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EFFECT OF ACUTE ETHANOL OR ACETALDEHYDE ADMINSTRATION ON THE UPTAKE, RELEASE, METABOLISM AND TURNOVER RATE OF NOREPINEPHRINE IN RAT BRAIN*

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Abstract—Administration of acetaldehyde to rats following an intracisternal injection of [^{3}H]norepinephrine (NE) produced a decrease in brain endogenous NE with a concurrent increase in disappearance rate of [^{3}H]NE. In contrast, acute ethanol administration appears to decrease both the rate of disappearance of [^{3}H]NE and endogenous NE in brain. The pattern of NE metabolites in the brain was changed by acetaldehyde but not by ethanol. In experiments where ethanol was given prior to i.c. injection of [^{3}H]NE, a small decrease in retention of [^{3}H]NE observed at 15 min was followed by an increase at 90 min exposure to [^{3}H]NE. There was a non-significant increase in [^{3}H]normetanephrine formation at 15 min. These results suggest ethanol has a dual action on NE, initially increasing the release of NE and at the same time decreasing the neuronal uptake which may decrease the turnover rate of NE. However, acetaldehyde affects only the release of NE, thus increasing its turnover rate in the brain.

Ethanol, in a large single dose, has been shown to cause increased urinary excretion of catecholamines and their metabolites in animals and man [1]. Studies with [^{14}C]norepinephrine (NE) [2, 3] and [^{14}C]serotonin [4, 5] have shown that the metabolism of these amines is shifted after ethanol from an oxidative towards a reductive pathway, probably because of the competitive action of acetaldehyde [6].

A decrease in brain endogenous levels of NE has been reported after acute ethanol administration [7, 8] but not substantiated by others [9–13]. However, inhibition of NE synthesis by disulfiram [14] or by α-methyl-tyrosine [15] revealed that a single moderate-sized dose of ethanol can cause a transient decrease in catecholamine levels in rat brain. A very large dose of ethanol (7 g/kg) given orally, produced an increase in accumulation of [^{3}H]catecholamines with a decrease in endogenous NE after about 2 hr but only in those animals showing the most severe intoxication [8]. However, studies with inhibition of aromatic amino-acid decarboxylase suggested that tyrosine hydroxylation was enhanced following ethanol [16], and acetaldehyde was not deemed responsible since the effect was potentiated by pyrazole inhibitors of alcohol dehydrogenase [17].

In previous studies, either labeled NE precursors or inhibitors of its biosynthesis have been used to measure the action of ethanol on catecholamine biosynthesis. However, these techniques provide only an indirect measure of turnover of catecholamines because biosynthesis is affected at several different steps. Therefore, we have elected to study the effects of ethanol and acetaldehyde on the uptake, release, metabolism, and turnover rate of NE by a direct method using an intracisternal injection of [7-^{3}H]*dl*-norepinephrine.

MATERIALS AND METHODS

Animals. Male, Wistar rats weighing 140–150 g were used. The animals were on hand at least 4–5 days prior to use and were maintained on Purina Chow diet and water *ad lib.*

Release and metabolism of [^{3}*H*]*norepinephrine.* The rats were anesthetized lightly with ether and injected i.c. with 0.114 μg [7-^{3}H]-*dl*-NE (Sp. act. 4.18 Ci/mM, obtained from New England Nuclear Corp.) in 20 μl Merlis solution by the method of Schanberg *et al.* [18]. At 1, 5 or 21 hr after i.c. injection of [^{3}H]NE, the rats were divided into two groups. The group of rats used for acute ethanol experiments were fasted for 2 hr and then given either an oral dose of ethanol, 4 g/kg, as 20% w/v in saline or an equal vol. of saline, whereas the second group of non-fasted rats were injected i.c. with two doses of either acetaldehyde, 300 mg/kg, as 2% solution in saline 30 min apart of saline. All animals were killed by decapitation 1 hr after the first dose of the drug, the brains removed quickly, rinsed in cold saline, blotted dry on filter paper and then frozen in liquid nitrogen. The blood was collected immediatedly following decapitation in cold heparinized centrifuge tubes for ethanol or acetaldehyde determination.

The frozen brains were weighed and homogenized twice in 10 ml cold 0.4 N perchloric acid. After centrifugation, an aliquot of supernatant was counted for

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total radioactivity in 10 ml Bray's solution in a Packard liquid scintillation counter. Additional aliquots were analyzed for [^{3}H]NE and [^{3}H]normetanephrine (NMN) by a dual column separation technique [2], and endogenous levels of NE were measured by a fluorimetric method [19]. The *O*-methylated deaminated metabolites were estimated by the difference between total radioactivity and the sum of [^{3}H]NE and [^{3}H]NMN. All samples were corrected for their respective recoveries and efficiency of counting. Blood levels of ethanol or acetaldehyde were determined by a modified gas–liquid chromatographic method [20].

Uptake and metabolism of [^{3}H]NE. Both the experimental and control rats were fasted 12–14 hr prior to the test day. Experimental rats received an oral dose of ethanol, 4 g/kg, as 20% w/v in saline, whereas, controls were given saline. At 30, 90 or 105 min after the oral dose, the lightly anesthetized rats were injected i.c. with 0.082 μg [7-^{3}H]-*dl*-NE (sp. act. 13.8 Ci/mM) in 20 μl Merlis solution. Two hr after the oral dose, all animals were killed by decapitation and the brains removed quickly, rinsed and frozen in liquid nitrogen. The brains were analyzed for endogenous NE, [^{3}H]NE and [^{3}H]NMN as described above. Blood levels of ethanol were measured by gas–liquid chromatography.

Statistics. Results are presented as means and standard errors and levels of significance were calculated by unpaired Student's *t*-test. The turnover rates were calculated as described by Iversen and Simmonds [21].

RESULTS

Effects of ethanol or acetaldehyde on the release and metabolism of [^{3}H]NE in brain. Administration of acetaldehyde to rats caused a decrease in brain endogenous NE with a concurrent increase in rate of disappearance of [^{3}H]NE given 2 or 6 hr previously but not when [^{3}H]NE was injected 22 hr previously (Fig. 1). The sp. act. of NE was significantly decreased only at 2 hr but not at 6 or 22 hr, thus the turnover rate of NE in acetaldehyde-treated rats was increased only between 2 and 6 hr (Table 1).

Table 1. Effect of acute ethanol or acetaldehyde on the turnover rate of NE in rat brain after i.c. injection of [^{3}H]NE

Treatment	*K*/hr* 2–6 hr	*K*/hr* 6–22 hr	Turnover rate (ng/g/hr) 2–6 hr	Turnover rate (ng/g/hr) 6–22 hr
Control	0.366	0.021	171	9.59
Acetaldehyde	0.940	0.020	329	7.5
Control	0.350	0.033	160	14.38
Ethanol	0.393	0.059	140	28.0

* K = rate constant = slope/0.434.

Table 2. Effect of a single oral dose of ethanol (4 g/kg) for 1 hr or two i.p. doses of acetaldehyde (300 mg/kg, 30 min apart) on the metabolites of [^{3}H]NE in rat brain 2 hr after i.c. injection of [^{3}H]NE

Treatment	[^{3}H]NMN* (μc/g)	*O*-Methylated deaminated (μc/g)
Control	0.006 ± 0.003 (8)	0.065 ± 0.015
Acetaldehyde	0.005 ± 0.003 (13)	0.022 ± 0.003†
Control	0.014 ± 0.004 (8)	0.073 ± 0.019
Ethanol	0.017 ± 0.003 (10)	0.077 ± 0.016

* Results are expressed as means ± standard errors of the number of animals in parentheses.

† Denotes significance difference vs. control ($P < 0.05$).

Acute ethanol administration to rats produced a small decrease both in brain endogenous NE and rate of disappearance of [^{3}H]NE injected 2 or 6 hr previously but not when [^{3}H]NE was administered 22 hr previously, compared to controls (Fig. 1). Even though the sp. act. was increased at both time points, it was significant only at 2 hr, and thus the turnover rate of NE was slightly decreased in ethanol-dosed rats (Table 1). At the earliest time, the ratio of the sp. act. of NE in ethanol-dosed animals to the sp. act. in control rats was about 2, but at later times this ratio was reduced to 1.4 and 0.8 at 6 and 22 hr, respectively, suggesting that the more recently bound NE is more resistant to the depleting action of ethanol.

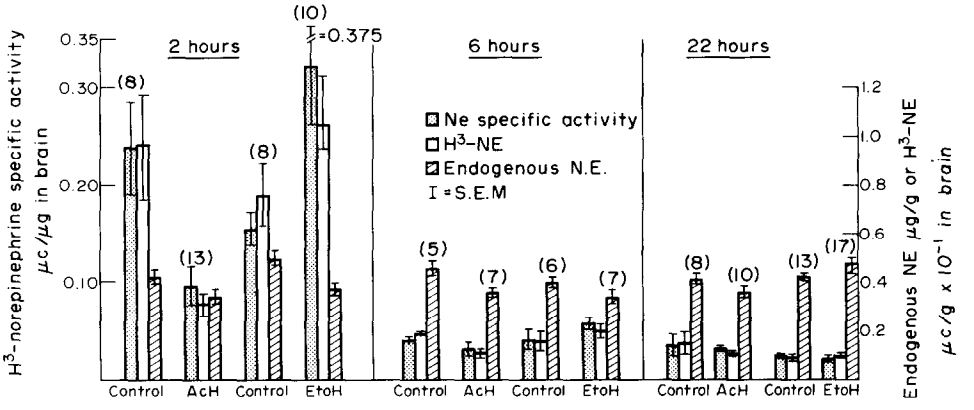

Fig. 1. Effect of a single oral dose of ethanol (4 g/kg) or two i.p. doses of acetaldehyde (300 mg/kg, 30 min apart) on the [^{3}H]NE content or rat brain at various times after i.c. injection of [^{3}H]NE. Number in parenthesis indicates number of animals used.

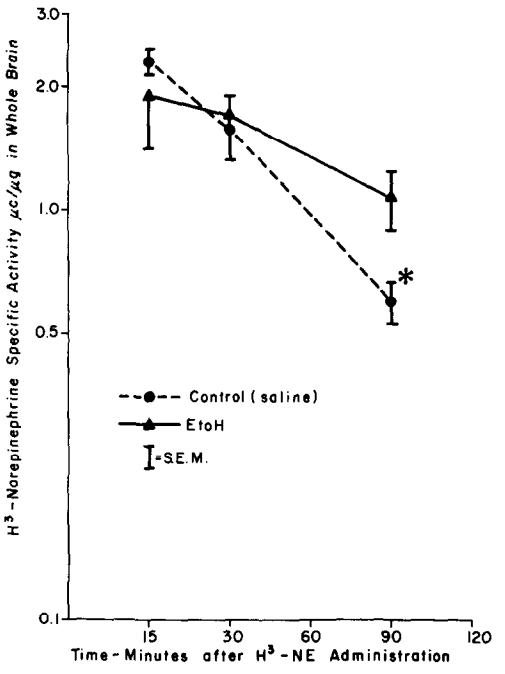

Fig. 2. Effect of exposure to an oral dose of ethanol, 4 g/kg, for 2 hr on the sp. act. of [^{3}H]NE in rat brain after i.c. injection of [^{3}H]NE. Points and bars represent means ± standard errors of 5–10 determination; asterisk denotes significance difference vs. control ($P < 0.05$).

Acetaldehyde produced a decrease in brain *O*-methylated deaminated metabolites but not in [^{3}H]NMN (Table 2). Both metabolites were unchanged after acute ethanol administration. These effects occurred at blood levels of 2.8 ± 0.3 mg/ml ethanol or 7.9 ± 1.0 μg/ml of acetaldehyde.

Effect of ethanol on [^{3}H]NE uptake and metabolism in the brain. A small decrease or no change was observed in [^{3}H]NE retained in brains of rats pretreated with ethanol and then exposed to [^{3}H]NE for 15 or 30 min (Table 3). However, a non-significant increase in retention of [^{3}H]NE in brain was observed, compared to the controls, after 90 min exposure to [^{3}H]NE. The endogenous NE level was unchanged in all the groups of rats pretreated with ethanol. The sp. act. of NE was decreased only 20% at 15 min, unchanged at 30 min, but increased significantly at 90 min exposure to [^{3}H]NE (Fig. 2). The blood ethanol levels in these experiments were approximately 3.8 mg/ml.

The rate of formation of [^{3}H]NMN in brain appeared to increase initially by 44% in ethanol pretreated rats compared to controls but was unchanged thereafter (Table 4). The concentration of *O*-methylated deaminated metabolites was similar to that of controls.

DISCUSSION

Studies have shown that a substantial portion of exogenous NE is rapidly and selectively taken up by brainstem NE-containing neurons [21–24]. The [^{3}H]NE content of brain after i.c. administration of

Table 3. Effect of exposure to an oral dose of ethanol (4 g/kg) for 2 hr on the uptake of [^{3}H]NE in the rat brain, allowing various time intervals after i.c. injection

Treatment	Time between [^{3}H]NE and kill (min)	[^{3}H]NE (μc/g)	Endogenous NE (μc/g)	Blood ethanol levels (μg/ml)
Control (5)*	15	1.08 ± 0.038†	0.40 ± 0.052	
Ethanol (8)		0.80 ± 0.176	0.51 ± 0.038	3748 ± 288
Control (6)	30	0.584 ± 0.036	0.39 ± 0.033	
Ethanol (8)		0.621 ± 0.049	0.38 ± 0.038	3937 ± 315
Control (7)	90	0.271 ± 0.032	0.46 ± 0.025	
Ethanol (10)		0.381 ± 0.051	0.39 ± 0.038	3603 ± 358

* Indicates number of animals used.
† Results are expressed as means ± standard errors.

Table 4. Effect of exposure to a single dose of ethanol (4 g/kg) for 2 hr on [^{3}H]NE metabolism in the rat brain after i.c. injection of [^{3}H]NE

Treatment	Time between [^{3}H]NE and kill (min)	[^{3}H]NMN (μc/g)	*O*-Methylated deaminated metabolites (μc/g)
Control (5)*	15	0.100 ± 0.028†	0.33 ± 0.05
Ethanol (8)		0.144 ± 0.061	0.223 ± 0.05
Control (6)	30	0.226 ± 0.040	0.103 ± 0.02
Ethanol (8)		0.160 ± 0.030	0.070 ± 0.02
Control (7)	90	0.059 ± 0.012	0.074 ± 0.02
Ethanol (10)		0.075 ± 0.015	0.120 ± 0.04

* Indicates number of animals used.
† Results are expressed as means ± standard errors.

[^{3}H]NE is a composite of neuronal uptake, release and metabolism. In drug pretreated animals, a short initial period (15 min) reflects mainly uptake, whereas when [^{3}H]NE is injected prior to drug administration, it measures both the release and re-uptake mechanisms. Thus, this technique was used in the present investigation to differentiate the action of ethanol on release and uptake mechanisms of NE in the brain.

The most unusual aspect of this study is the marked difference between ethanol and its metabolite, acetaldehyde, on catecholamine disposition. Both acetaldehyde and ethanol decreased endogenous NE in the brain but their effects on the rate of disappearance of [^{3}H]NE differed. This suggests that either these drugs released NE from different pools in the brain or that NE is released differently by these drugs. Since acetaldehyde caused a greater increase in the release and turnover rate of NE at a shorter rather than longer time period, this indicates that NE is released from the "labile pool." Thus, the central action of acetaldehyde on the release of NE appears to be similar to tyramine's action in the peripheral nervous system [25, 26] and to the central action of amphetamine [24] and in agreement with previous findings [14].

In contrast to acetaldehyde, ethanol appears to slow the disappearance rate of [^{3}H]NE at shorter time periods, suggesting a decrease in release of recently bound NE and in turnover rate and confirming previous findings [11, 27]. Studies *in vitro* have also shown that ethanol inhibited release of NE from electrically stimulated brain slices [28].

As the disappearance rate of [^{3}H]NE is a consequence of release, uptake and metabolism, a decrease in disappearance rate of [^{3}H]NE cannot be taken to indicate that only the release is affected since uptake may also be changed. In the present study, the results on [^{3}H]NE uptake after acute ethanol administration were not clear-cut. Initially, a small decrease in [^{3}H]NE retention was accompanied by an increase in NMN formation, suggesting that reuptake of NE may be affected by ethanol. Since an increase in retention of NE was observed at 90 min exposure, it suggests that ethanol may have a biphasic effect on NE turnover. Hunt and Majchrowicz [13] also reported a biphasic effect on NE turnover rate after acute ethanol administration but used the catecholamine synthesis inhibitor, α-methyl-tyrosine, to measure turnover. The decreased neuronal uptake of [^{3}H]NE has also been observed in *in vitro* studies [29, 30].

After both ethanol and acetaldehyde, the major metabolites present in the brain are *O*-methylated deaminated products, in agreement with previous findings with other drugs like imipramine and tricyclic antidepressants [31, 32]. Acetaldehyde caused a decrease in these metabolites without any change in NMN formation, suggesting that either release NE is inactivated i.e. or the transport of these metabolites from brain is altered.

In summary, our data suggest that ethanol affects both the uptake and release of NE in brain, whereas acetaldehyde causes only an increase on the release of NE, thereby its turnover rates. Thus, acetaldehyde may play a role in only one phase of ethanol's action on catecholamine disposition.

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