0.1* | 7.5 ± 0.1 | 4.3 ± 0.1* |
| Tolerant | EtOH (N=17) | 43 ± 1 | 5.6 ± 0.3† | 2.2 ± 0.1*† | 7.6 ± 0.1 | 3.9 ± 0.1*† |

*$p<0.05$ vs. saline-injected group and †$p<0.05$ for differences between the two ethanol-injected groups. See legend for Table 3.

at blood alcohol concentrations in the range of 220–350 mg% [11].

The effects of acute ethanol administration on the levels of DOPAC and HVA in the three forebrain regions and of 5-HIAA in the nucleus accumbens of the nontolerant P rats were significantly attenuated in the tolerant group (Tables 2–5). The difference in the levels of DOPAC, HVA and 5-HIAA between the nontolerant and tolerant P rats is not likely due to a difference in the alcohol elimination rate since, at the high dose of ethanol used and within the time frame of the experiment, the blood alcohol concentrations of the two groups were not statistically different. Therefore, these data indicate that, as a result of chronic alcohol consumption, neuronal tolerance developed in specific dopaminergic and serotonergic nuclei and/or in pathways which regulate the activity of these monoaminergic nuclei.

Since the frontal cortex and nucleus accumbens are considered to be part of the brain reward system [27,36], the present data suggest that chronic alcohol drinking by the P rats may alter part of a neuronal network involved in the reinforcing actions of ethanol. Behavioral experiments have indicated that alcohol is reinforcing to the P rat [33] and that 5-HT may be involved in this action [22]. In addition, the improved behavioral performance observed for the tolerant rats in the jumping apparatus may reflect the development of neuronal tolerance in the nigrostriatal DA pathway and/or in circuits which regulate it.

In conclusion, although there are some reports that increased DOPAC, HVA and 5-HIAA tissue levels could reflect enhanced intraneuronal metabolism unrelated to unit firing rate [4, 16, 37], interpretation of the present neurochemical data favors enhanced activity since the effects are not widespread but appear to be specific to certain pathways. Furthermore, the neurochemical data suggest that chronic ethanol consumption by the P-rat alters certain dopaminergic and serotonergic pathways in CNS regions reportedly involved in the brain reward system and dopaminergic pathways involved in motor functions.

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