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Acute and chronic ethanol-induced alterations in brain norepinephrine metabolites in the rat*

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Ethanol, in a large single dose, has been shown to cause a decrease in urinary output of 5-hydroxyindolacetic acid (5-HIAA) [1] and 3-methoxy-4-hydroxy-mandelic acid (VMA) in man [2]. Studies using [^{14}C]norepinephrine (NE) [3, 4] and [^{14}C]serotonin (5-HT) [2, 5] also revealed a decreased excretion of labeled VMA and 5-HIAA, respectively, after ethanol ingestion. These changes in the oxidative pathway of biogenic amines were accompanied by an increase in urinary excretion of labeled 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxytrypotophol (5-HTP). Similar alterations in NE metabolism have been shown in rats after acute acetaldehyde administration [6].

Since both the brain [7] and peripheral sympathetic nervous system contribute to urinary MHPG, ethanol-induced changes in the latter may or may not provide an index for the synthesis and release of NE in the brain. Ethanol, administered acutely, has been reported to decrease brain endogenous NE and 5-HT levels [8, 9]; however these results have not been confirmed by other investigators [10–13]. Studies using [^{3}H]NE have revealed that acute ethanol administration caused a decrease in NE turnover in the rat brain [14],† whereas chronic ethanol intake in a nutritionally complete diet accelerated the disappearance rate of [^{3}H]NE in the brain [14]. The present study was undertaken to extend these investigations and to determine whether ethanol does, in fact, produce a change in brain NE metabolism.

Male, Wistar rats, weighing 170–190 g, were divided into seven groups with 6–14 rats per group. Two of these groups were maintained on Purina Chow diet and then used for acute ethanol experiments, while rats in the remaining five groups were housed individually and given a nutritionally complete liquid diet (commercial Metrecal) for 2 days before being used for chronic or acute ethanol experiments.

For chronic ethanol experiments, two groups of Metrecal-fed rats were given ethanol (6%, w/v) in their liquid diet for either 4 or 8 days, while the control group was maintained on Metrecal supplemented with an isocaloric amount of sucrose for 6 days. The remaining two groups were also maintained on Metrecal supplemented with sucrose for 6 days and then used for acute ethanol experiments. The food consumption was measured daily for all groups and the animals were weighed every third day. We chose these two days for chronic ethanol because our previous studies have shown that the dramatic changes in NE uptake occur between days 5 and 9 of chronic ethanol ingestion [14].

On the scheduled days, rats were lightly anesthetized with ether and injected intracisternally with 6·6 μg *dl*[7-^{3}H]NE (0·12 μg, sp. act. 10 Ci/m-mole, obtained from New England Nuclear Corp.) in 20 μl Merle's solution by the method of Schanberg *et al.* [15]. All animals used for acute ethanol experiments were fasted for 2 hr prior to injection of [^{3}H]NE and then received either ethanol, 4 g/kg (p.o.), or saline, 30 min later. All animals were killed by decapitation 90 min after [^{3}H]NE. The brains were quickly removed, rinsed in cold saline, blotted dry on filter paper, and then frozen in liquid nitrogen. The blood was collected during decapitation, in cold heparinized centrifuge tubes for plasma ethanol determination.

The frozen brains were weighed and homogenized in cold 0·4 N perchloric acid. After centrifugation, an aliquot of supernatant fluid was counted for total radioactivity in 10 ml Bray's solution on a Beckman model no. LS230 liquid scintillation counter. Additional aliquots were analyzed for [^{3}H]NE and [^{3}H]normetanephrine (NMN) by a dual column technique [3]; labeled glycols (both bound and free) were separated by the method of Eccleston and Ritchie [16]. The results were not corrected for percentage recoveries of NE, NMN (Dowex recoveries varied from 60 to 75 per cent) and MHPG (hydrolysis was 55–70 per cent complete). The total radioactivity for 3,4-dihydroxyphenyl glycol was less than 1 per cent in the brain. Plasma ethanol was determined by gas–liquid chromatography [17]. An unpaired Student *t*-test was used to calculate the level of significance.

Table 1 shows the effect of acute and chronic ethanol on brain NE metabolism. Acute ethanol administration (4 g/kg) to rats increased the accumulation of [^{3}H]MHPG and [^{3}H]NMN in the brain with a concurrent decrease in the disappearance rate of [^{3}H]NE.

To determine whether the Metrecal liquid diet (used for chronic ethanol experiments) had any effect on the brain NE metabolism, the rats fed this diet were given either an oral dose of saline or ethanol (4 g/kg). The pattern of [^{3}H]NE metabolism after ethanol was similar to that observed in rats maintained on Purina Chow. The mean plasma ethanol level after an oral dose of ethanol was 363 ± 29 mg/100 ml. Two-way analysis of variance showed that the diet had no significant effect on brain NE metabolism, whereas on either diet acute ethanol administration did.

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Table 1. Effect of acute and chronic ethanol on [^{3}H]norepinephrine and its metabolites in the rat brain after an intracisternal injection of 6·6 μCi *dl*[7-^{3}H]norepinephrine*

Treatment	[^{3}H]NE	[^{3}H]NMN	[^{3}H]MHPG Free	[^{3}H]MHPG Bound	Total radioactivity
Saline (9)†	54·1 ± 12·8	23·9 ± 5·9	1·2 ± 0·2	22·0 ± 7·9	137·4 ± 37·4
Acute, ethanol (11)	113·8 ± 22·5‡	48·0 ± 8·3‡	3·2 ± 0·6‡	35·3 ± 6·0	294·0 ± 54·2‡
Control (8)	64·9 ± 14·8	20·1 ± 4·1	3·4 ± 0·7	40·8 ± 9·5	206·0 ± 44·6
Ethanol-day 5 (11)	34·3 ± 6·8§	11·2 ± 3·3	1·3 ± 0·3‖	15·7 ± 4·3‖	97·0 ± 23·3‖
Ethanol-day 9 (14)	50·5 ± 10·7	20·6 ± 4·4	3·2 ± 0·6	29·0 ± 5·8	178·8 ± 37·7

* Results are expressed as nCi/g of tissue.
† Number of animals.
‡ $P < 0·05$ vs saline group.
§ $P < 0·06$ vs control group.
‖ $P < 0·05$ vs control group.

Rats which were given an ethanol-containing liquid diet consumed 12 and 13 g/kg/day of ethanol on days 5 and 9 respectively. At sacrifice, plasma ethanol ranged from 0 to 110 mg/100 ml in these rats. On day 5, the brain levels of [^{3}H]NE metabolites were decreased compared to controls, and an increase in the rate of disappearance of [^{3}H]NE was also observed (Table 1). In contrast, on day 9 of chronic ethanol intake, little or no change compared to controls was observed in the levels of labeled NE or its metabolites (Table 1).

The present study demonstrates that acute or chronic ethanol, in a nutritionally complete diet, affects the release and metabolism of NE. However, the effects of acute vs chronic administration are quite different from each other. After a single dose of ethanol, the concentration of [^{3}H]NE remaining in the brain was increased indicating a decrease in the release of NE, confirming previous findings [13, 14]. Acute ethanol treatment also caused an increase in the formation of both labeled NMN and MHPG. Pohorecky [13] reported a decrease in NE metabolites in the brain when [^{14}C]tyrosine was injected rather than [^{3}H]NE. This discrepancy may be due to the use of a different precursor. Our data suggest that ethanol may cause either an initial increase in the release of NE or a decrease in the reuptake of NE, resulting in an increased formation of NMN. Since the level of [^{3}H]MHPG was also increased after acute ethanol treatment, ethanol may cause a block in the transport of neutral metabolites of NE from the brain.

Upon chronic ethanol intake, the opposite effect is observed. The decreased concentration of [^{3}H]NE remaining in the brain by day 5 of chronic ethanol ingestion suggests an acceleration in the release of NE, as previously reported [13, 14, 18]. Additionally, a decrease in the brain levels of [^{3}H]NMN and [^{3}H]MHPG was seen. On the other hand, Pohorecky [13] reported increased brain levels of [^{3}H]NE metabolites when [^{14}C]tyrosine was injected into rats fed an ethanol-containing liquid diet for 2 weeks during which the blood ethanol levels were high. In the present study, the plasma ethanol concentration ranged from 0 to 110 mg/100 ml for the animals deprived of ethanol-containing liquid diets for 90 min prior to sacrifice. This suggests that the plasma ethanol levels may be partially responsible for the discrepancy in [^{3}H]NE metabolism of the two studies.

In contrast to the reports of other investigators [3, 4] who described a shift from acidic to neutral metabolites in the urine, no alteration in the general pattern of brain NE metabolism was observed. Since the alteration in [^{3}H]NE and its metabolites occurs on day 5 of chronic ethanol ingestion but not on day 9, adaptive changes may occur as the tolerance to ethanol develops.

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