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Alteration of Endogenous Catecholamine Metabolism by Ethanol Ingestion.* (32298)

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Recent work in this laboratory has demonstrated that ethanol ingestion in man evoked marked alterations of C^{14}-norepinephrine metabolism. Following the ingestion of ethanol, a significant portion of intravenously administered C^{14}-norepinephrine was diverted from the major oxidative route of metabolism which results in the formation of C^{14}-3-methoxy-4-hydroxymandelic acid to a reductive route as evidenced by an increased excretion of C^{14}-3-methoxy-4-hydroxyphenylglycol(1, 2).

In this study chemical determinations of 3-methoxy-4-hydroxymandelic acid (MHMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were performed on human urine collected under the same experimental design used in the isotope study. These assays were necessary to determine if the excretion of endogenous 3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol derived from the metabolism of both epinephrine and norepinephrine was also markedly altered by ethanol ingestion. Administration of moderate amounts of ethanol has been reported to result in increased urinary excretion of epinephrine and norepinephrine in man and experimental animals(3, 4,5). Therefore, total 0-methylated catecholamine (metanephrine and normetanephrine) levels were also assayed in order to determine if ethanol ingestion resulted in increased excretion of the total 0-methylated catecholamine metabolites.

TABLE I. General Outline of Experimental Program.

	Collection period	Time of collection	Treatment
Day 1	I	8 AM– 4 PM	Ethanol*
	II	4 PM–12 MN	—
	III	12 MN– 8 AM	Ethanol*
Day 2	I	8 AM– 4 PM	—
	II	4 PM–12 MN	Ethanol*
	III	12 MN– 8 AM	—

* Each subject ingested 60 ml of absolute ethanol diluted with orange juice at the beginning of these collection periods.

Materials and methods. The study was conducted using 2 normal adult males weighing 75 and 87 kg. Urine was collected for 48 hours using six 8-hour collections which were stored in the refrigerator. At the end of each 8-hour collection period the urine was diluted to 1000 ml with deionized water and stored frozen until analyzed. At the beginning of every other collection period each subject drank 60 ml of absolute ethanol diluted in orange juice. Thus, during the 2-day study there was a urine collection for a given 8-hour period with and without ethanol treatment. Details of the time of ethanol administration and urine collections are given in Table I.

The 0-methylated acid, glycol, and amine metabolites of the catecholamines were isolated from urine, oxidized with periodate to vanillin, and the vanillin was determined spec-

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trophotometrically. 3 - Methoxy - 4 - hydroxymandelic acid was measured in unhydrolyzed urine by the method of Pisano and associates (6). 3-Methoxy-4-hydroxyphenylglycol and total metanephrines (metanephrine and normetanephrine) were determined in Glusulase-hydrolyzed urine essentially as described by Ruthven and Sandler(7). However, after the enzymatic hydrolysis, the urine was passed through a column of the weakly basic anion exchange resin, Amberlite CG-4B, 100-200 mesh, acetate form, pH 4.5, prepared as described previously(8). This modification effectively removed all the 3-methoxy-4-hydroxymandelic acid and obviated any carry over of the acid metabolite during the extraction of the glycol. The strongly acidic cation exchange resin, Dowex-50 x 12, 200-400 mesh, ammonium form, was substituted for the weakly acidic cation exchange resin, Amberlite CG-50, for the isolation of the total metanephrines because of the improved recoveries obtained.

TABLE II. Effect of Ethanol Ingestion on Urinary Excretion of 0-Methylated Catecholamine Metabolites.

Subject	Collection period	Time of collection	0-Methylated catecholamine metabolites (mg/collection period)							
			MHMA		MHPG		Metanephrines		Total	
			Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
A	I	8 AM– 4 PM	1.48	.99	.81	1.49	.24	.41	2.53	2.89
	II	4 PM–12 MN	1.52	1.09	.81	1.67	.17	.42	2.50	3.18
	III	12 MN– 8 AM	1.15	.46	.55	1.09	.21	.17	1.91	1.72
	I–III	8 AM– 8 AM	4.15	2.54	2.17	4.25	.62	1.00	6.94	7.79
B	I	8 AM– 4 PM	2.53	1.77	1.39	2.43	.16	.20	4.08	4.40
	II	4 PM–12 MN	2.15	1.46	1.38	2.12	.22	.27	3.75	3.85
	III	12 MN– 8 AM	2.08	.94	.83	1.73	.21	.22	3.12	2.89
	I–III	8 AM– 8 AM	6.76	4.17	3.60	6.28	.59	.69	10.95	11.14

Results and discussion. The quantities of the major catecholamine metabolites, 3-methoxy-4-hydroxymandelic acid, 3-methory-4-hydroxyphenylglycol, and total metanephrines (metanephrine and normetanephrine), in the urine of both subjects in the control and ethanol treatment periods are given in Table II. Although the subjects differed in the absolute amounts of the 0-methylated catecholamine metabolites excreted, the per cent distribution of the individual 0-methylated metabolites was remarkably similar for both subjects (Fig. 1).

Although there was a modest increase in the excretion of total metanephrines following ethanol ingestion for the daytime collection periods, no increase was noted for the nocturnal collection. A similar pattern was observed for the excretion of the total 0-methylated metabolites.

A diurnal variation in the excretion of 3-methoxy-4-hydroxymandelic acid, 3-methoxy-4-hydroxyphenylglycol and total 0-methylated catecholamine metabolites was found in both the control and ethanol treatment periods for both subjects. The acid and glycol 0-methylated metabolites excreted from 12:00 midnight to 8:00 A.M., at which time the subjects were sleeping, were always less than during the other periods.

The cardinal effect of ethanol ingestion on catecholamine metabolism in every collection period was a marked decrease in excretion of 3-methoxy-4-hydroxymandelic acid and a compensatory increase in excretion of

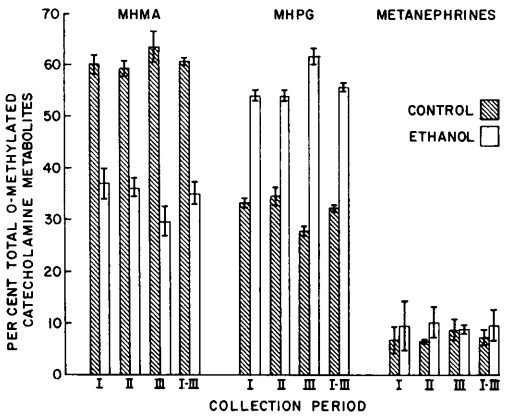

FIG. 1. Ethanol-induced alterations in excretion of 3-methoxy-4-hydroxymandelic acid (MHMA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and total metanephrines. Results are expressed as the mean per cent of the total acid, glycol, and amine O-methylated metabolites of the catecholamines. The bar represents range of results obtained from the 2 subjects. Collection periods: I (8 AM - 4 PM), II (4 PM - 12 MN), III (12 MN - 8 AM), I-III (8 AM - 8 AM).

3-methoxy-4-hydroxyphenylglycol. The per cent of the total O-methylated catecholamine metabolites excreted, during the 24-hour period, as 3-methoxy-4-hydroxymandelic acid decreased from control values of 60.8 to 35.0 when ethanol was ingested while the amount excreted as 3-methoxy-4-hydroxyphenylglycol increased from 32.1 to 55.5.

Epinephrine and norepinephrine are metabolized sequentially by both monoamine oxidase and catechol-O-methyl transferase(9, 10,11). Both of these major routes of metabolism have in common the oxidative deamination of the catecholamine or the O-methylated catecholamine to form the corresponding glycol aldehyde. The mechanism by which ethanol ingestion diverts the metabolism of the glycol aldehyde intermediate of the catecholamines from the normal predominant oxidative route resulting in 3-methoxy-4-hydroxymandelic acid formation to a reductive pathway yielding 3-methoxy-4-hydroxyphenylglycol is open to speculation. As postulated previously, the alteration could be mediated by the elevated levels of reduced nicotinamide adenine dinucleotide effected by ethanol metabolism and/or by competitive inhibition of aldehyde dehydrogenase by acetaldehyde formed during the metabolism of ethanol (1,2).

The striking decrease in excretion of 3-methoxy-4-hydroxymandelic acid and compensatory increase in excretion of 3-methoxy-4-hydroxyphenylglycol induced by ethanol ingestion points out a possible error in interpretation of data in which only 3-methoxy-4-hydroxymandelic acid excretion is used as an index of catecholamine release. As demonstrated in this study, a decreased excretion of 3-methoxy-4-hydroxymandelic acid does not necessarily reflect decreased catecholamine release but may represent a modification in the intermediary metabolism of the catecholamine because of the multiple and alternate channels available for catecholamine metabolism.

The present studies complement and extend our recent observations on the alteration of C^{14}-norepinephrine metabolism induced by ethanol ingestion in man(2). Previous studies from our laboratory showed a marked ethanol-evoked diversion of C^{14}-serotonin metabolism from the usual oxidative pathway to C^{14}-5-hydroxyindoleacetic acid, to a reductive route with an increase in excretion of C^{14}-5-hydroxytryptophol(12). These observations strongly suggest that ethanol ingestion could similarly alter the metabolism of the intermediate aldehydes derived from other biogenic amines. The intriguing possibility that a causal relationship exists between the ethanol-induced alterations of amine metabolism and the physiological consequences of ethanol ingestion has yet to be established.

Summary. Ingestion of moderate amounts of ethanol by normal human subjects results in a striking decrease in excretion of 3-methoxy-4-hydroxymandelic acid and a compensatory increase in excretion of 3-methoxy-4-hydroxyphenylglycol. These findings are supported by previous studies using C^{14}-norepinephrine and indicate that ethanol ingestion diverts the intermediate metabolism of both epinephrine and norepinephrine from the normal oxidative route to a reductive pathway.

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Effect of Hypothalamic Extracts on RNA Synthesis in Rat Anterior Pituitary Tissue.* (32299)

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Addition of crude acid extracts of rat hypothalamus to rat anterior pituitary glands in organ culture results in release of biologically detectable quantities of trophic hormones (*e.g.*, TSH, FSH) within 2 to 6 hours of incubation(1-5). This observation has provided experimental evidence for the concept of neuroendocrine regulation of anterior pituitary gland function. Thus, peptide "releaser" hormones, produced within specific neurosecretory cells of the hypothalamus, reach cells of the anterior pituitary gland by way of the hypophyseal portal circulatory system. These substances then cause the *release* of preformed hormone and, probably, the *synthesis* of new hormone(1,2).

It is generally believed that some hormones may, as a primary event, activate transcriptive mechanisms within the nucleus of the target cell(6-8). A study of RNA synthesis in explants of rat anterior pituitary tissue *in vitro* was undertaken in an effort to determine if, in addition to their primary function of release, hypothalamic extracts affect the rate of RNA synthesis in pituitary cells. The results presented here indicate that molecules of the ribosomal precursor and ribosomal type (as well as populations of heterogeneously labeled molecules) are actively synthesized in the pituitary organ culture system. They further show that the rate of incorporation of uridine-H^3 into RNA is not increased above control levels in glands exposed to hypothalamic extracts *in vivo* or *in vitro*.

Materials and methods. Preparation of pituitaries. Adult male rats (170-300 g), obtained from the Holtzman Co., Madison, Wis., were used in these studies. They were maintained on a 12-hour light, 12-hour dark cycle with feeding *ad lib*. Animals were sacrificed by cervical dislocation, pituitary glands removed and the posterior lobes discarded. Twelve to fourteen animals were usually required for each experiment. Incubation conditions were essentially the same as those used by Meites and his co-workers(1-4). Synthetic medium 199 (Difco Labs), buffered with $NaHCO_3$ to pH 7.2-7.4, was used. One or two anterior pituitary glands (halves or quarters) were incubated (Dubnoff shaker, 60 cycles/minute, 37°C) in 10 ml Erlenmeyer flasks containing 1.5 ml of medium/gland under a 95% O_2-5% CO_2 atmosphere.

Preparation of hypothalamic extracts. Fresh whole hypothalamic tissue from 12-14 rats was placed on ice, pooled, and homogenized in 1.2 ml of cold 0.1 N HCl with a ground glass homogenizer. Time from removal of fresh tissue to homogenization was 15 minutes. Homogenates were centrifuged in 0.6 ml

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